

Safety and Health Policy and Procedure Manual

Biological Safety Manual Section 280

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I. Policy Statement

The use of biohazardous materials shall comply with the *NIH Guidelines*, the recommendations of the *BMBL*, and the American Biological Safety Association (ABSA) best practices as well as all federal, state, and local regulations.

II. Responsibilities

The various responsibilities of the parties listed below are stated in more detail in the [Policy on the use of Biohazardous Agents and Recombinant DNA in Research and Teaching Laboratories at the University of North Carolina at Greensboro](#), which is the official statement of such responsibilities. Some of the major responsibilities are as follows:

Department:

- Supervising department shall provide laboratory space suitable for work being conducted
- Under the Office of Research Compliance, establish and manage an Institutional Biosafety Committee according to Federal guidelines. The IBC is responsible for review and oversight of research with recombinant DNA, review and approval of the Laboratory Biosafety Manual, and related duties

Office of Safety:

- Develop and maintain the Laboratory Biosafety Manual
- Assist Principal Investigator with risk assessment
- Assist Principal Investigator with biohazard incident response (in case of major spill or environmental release)

Principal Investigator (PI):

- Assess risks
- Respond to biohazard incident
- Complete Laboratory Biosafety Form as part of a Laboratory Safety Plan
- Provide laboratory specific training to all personnel
- Provide or arrange bloodborne pathogens (BBP) training if research activity includes human cells
- Biohazardous waste disposal when other options (e.g., autoclave) are not available
- Other responsibilities as listed in the policy on biohazardous agents and rDNA

Laboratory Personnel:

- Conduct all procedures in accordance with the Laboratory Biosafety Manual and all other applicable university policies and procedures

III. Biosafety Level Information

Biosafety Level 1 is the basic level of protection and is appropriate for agents that are not known to cause disease in normal, healthy humans. Standard microbiological practices are to be used. No special primary or secondary barriers are required within the laboratory beyond normal open bench operations other than a sink for hand washing.

Biosafety Level 2 is appropriate for handling moderate-risk agents that cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure. BSL-1 practices are to be used as well as biohazard warning signs on doors and equipment, limited

access to laboratory, and autoclave all biological waste and lab coats. Procedures that involve aerosols or high splash potential that may increase the risk of personnel exposure shall be conducted in primary containment equipment (Class I or Class II biosafety cabinet (BSC)) or in devices such as a BSC or safety centrifuge cup. PPE should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

Biosafety Level 3 is appropriate for agents with a known potential for aerosol transmission, for agents that may cause serious and potentially lethal infections, and for agents that are indigenous or exotic in origin.

Biosafety Level 4 is appropriate for agents that pose a high individual risk of life-threatening disease by infectious aerosols and for which no treatment is available.

Microorganisms listed by Biosafety Level can be found [HERE](#), and a list of organisms and toxins classified as “Select Agents” (having the potential to be diverted for use in bioterrorism or biowarfare) can be found [HERE](#).

BSL	Agent Group	Minimum Practices	Primary Barriers	Secondary Barriers
1	Agents not known to cause disease in normal, healthy humans	Standard Microbiological Practices	Laboratory coats, gowns, or uniforms; protective eyewear; gloves; other PPE as required by Hazard Risk Assessment	Sink, impervious bench tops,- Non-porous covered chairs, windows that open fitted with screens, other items as described in Biosafety Manual
2	Moderate-risk agents that cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure	BSL-1 plus biohazard warning signs on doors and equipment, limited access to laboratory, autoclave all biological waste and lab coats	BSC used for all manipulations of agents that cause splashes or aerosols; lab coats, gowns, smocks, or uniforms; goggles, face shield, or other splatter guard; gloves; respirator as an option; other PPE as required by Hazard Risk Assessment	Self-closing doors and locks, sink, impervious bench tops, non-porous covered chairs, windows that open fitted with screens, BSC, eyewash station, autoclave, other items as described in Biosafety Manual
3	Agents with known potential for aerosol transmission; agents that may cause serious and potentially lethal infections	BSL-2 plus controlled access, all procedures involving manipulation of infectious materials conducted in BSC	Biosafety cabinet used for all manipulations of agents that cause splashes or aerosols; lab coats, gowns, smocks, or uniforms; goggles, face shield, or other splatter guard; gloves; respirator; other PPE as required by Hazard Risk Assessment	Self-closing doors and locks, laboratory separated from areas open to unrestricted traffic, access restricted to entry by series of two self-closing doors, hands free sink in each zone, impervious bench tops, non-porous covered chairs, all windows sealed, BSC, vacuum lines protected by HEPA filter, ducted ventilation system, eyewash station, autoclave, other items as described in Biosafety Manual
4	N/A	N/A	N/A	N/A

Table 1. Summary of defining characteristics and requirements for Biosafety Levels 1-4.

IV. Minimum Requirements

All researchers using potential biohazards shall, at a minimum, follow the Standard Microbiological Practices as listed below.

Standard Microbiological Practices (apply to all Biosafety Levels)

- Laboratory supervisor (PI) shall enforce all institutional policies and procedures that control access to the laboratory.
- Persons shall wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption shall not be permitted in laboratory areas. Food shall be stored

outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

- Mouth pipetting is prohibited.
- All procedures shall be performed to minimize the creation of splashes and / or aerosols.
- All work surfaces shall be decontaminated after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
- All cultures, stocks, and other potentially infectious materials shall be decontaminated before disposal using an effective method. Depending on where the decontamination will be performed, the following methods shall be used prior to transport:
 - Materials to be decontaminated outside of the immediate laboratory shall be placed in a durable, leak proof container and secured for transport.
 - Materials to be removed from the facility for decontamination shall be packed in accordance with applicable local, state, and federal regulations.
- A sign shall be installed at each entrance to the lab area that contains all required information.
- An effective integrated pest management program is required.
- Laboratory supervisor (PI) shall ensure that all laboratory personnel receive appropriate training.

Biosafety Level 1

Safety Equipment (Primary Barriers and Personal Protective Equipment)

- Special containment devices or equipment, such as Biological Safety Cabinets (BSCs), are not generally required.
- Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
- Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
- Gloves shall be worn to protect hands from exposure to hazardous materials.
 - Glove selection shall be based on an appropriate risk assessment. Alternatives to latex gloves shall be available.
 - Wash hands prior to leaving the laboratory.
 - In addition, BSL-1 workers shall:
 - Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
 - Remove gloves and wash hands when work with potentially hazardous materials has been completed and before leaving the laboratory.
 - Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols shall be rigorously followed.

Laboratory Facilities (Secondary Barriers)

- Laboratories shall have doors for access control.
- Laboratories shall have a sink for hand washing.

- The laboratory shall be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not permitted.
- Laboratory furniture shall be capable of supporting anticipated loads and uses.
- Spaces between benches, cabinets, and equipment shall be accessible for cleaning.
- Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
- Chairs used in laboratory work shall be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
- Laboratory windows that open to the exterior shall be fitted with screens.

Biosafety Level 2

Safety Equipment (Primary Barriers and Personal Protective Equipment)

- Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices shall be used whenever:
 - Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
 - High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
- Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use shall be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., library, administrative offices, etc.). Dispose of protective clothing appropriately, or deposit it for laundering by the department. Laboratory clothing shall not be taken home.
- Eye and face protection (goggles, mask, face shield, or other splatter guard) shall be used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection shall be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.
- Gloves shall be worn to protect hands from exposure to hazardous materials. Glove selection shall be based on an appropriate risk assessment. Alternatives to latex gloves shall be available. Gloves shall not be worn outside the laboratory. In addition, BSL-2 laboratory workers shall:
 - Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
 - Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols shall be rigorously followed.

- Eye, face, and respiratory protection shall be used in rooms containing infected animals as determined by the risk assessment.

Laboratory Facilities (Secondary Barriers)

- Laboratory doors shall be self-closing and have locks in accordance with UNCG policies.
- Laboratories shall have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
- The laboratory shall be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
- Laboratory furniture shall be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment shall be accessible for cleaning.
 - Bench tops shall be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - Chairs used in laboratory work shall be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
- Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they shall be fitted with screens.
- BSCs shall be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs shall be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
- Vacuum lines shall be protected with High Efficiency Particulate Air (HEPA) filters or their equivalent. Filters shall be replaced as needed. Liquid disinfectant traps may be required.
- An eyewash station shall be readily available.
- There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
- HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified in accordance with this document and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation shall be verified.
- A method for decontaminating all laboratory wastes shall be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

Biosafety Level 3 – Not Currently Approved

Research requiring BSL3 containment is not currently permitted at UNCG, according to the [Policy on the use of Biohazardous Agents and Recombinant DNA in Research and Teaching Laboratories at the University of North Carolina at Greensboro](#). Following the appropriate

petitions and approvals, that policy could be amended in the future to permit BSL3 work, if satisfactory physical facilities and research expertise exist. The following details are provided for information and planning purposes.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

- All procedures involving the manipulation of infectious materials shall be conducted within a BSC (preferably Class II or Class III) or other physical containment devices.
- Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls shall be worn by workers when in the laboratory. Protective clothing shall not be worn outside of the laboratory. Reusable clothing shall be decontaminated with appropriate disinfectant before being laundered. Clothing shall be changed when contaminated.
- Eye and face protection (goggles, mask, face shield or other splatter guard) shall be used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection shall be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories shall also wear eye protection.
- Gloves shall be worn to protect hands from exposure to hazardous materials. Glove selection shall be based on an appropriate risk assessment. Alternatives to latex gloves shall be available. Gloves shall not be worn outside the laboratory. In addition, BSL-3 laboratory workers shall:
 - Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
 - Wear two pairs of gloves when appropriate.
 - Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
 - Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols shall be rigorously followed.
- Eye, face, and respiratory protection shall be used in rooms containing infected animals.

Laboratory Facilities (Secondary Barriers)

- Laboratory doors shall be self closing and have locks in accordance with UNCG policies.
- The laboratory shall be separated from areas that are open to unrestricted traffic flow within the building.
- Access to the laboratory shall be restricted to entry by a series of two self-closing doors.
- A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.
- Laboratories shall have a sink for hand washing. The sink shall be hands-free or automatically operated. It shall be located near the exit door.
- If the laboratory is segregated into different laboratories, a sink shall also be available for hand washing in each zone.

- Additional sinks may be required as determined by the risk assessment.
- The laboratory shall be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces shall be sealed. Spaces around doors and ventilation openings shall be capable of being sealed to facilitate space decontamination.
 - Floors shall be slip resistant, impervious to liquids, and resistant to chemicals. Consideration shall be given to the installation of seamless, sealed, resilient, or poured floors with integral cove bases.
 - Walls shall be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
 - Ceilings shall be constructed, sealed, and finished in the same general manner as walls.
- Decontamination of the entire laboratory shall be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory shall be based on the risk assessment of the biological agent(s) in use.
- Laboratory furniture shall be capable of supporting anticipated loads and uses.
- Spaces between benches, cabinets, and equipment shall be accessible for cleaning.
 - Bench tops shall be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
 - Chairs used in laboratory work shall be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
- All windows in the laboratory shall be sealed.
- BSCs shall be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs shall be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
- Vacuum lines shall be protected with HEPA filters or their equivalent. Filters shall be replaced as needed. Liquid disinfectant traps may be required.
- An eyewash station shall be readily available in the laboratory.
- A ducted air ventilation system is required. This system shall provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that, under failure conditions, the airflow will not be reversed.
 - Laboratory personnel shall be able to verify directional air flow. A visual monitoring device that confirms directional air flow shall be provided at the laboratory entry. Audible alarms shall be installed to notify personnel of air flow disruption.
 - The laboratory exhaust air shall not re-circulate to any other area of the building.
 - The laboratory building exhaust air shall be dispersed away from occupied areas and from building air intake locations or the exhaust air shall be HEPA filtered.
- HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified in accordance with

this document and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation shall be verified. Supply air shall be provided in such a manner that prevents positive pressurization of the cabinet.

- A method for decontaminating all laboratory wastes shall be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
- Equipment that may produce infectious aerosols shall be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters shall be tested and / or replaced at least annually.
- Facility design consideration shall be given to means of decontaminating large pieces of equipment before removal from the laboratory.
- Enhanced environmental and personal protection may be required by the agent summary statement; risk assessment; or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings shall have gas-tight isolation dampers; decontamination ports; and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing shall allow for leak testing of each filter and assembly. The filters and the housing shall be certified at least annually.
- The BSL-3 facility design, operational parameters, and procedures shall be verified and documented prior to operation. Facilities shall be re-verified and documented at least annually.

Biosafety Level 4 – Not Currently Approved

Research requiring BSL4 containment is not currently permitted at UNCG, or anticipated in the future, according to the [Policy on the use of Biohazardous Agents and Recombinant DNA in Research and Teaching Laboratories at the University of North Carolina at Greensboro](#). Details about BSL4 requirements can be found in the [BMBL](#) handbook and other online sources.

V. Cell and Tissue Culture

Cell and tissue may contain viruses. It is prudent to consider all cell lines to be potentially infectious. Most cell and tissue cultures can be safely manipulated using BSL-2 practices and containment.

- All primary and permanent human or other primate cell lines or tissue cultures shall be handled using BSL-2 practices and containment.
- Personnel handling human cell and tissue cultures shall participate in the UNCG Bloodborne Pathogen Exposure Control program.

- If any cell or tissue cultures are known or suspected to contain a specific pathogen or oncogenic virus, appropriate biosafety practices for handling that virus shall be used when working with the cell or tissue culture.
- BSL-1 practices and containment may be used for cell lines that meet all of the following criteria. Cells shall:
 - Not be of human or other primate origin.
 - Be confirmed not to contain human or other primate pathogens, including viruses, pathogenic bacteria, mycoplasma, or fungi.
 - Be well-characterized in regard to low risk.

VI. Training

All laboratory and support personnel shall be trained on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. The Principal Investigator shall be knowledgeable of all the hazards associated with their research and introduce the controls necessary to ensure safe operation.

The Principal Investigator shall provide initial (prior to work) and annual biosafety training to all laboratory personnel (students, graduate students, and other researchers) conducting biohazardous work. The training shall include, at a minimum, appropriate sections of the HHMI's DVD *Safety in the Research Laboratory* and *Practicing Safe Science* and the following:

- Risk factors of specific biohazardous agents used in the laboratory
- Routes of exposure
- Hand washing and other hygiene procedures
- Containment facilities and hazard(s) associated with laboratory equipment
- Personal Protective Equipment
- Use of Biological Safety Cabinet
- Decontamination sterilization procedures
- Emergency procedures and handling of biohazardous agents release
- Disposal procedures for biohazardous waste

VII. Signs

Anyone entering areas where biohazardous materials are used shall be aware of the potential hazards. Specific door signs for each laboratory can be requested from the Office of Safety for each entry door to the laboratory.

VIII. Sharps Procedures

A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

- Needles and Syringes:
- Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware shall be substituted for glassware whenever possible.

- Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles shall not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they shall be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps shall be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving. All containers shall be labeled with warning labels.
- Syringes which re-sheath the needle, needleless systems, and other safety devices are used when appropriate.
- Broken glassware shall not be handled directly by hand but shall be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass shall be decontaminated by autoclaving and then labels removed before disposal.

IX. Equipment

Biosafety Cabinets

Biosafety cabinets (BSC) are one type of primary safety device within an overall biosafety program. BSCs are designed to provide protection of personnel, environment, and product (Class I does not offer product protection) and are available in three classes: Class I, Class II, and Class III. Most BSCs utilize high efficiency particulate air (HEPA) filters in the exhaust and supply (Class I does not have HEPA filtered supply air).

All BSCs shall be certified by a factory certified specialist at least annually and whenever the BSC is moved or serviced. This shall be completed by the Principal Investigator and the certification forwarded to the Office of Safety upon completion.

HEPA filters provide a control of airborne particulate materials (including bacteria, spores, and viruses) by removing at least 99.7% of the most penetrating particle size (MPPS) of 0.3 μm . Particles smaller and larger than the MPPS are removed with greater efficiency.

Class I BSC (Figure 1):

- Provides protection for personnel and environment – Does not protect product.
- Similar in air movement (unfiltered room air is drawn across the work surface) to a chemical fume hood with the addition of a HEPA filter in the exhaust system to provide environmental protection.
- A minimum velocity of 75 linear feet per minute (lfpm) through the front opening shall be maintained to provide personnel protection.
- May be ducted to the building exhaust system or may be recirculated into the room after HEPA filtration.

Class II BSC (Figures 2, 3, and 4):

- Provides protection for personnel, environment and product.

- Airflow is drawn into the front grille of the cabinet, providing personnel protection.
- Downward laminar flow of HEPA-filtered air provides product protection.
 - Utilizes laminar flow principle – Unidirectional air moving at a fixed velocity along parallel lines, which aids in the capture and removal of airborne contaminants from the air stream. If the air curtain is disrupted (e.g., movement of materials in and out of a cabinet, rapid or sweeping movement of the arms), the potential for contaminant release into the laboratory work environment is increased as is the risk of product contamination.
- Exhaust air passes through a HEPA filter, providing environmental protection.
- Utilizes HEPA filters on the supply and exhaust air.
- Designed for work involving microorganisms assigned to biosafety levels 1, 2, and 3.
- Divided into four types (Type A1, Type A2, Type B1, and Type B2):
 - Type A1:
 - Exhaust air may be recirculated to the laboratory.
 - Not to be used with work involving volatile, toxic chemicals.
 - Type A2:
 - Exhaust air may be recirculated to the laboratory.
 - May be used when working with volatile, toxic chemicals only if exhausted to the outside.
 - Type B1:
 - Exhaust air shall be discharged to the outside via a hard connection.
 - May be used when working with volatile, toxic chemicals.
 - Meets the specification requirements of the National Cancer Institute (NCI) for a General Purpose Clean Air Biological Safety Cabinet
 - Type B2:
 - Exhaust air shall be discharged to the outside via a hard connection.
 - May be used when working with volatile, toxic chemicals.

Class III (Figure 5):

- Designed for work with highly infectious microbiological agents and for the conduct of hazardous operations.
- Provides maximum protection for the environment and worker.
- Gas-tight enclosure with a non-opening window.
- Supply and exhaust air are HEPA filtered – exhaust air must pass through two HEPA filters or a HEPA filter and an incinerator before discharge outdoors.

Figure 1.

Class I BSC. A. front opening, B. sash, C. exhaust HEPA filter, D. exhaust plenum. Note: The cabinet needs to be hard connected to the building exhaust system if toxic vapors are to be used.

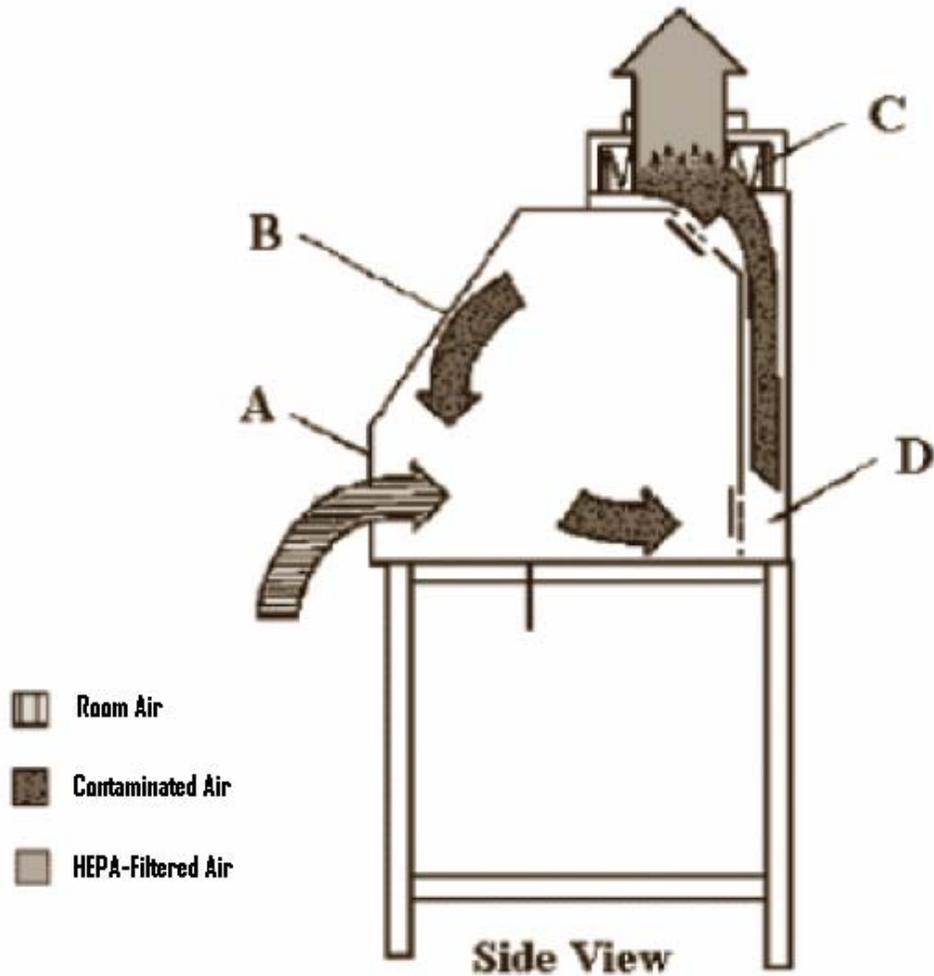


Figure 2.

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

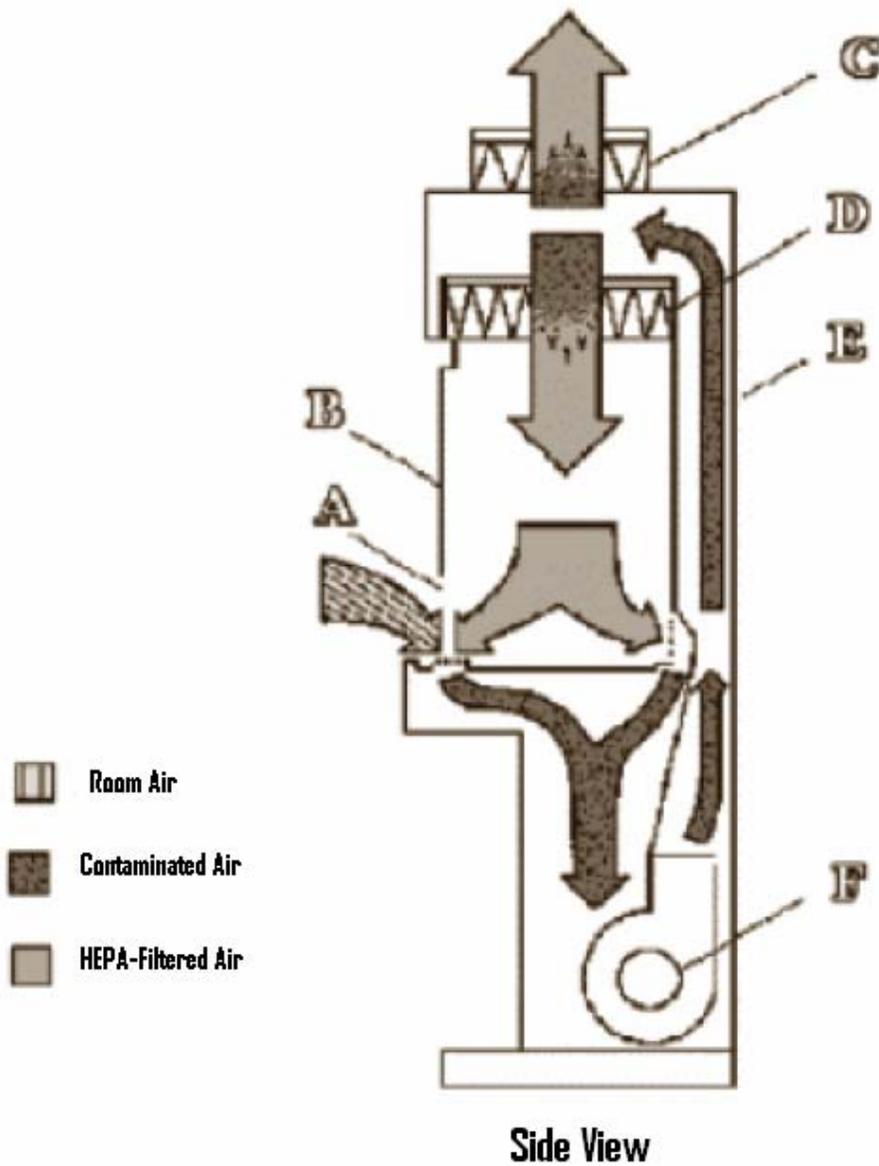


Figure 3.

Class II, Type B1 BSC. 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. Note: The cabinet exhaust needs to be hard connected to the building exhaust system.

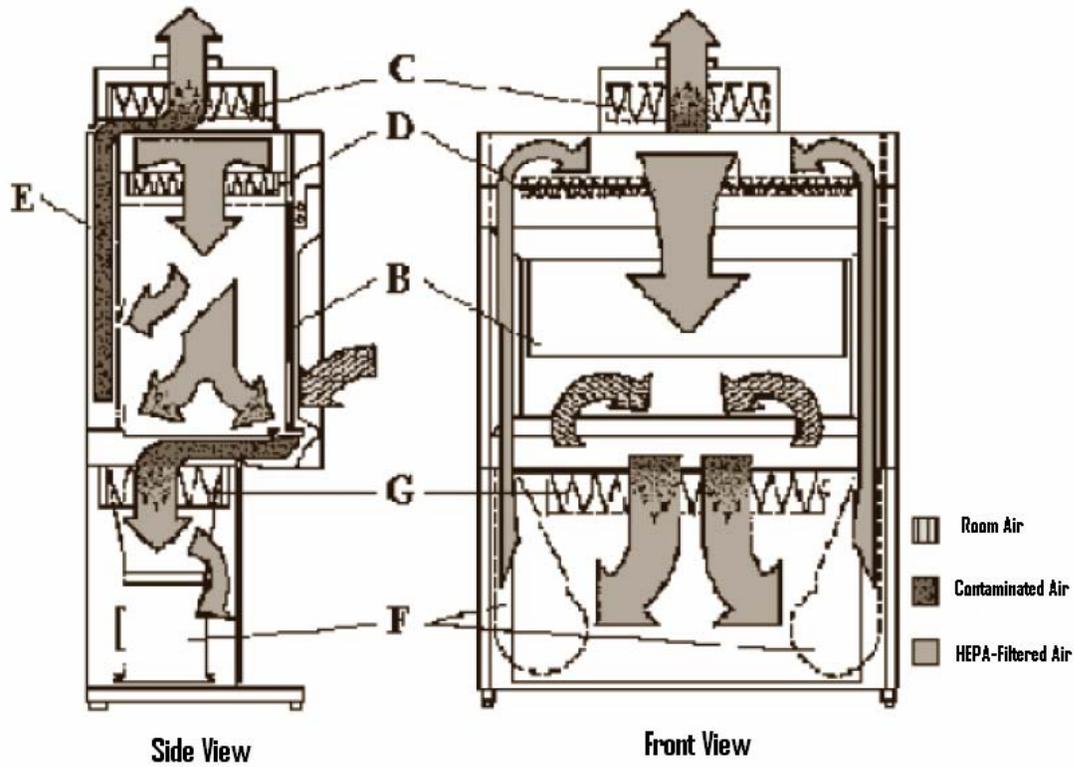


Figure 4.

Class II, Type B2 BSC. A. front opening, B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. negative pressure exhaust plenum, F. filter screen. Note: The carbon filter in the exhaust system is not shown. The cabinet needs to be hard connected to the building exhaust system.

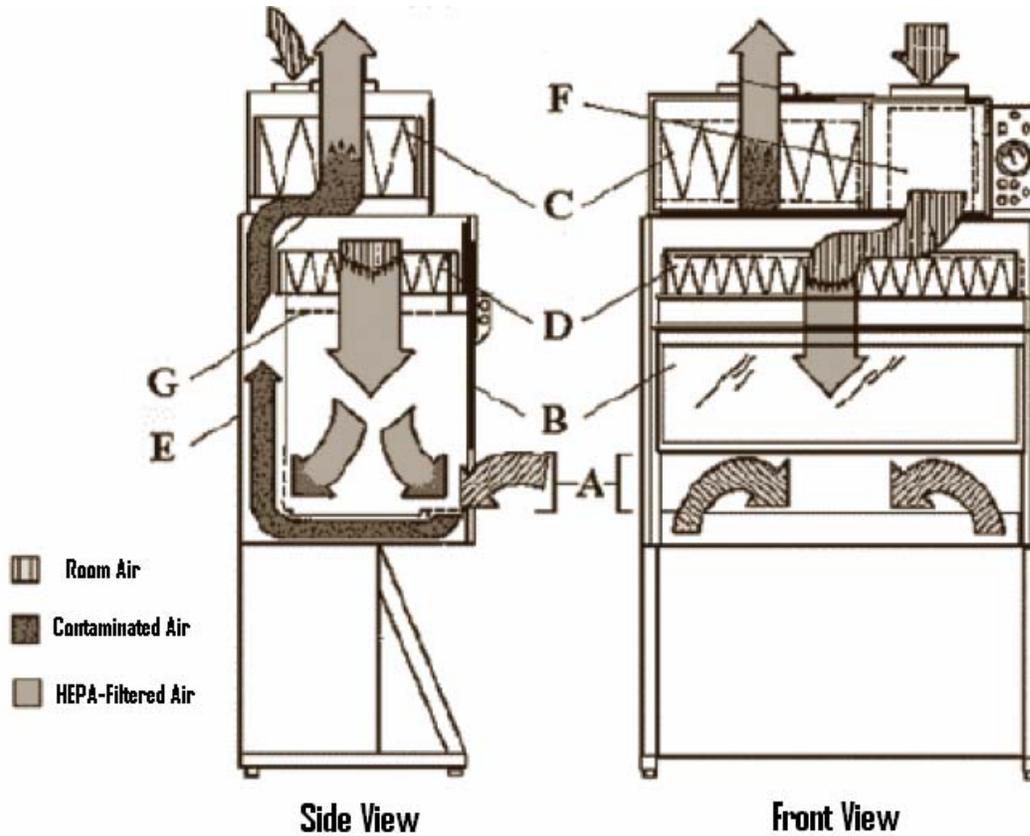
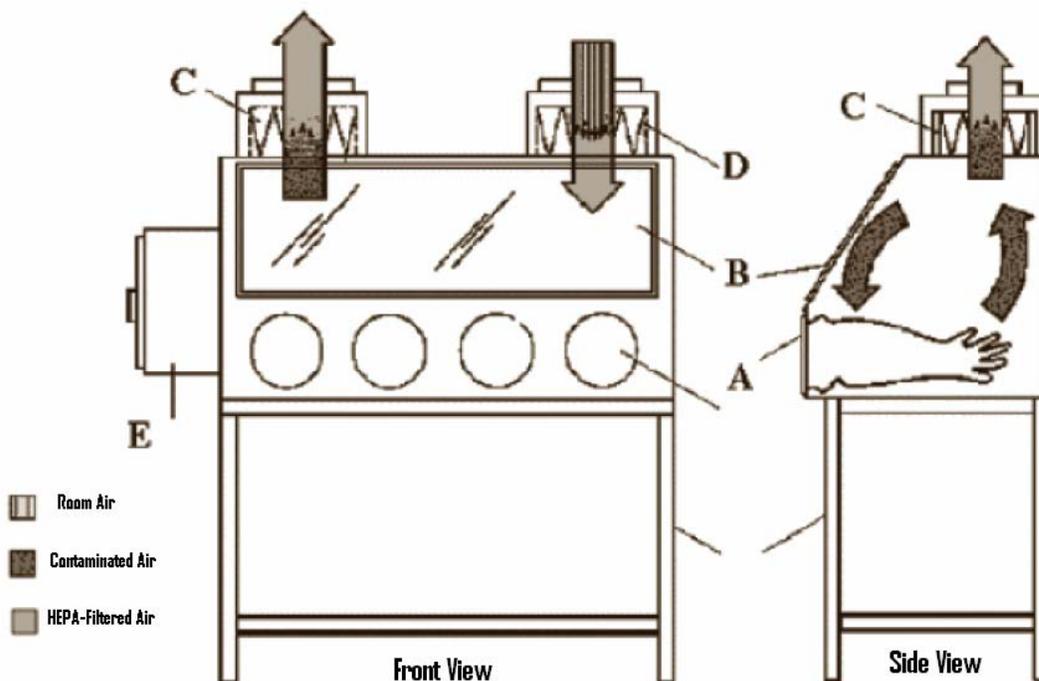


Figure 5.

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 independent dedicated exhaust system. The exhaust air must be double HEPA filtered or HEPA filtered and incinerated.



X. Personal Protective Equipment:

- Shall be based on risk assessment and hazards present and shall be listed in the Laboratory Safety Plan.
- Shall be provided without cost to all laboratory personnel with appropriate training in storage, use, limitations, and disposal.
- Shall be maintained in good operating or useable condition.

Specific examples of Personal Protective Equipment

Lab Coats, Gowns, Smocks, and Uniforms

- Include:
 - Long-sleeved lab coat
 - Closed-toe shoes
- May include:
 - Shoe Covers
 - Hair nets
- Prevent exposure to skin and street clothing.

- Prevent contamination of culture from workers.
- Shall remain in laboratory.
- Follow commercial laundering service's guidelines.
- When required, shall be autoclaved prior to laundering by a commercial laundering service.

Eye and Face Protection

- Include:
 - Safety glasses (including prescription safety glasses)
 - Goggles
 - Face Shield
- Prevent exposure to eyes, nose, mouth, and skin.

Gloves

- Include:
 - Disposable
 - Utility
- Prevent exposure to skin.
- Cuff of glove shall overlap lower sleeve of lab coat.
- Disposable gloves shall not be reused.

Respirators

- Include:
 - N-95
 - Half-face
 - Full-face
- Prevent inhalation of aerosolized microorganisms.
- Requires adherence to UNCG Respiratory Protection Program.

XI. Transport and Shipping:

Shipment of infectious agents, biological products, and clinical specimens is regulated by many agencies, and requirements are not always uniform. In addition, regulations are continually modified and new ones are added. A summary of current requirements is presented here, but it is recommended that the investigator check with the various agencies before shipping any material that may be regulated. Furthermore, the shipping of any biohazardous material shall be coordinated with the Office of Safety to ensure compliance with Department of Transportation requirements.

In general, first determine whether the material you wish to ship requires a permit and begin the application process, if required. Second, decide on a carrier, and learn the packaging and labeling requirements of that carrier.

Permits

- Permits are required from the Centers for Disease Control and Prevention (CDC) to import or transport: 1) any microorganism that causes disease in humans; 2) biological materials, such as blood and tissues, when known or suspected to contain an infectious agent; 3) live insects, such as mosquitoes, known or suspected of being infected with any disease transmissible to humans; and 4) any animal known or suspected of being infected with any disease transmissible to humans. Importation permits are issued only to the importer, who must be located in the U.S. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the U.S. Public Health Service Division of Quarantine and release by U.S. Customs. Transfers of previously imported material within the U.S. also require a permit. Application for the permit should be made at least 10 working days in advance of the anticipated shipment date. Further information and application forms may be obtained at <http://www.cdc.gov/od/ohs/biosfty/inportper.htm>.
- Permits are also required from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for import or transport of organisms infectious to livestock and of biological reagents containing animal, particularly livestock, material (this includes tissue culture media containing growth stimulants of bovine origin, such as calf serum). Further information and application forms may be obtained at http://www.aphis.usda.gov/animal_health/permits/.
- Permits are also required from the USDA/APHIS for interstate movement, importation, or release into the environment (e.g., field tests) of genetically engineered organisms that are plant pests or that contain portions (plasmids, DNA fragments, etc.) of plant pests. Applications should be made at least 120 days in advance of the anticipated release or shipment date. Information and applications may be obtained at http://www.aphis.usda.gov/brs/regulatory_activities.html.
- Facility registration and completion of the CDC Form EA-101 are required by the CDC and USDS/APHIS prior to transfer of select agents and toxins (42 CFR Part 73).
- A validated license is required by the Department of Commerce for export of certain microorganisms and toxins to all destinations except Canada. Information may be obtained by calling the Office of Safety at 336-334-4357.

Packaging

Various carriers (FedEx, UPS, U.S. Postal Service, or others) have different requirements for packaging and labeling infectious substances. In addition, various agencies, such as the International Air Transport Association (IATA) and the Department of Transportation (DOT), have developed guidelines and procedures to facilitate the safe shipment of infectious substances. The Office of Safety is required to be contacted to assist with compliance. Also, check with the carrier you have chosen to determine their specific requirements for shipping infectious agents. In addition to the materials listed above that require permits, the following materials are likely to require special packaging and / or labeling:

- Infectious Substance is a viable microorganism or its toxin, which causes or may cause disease in humans.

- Diagnostic Specimen is any human or animal material, including blood, tissue, and tissue fluids, shipped for the purpose of diagnosis.
- Biological Product is a product for human or veterinary use, such as vaccines and investigational new drugs.

The basic component of all shipping requirements, with various minor modifications, is triple packaging, as follows:

- A primary container that contains the specimen;
- A secondary container that contains the primary container and packaging capable of absorbing the specimen;
- An outer rigid shipping container that contains the secondary container and other material.

Genetically Modified Microorganisms

The *NIH Guidelines for Experiments Involving Recombinant DNA Molecules* (April 2002) states that:

- Host organisms should be shipped as etiologic agents, regardless of whether they contain recombinant DNA (rDNA), if they are regulated as human pathogens, animal pathogens, or plant pests.
- Host organisms should be shipped as etiologic agents if they contain 1) rDNA that includes the complete genome of an organism that is a human pathogen, animal pathogen, or plant pest; 2) rDNA that codes for a toxin involved in eliciting human, animal, or plant disease and is carried on an expression vector or within the host chromosome; or 3) rDNA from an organism regulated as a human pathogen, animal pathogen, or a plant pest that has not been adequately characterized.

Human Blood and Tissue

- The OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030(g)) requires that all packages containing human blood and other potentially infectious materials be labeled with the universal biohazard symbol or color coded red. Various carriers may have additional requirements.

On-campus Transport Between Laboratories or Buildings

When moving infectious substances between labs or buildings on campus, the following minimum procedures shall be followed:

- Sample shall be in sealed primary container. Utilize plastic containers whenever possible.
- Place primary container in sealed secondary container with absorbent (paper towels) between primary and secondary container suitable for the volume transported.
- If dry ice is needed, the secondary container shall be placed in an outer container, with the dry ice placed between the secondary container and tertiary container (never place dry ice in a sealed container).
- Place biohazard label with agent name, lab address, and phone number on outer container.

XII. Storage

Biological Materials Inventory

Biological materials that are used or stored shall be inventoried annually. One copy of the inventory shall be kept with the Lab Safety Plan and another copy provided to the Office of Safety for centralized record keeping to comply with Federal regulations regarding biological agents and toxins.

XIII. Disposal and Disinfection

University Policies

Disposal shall be determined based on biohazardous agent and biosafety level in accordance with [UNCG Safety and Health Policy 260 – Regulated Medical Waste](#).

What If I Do Not Have Waste Handling Facilities?

If facilities for decontaminating biohazardous waste, such as autoclaves, are not available in a given work area, arrangements shall be made with a third party or other university department with the required facilities. Either action requires the notification of the Office of Safety.

Autoclaves

Elements Required for Effective Autoclave Use

Autoclaves shall be properly used to effectively sterilize their contents. Autoclave use for microbiological media preparation requires various time and temperature settings for sterilization. Individual trials should be done to determine the proper loading and time settings to determine adequate sterilization.

Autoclaving biohazardous waste shall take into account the volume of waste and the ability of steam to penetrate the load. Minimum autoclave cycle time for biohazardous waste is 45 minutes at 121°C (250°F). The following elements all contribute to autoclave effectiveness.

- **Temperature:** Unless specifically instructed by media manufacturers' directions, autoclave chamber temperature shall be at least 121°C.
- **Time:** Autoclave cycle time will vary according to the contents of the autoclave. If media is to be prepared, then the manufacturers' instructions should be followed. Adequate autoclaving time for biohazardous waste is a minimum of 45 minutes, measured after the temperature of the material being sterilized reaches 121°C and 15 PSI pressure. The tighter the autoclave is packed, the longer it will take to reach 121°C in the center of the load. It is important to assure that the material you are autoclaving is properly inactivated.
- **Contact:** Steam saturation of the load is essential for effective decontamination. Air pockets or insufficient steam supply will prevent adequate contact. To ensure adequate steam contact, leave autoclave bags partially open during autoclaving to allow steam to penetrate into the bag. Add a small amount of water inside the bag

to help ensure heat transfers to the items being decontaminated (do not add water if it will cause biohazard materials to splash out of the bag).

- **Containers:** Use leak proof containers for items to be autoclaved. Wherever possible, all considerations should be given to non-glass containers. Plastics such as polypropylene, polypropylene copolymer or fluoropolymer products are capable of being autoclaved repeatedly. Place non-borosilicate glass bottles in a tray of water to help prevent heat shock. Place plastic bags inside a secondary container in the autoclave in case liquids leak out. Plastic or stainless steel containers are appropriate secondary containers. Make sure plastic bags and pans are autoclavable, to avoid having to clean up melted plastic.
- **Indicators:** Tape indicators can only verify that the autoclave has reached normal operating temperatures for decontamination. Most chemical indicators change color after being exposed to 121°C but cannot measure the length of time spent at 121°C. Biological indicators (e.g., *Geobacillus stearothermophilus* spore strips or spore suspension) and certain chemical indicators (e.g., Sterigage) verify that the autoclave reached adequate temperature for a long enough time to kill microorganisms.
- Use autoclave tape on all bags of biohazardous waste. Before autoclaving bags of biohazardous waste, place an “X” with autoclave indicator tape over the biohazard symbol. Autoclave tape can also be used to indicate if media or equipment has been autoclaved.
- Once a month, use a biological indicator (e.g., *Geobacillus stearothermophilus* spore strips or spore suspension). Bury the indicator in the center of the load to validate adequate steam penetration. Document the biological indicator results in a log book.

Autoclave Safety

Autoclaves use saturated steam under high pressure to achieve sterilizing temperatures. Proper use is important to ensure operator safety. Prevent injuries when using the autoclave by observing the following rules:

- Wear heat resistant gloves, eye protection, closed-toe shoes, and a lab coat, especially when unloading the autoclave.
- Prevent steam burns and shattered glassware by making sure that the pressure in the autoclave chamber is zero before opening the door at the end of a cycle. Slowly open the autoclave door and allow any residual steam to escape gradually.
- Allow items to cool for at least 10 minutes before removing them from the autoclave. Be careful with glass contains that contain liquids. Superheating is a condition that occurs often in autoclaves. Superheating occurs when liquids are at a temperature above their normal boiling point but do not appear to be boiling. In situations where personnel are in a hurry removing flasks or bottles from the autoclave, these superheated containers can explode.
- Never put sealed containers in an autoclave. They can explode. Large bottles with narrow necks may boil over violently if filled too full of liquid.
- Never put solvents, volatile or corrosive chemicals (e.g., phenol chloroform, bleach, formalin, fixed tissues, etc.), or radioactive materials in an autoclave. Call

the Office of Safety at 334-4357 if you have questions about proper disposal of these materials.

Pressure Vessel Monitoring

Inspect your autoclave components regularly. Do not operate an autoclave until it has been properly repaired. Repair or service of autoclaves can generally be decontaminated using a chemical disinfectant. Choosing the appropriate chemical disinfectant depends on the surface or item needing decontamination, as well as the particular organism requiring inactivation.

Chemical Disinfection

Choosing a Chemical Disinfectant

When choosing a chemical disinfectant, the MSDS of the Public Health Agency of Canada (if available) for the agent needing inactivation, the categories of disinfectants listed in this section, and the disinfectant product label shall be reviewed.

Note: Be sure to wear eye all PPE when using any chemical disinfectant.

Personnel in the process of choosing a disinfectant shall also keep the following considerations in mind:

- How effective is the disinfectant for the particular application?
 - What is the organism requiring inactivation (Different disinfectants are more effective against different types of organisms.)?
 - How many of the organisms are present (The more organisms present, the more disinfectant required and/or the longer the application time will be.)?
- What needs decontamination (The disinfectant shall be compatible with the item to be decontaminated.)?
- Work surfaces (e.g., metal, tile, plastic, wood, concrete)
- Glassware
- Equipment (e.g., biosafety cabinet, surgical tools, cages)
- Liquids for disposal
- Does organic matter inactivate the disinfectant (Proteins in organic matter can inactivate or slow down the activity of certain disinfectants, such as bleach.)?
- What is the shelf life of the disinfectant?
- How hazardous is the disinfectant? Refer to the MSDS and the product label for this information.
 - Perform a risk assessment on the disinfectant to determine required PPE.
 - Is the disinfectant corrosive to equipment or work surfaces?
 - Does the disinfectant leave a residue?

Types of Chemical Disinfectants

The following are outlines of the basic properties and examples of the most common categories of chemical disinfectants, including alcohols, chlorine compounds, liquid formaldehyde, glutaraldehyde, iodophors, peracetic acid, phenolic compounds, and

quaternary ammonium compounds. Adequate contact time is very important to ensure complete disinfection. Contact time varies with the type of material being disinfected.

- Alcohols (e.g., ethanol, isopropanol)
 - Alcohols are the most effective against lipophilic viruses, less effective against non-lipid viruses, and ineffective against bacterial spores.
 - Optimal disinfection is attained by using 70% ethanol for 15 minutes.
 - These types of disinfectants evaporate quickly, so sufficient contact time may be difficult to achieve. Concentrations above 70% are less effective because of increased evaporation rate.
- Chlorine compounds (e.g., household bleach – 5.25% sodium hypochlorite)
 - Chlorine compounds are effective against vegetative bacteria and most viruses in solutions of 50 – 500 ppm available chlorine. Bacterial spores require concentrations of 2,500 ppm with extended exposure time. Prions require 20,000 ppm with extended exposure time.
 - A 5,000 ppm available chlorine solution is preferred for general use because excess organic materials inactivate chlorine compounds. This concentration of solution is made by diluting household bleach 1:10 with water. Shelf life for diluted bleach is approximately 24 hours, if kept in a clear container.
 - Air and light inactivate diluted solutions, so solutions must be freshly made in order to maintain adequate available chlorine concentrations. These solutions should be stored in an airtight, opaque container out of the light. Shelf life is approximately seven days. Otherwise, make up a new solution everyday.
 - Strong oxidizers are very corrosive to metal surfaces, as well as to the skin, eyes, and respiratory tract.
- Formalin – Requires initial monitoring prior to use. Contact the Office of Safety at 334-4357 to schedule monitoring.
 - Formalin is effective against vegetative bacteria, spores, and viruses.
 - Effective concentration is a 5 – 8% solution of formalin (formaldehyde in water; made by diluting a 37% solution).
 - Formaldehyde is a suspected human carcinogen and can cause respiratory problems at very low concentrations. Inhalation limits are 2 ppm for 15 minutes, 0.75 ppm for 8 hours of exposure.
 - Formaldehyde has an irritating odor and is a sensitizer, so a potential exists for developing allergic reactions.
- Glutaraldehyde mixtures (e.g., Cidex, Sporidex, and 3M Glutarex) – Requires initial monitoring prior to use. Contact the Office of Safety at 334-4357 to schedule monitoring.
 - Glutaraldehyde mixtures are effective against vegetative bacteria, spores, and viruses (more so than formaldehyde).
 - Effective concentration is 2%.
 - Chemically related to formaldehyde, vapors are irritating to the eyes, nasal passages, and upper respiratory tract.
- Iodophors – organically bound iodine compounds (e.g., Wescodyne diluted 1:10 is a popular hand washing disinfectant)

- Iodophors are effective against vegetative bacteria and viruses but not against bacterial spores.
- Effective concentration is 75 – 150 ppm.
- Iodophors are relatively nontoxic to humans, so they are often used as general disinfectants in antiseptics and surgical soaps.
- These disinfectants have built-in indicators: if the solution is brown or yellow, it is active. Sodium thiosulfate solution can be used to readily inactivate iodophors and remove iodophor stains.
- Peracetic acid
 - Peracetic acid is used most commonly to sterilize gnotobiotic animal holding chambers and equipment.
 - Peracetic acid is effective against bacteria, viruses, fungi, and bacterial spores. It is very powerful and fast acting.
 - Effective concentration is 2% in water, or 0.08% solution in 10-20% ethanol. The ethanol solution has fewer adverse properties than the 2% solution in water.
 - Peracetic acid is received as a 40% concentrated solution, which can explode if contaminated with heavy metals or reducing agents or if rapidly heated. It is also flammable and must be refrigerated. It is a potent respiratory irritant and requires a respirator for use – Contact the Office of Safety prior to use.
 - Peracetic acid is corrosive to metal surfaces.
 - Diluted solution degrades rapidly, so it must be freshly prepared for use.
- Phenolic compounds (e.g., Amphyl, Vesphene II)
 - Phenolic compounds are commonly used for disinfecting contaminated walls, floors, and bench tops.
 - Phenolic compounds are effective against vegetative bacteria, including mycobacterium tuberculosis, fungi, and lipophilic viruses. They are not effective against spores and non-lipid viruses.
 - Effective concentrations are 0.5 – 2%.
 - Phenolic compounds produce an unpleasant odor and are toxic.
 - These are irritants to the eyes, skin, respiratory tract, and gastric tract.
- Quaternary Ammonium compounds – cationic detergent (surfactant) with strong surface activity, commonly referred to as “Quats”
 - Quats are effective against fungi, Gram-positive bacteria, and lipophilic viruses but less effective against Gram-negative bacteria. They are ineffective against hydrophilic viruses or bacterial spores. Quats mixed with phenolics are very effective against disinfectants, as well as cleaners.
 - Usual effective concentration is 1:750.
 - Quats are relatively nontoxic and acceptable as a general disinfectant, such as for decontaminating food equipment or for general cleaning.
 - Quats are easily inactivated by organic materials, anionic detergents (soaps), or salts of metals found in hard water.

Procedures for Inactivation and Safety Containment of Toxins

For more information on procedures for inactivation and safety containment of toxins, please refer to the current BMBL for Guidelines for Working with Toxins of Biological Origin.

XIV. Spill / Release Info:

The following protocol is generic and is intended for use with microorganisms classified as BSL-2 or lower. The right protocol for any situation depends on the specific biohazardous material used, quantity of material spilled, and location of the spill. Spill plans shall be included in the Laboratory Safety Plan and shall include, but not be limited to, procedures, training, and the names and contact information of trained personnel from the laboratory or department.

Biohazard Spill Clean-Up Protocol

1. Biohazardous spill outside the laboratory:

- Evacuate the immediate area for at least 30 minutes to allow any potential aerosols to settle. If outdoors, personnel should remain upwind from the spill, if at all possible.
- The Office of Safety and the UNCG Police Department are available to assist in evacuation perimeter control. Laboratory personnel should secure the site while someone else is sent for help.
- The Principle Investigator or their trained designee shall clean up the spill per the biohazard spill clean-up protocol located in the Laboratory Safety Plan.

Biohazardous spill within a laboratory:

- Outside of a BSC: evacuate the laboratory for at least 30 minutes to allow any potential aerosols to settle. It is the responsibility of the last person out to ensure that all doors have been closed.
 - Within a centrifuge: the centrifuge should be closed as soon as the spill is noticed. Wait 30 minutes to allow aerosol to settle before opening to clean and disinfect.
 - Within a BSC: the BSC shall remain running. The Principle Investigator or their trained designee shall clean up the spill per the biohazard spill clean-up protocol located in the Laboratory Safety Plan.
2. Everyone not needed for spill clean up shall be cautioned to stay away from the spill area until clean up has occurred. Signs may be posted if necessary.
 3. Any potentially contaminated clothing shall be removed and placed in a biohazard waste bag for decontamination.
 4. Hands and any other contaminated skin shall be washed thoroughly with soap and water.
 5. Appropriate PPE shall be worn. At a minimum, nitrile gloves, eye protection, and a lab coat shall be worn. Face shield or mask (splash protection) is advised for spills greater than ~10 ml outside a BSC or any spill inside a centrifuge. If there is a potential for aerosolization of the spilled material, use a respirator (see the Office of Safety Respiratory Protection Manual).

6. Any sharp, contaminated objects shall be removed from the spill area using mechanical means, never with hands.
7. Paper towels shall be placed on the spilled material and disinfectant poured carefully around the edges of the spill with care taken to avoid splashing. Working from the outside of the spill toward the center avoids spreading the contamination. Place discarded paper towels into a biohazard bag for disposal.
 - o Note: Alcohol is not recommended as a disinfectant for large spills, especially inside a BSC, because large amounts of alcohol pose an explosion hazard and small amounts evaporate too quickly to ensure disinfection.
8. If the spill is inside a centrifuge, the rotor and its contents should be moved to a BSC, if possible. The external surfaces should be decontaminated prior to moving to the BSC.
9. If the spill is inside a BSC, the spill tray underneath the work area and the trough below the air intake grill shall be cleaned as well as the work area itself. These are likely to be contaminated when the spill is large. The cabinet shall be left running for at least 10 minutes before resuming use.
10. After initial clean up, paper towels shall again be placed on the spill area, flooded with disinfectant, and left to soak for at least 15 minutes or according to manufacturers' instruction. Adequate contact time is important to ensure complete decontamination.
11. A final wipe-down shall be done with clean paper towels soaked with disinfectant. Laboratory personnel shall disinfect any equipment, walls, or other areas likely to have been splashed by the spill.
12. If radioactive material is involved in the spill, also wash the surface with detergent according to radioactive spill guidelines.
13. All contaminated waste shall be disposed of properly.
14. Hands shall be washed thoroughly with soap and water.
15. Complete spill report and forward a copy to the Office of Safety.

Biohazard Spill Kit

Each laboratory using biohazardous materials shall have appropriate equipment and supplies on hand for managing spills and incidents involving biohazardous materials. Permanent equipment should include a safety shower, eyewash, and a hand-washing sink and supplies. The materials required for the Biohazard Spill Kit shall be available (though not necessarily consolidated in one location) in the areas where work is being conducted with biohazardous materials. The supplies available in a Biohazard Spill Kit should include, but are not limited to:

- A copy of the biohazard spill clean-up protocol for the laboratory (located in Laboratory Safety Plan)
- Nitrile disposable gloves (8 mil)(check for holes or deterioration; replace box of nitrile gloves every two years)
- Lab coat(s) or gowns
- Goggles or safety glasses with side shields
- Face masks
- Disposable shoe covers (booties)

- Absorbent material, such as absorbent paper towels, granular absorbent material, etc. (a disposable or cleanable scoop will be needed for granular absorbent)
- All-purpose disinfectant, such as normal household bleach (freshly diluted 1:10) or an iodophor (e.g., Wescodyne) or a quaternary ammonia preparation (e.g., EndBac II)
- Autoclavable bucket for diluting disinfectant (this can be used to store the kit contents when not in use)
- Something disposable or easily disinfected such as tongs, forceps, manila folders, etc. for picking up broken glass, other contaminated sharps, or contaminated absorbent material
- Biohazard sharps waste container(s)
- Autoclavable biohazard waste bags
- Biohazard spill warning signs

All non-disposable items shall either be autoclavable or compatible with the disinfectant to be used.