
Biological Safety Manual

UNC Greensboro

(Revised September, 2019)



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Policy Statement

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

Responsibilities

The responsibility for ensuring that work with biological materials is conducted safely extends from the administration to the end user, as outlined below.

Institutional Biosafety Committee:

- Establish guidelines, procedures, and application forms for the registration, use, and containment of rDNA and BSL-2 agents and/or procedures;
- Review, approve, and oversee rDNA research conducted at or sponsored by the University in accordance with the NIH Guidelines, regardless of the source of funding;
- Review of any experiments that introduce or expose biohazardous agents to plants, animals, or human participants;
- Assemble and approve the University's Biosafety Policy Manual which contains biosafety standards from the National Institutes of Health (NIH), Centers for Disease Control and Prevention (CDC), and other applicable regulations.

Departments:

- Provide laboratory facilities suitable for work being conducted;

Environmental Health & Safety:

- Develop and maintain the Laboratory Biosafety Manual;
- Conduct semiannual safety and compliance review of laboratories;
- 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
- Submit protocols for IBC approval for work with rDNA and other non-exempt materials;
- Identify all biological hazards (BSL \geq 1, rDNA, etc.) in Laboratory Safety Plan;
- Provide laboratory specific training to all personnel;

- Provide or arrange bloodborne pathogens (BBP) training and vaccinations if research activity includes human blood, unfixed tissues/cells, or other potentially infectious materials;
- Proper disposal of biological waste;
- Report incidents (spills, exposures, etc.) to EH&S (336-334-4357);
- 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;
- Report incidents or safety concerns to principal investigator or EH&S.

Institutional Biosafety Committee Approval

To ensure that UNCG operates its laboratories in compliance with all applicable biosafety regulations and good laboratory practices, all researchers and lab directors who plan to use rDNA must have a protocol approved by the [Institutional Biosafety Committee \(IBC\)](#) before the work can be initiated. Guidelines for the preparation of this protocol will be established by the IBC and can be found, in the [IBC section](#) of the Office of Research Integrity website. Researchers or lab directors who plan to use rDNA are responsible for knowledge of the University's procedures.

Some rDNA projects and all BSL1 agents are exempt from IBC review. Work with BSL-2/RG2 materials may be reviewed by the IBC on a case by case basis. Currently, BSL-3 and BSL-4 agents are not approved for use at UNCG. Furthermore, the procurement and use of any agent designated by the Federal Government as a Select Agent is currently prohibited at UNCG. Any UNCG investigator desiring to initiate work with BSL-3 or Select Agents must discuss this with the Associate Provost for Research and Public / Private Sector Partnerships. Registration of Select Agents with the Federal Government must be done through the university's Responsible Official (Vice Chancellor for Research and Economic Development) or their designee.

Biosafety Level Information

Four Biosafety Levels are described in this section, which outline the appropriate laboratory facilities and practices for work with biological agents or material which may carry them. Biological agents are assigned BSL's based on the risk they pose to human health and the environment. The likelihood to cause disease, severity of disease, and routes of transmission are considered when determining the appropriate BSL. All biological agents or materials used in the laboratory shall be listed on the Biological Material ID Form in the Laboratory Safety Plan.

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard microbiological practices	PPE: Lab coats and gloves recommended; eye and face protection as needed	<ul style="list-style-type: none"> • Non-porous surfaces (bench tops, floors, chairs) • Sink for hand washing • Doors for access control • Windows that open should have screens
2	Associated with human disease. Routes of transmission: percutaneous, ingestion, mucous membrane	BSL-1 practice plus: <ul style="list-style-type: none"> • Limited access • Biohazard warning signs • Sharps precautions • Written procedures for waste decontamination or medical surveillance 	Primary barriers: Class I or II BSC for manipulations that may cause splashes or aerosols, vacuum lines protected with liquid disinfectant traps; PPE: Lab coat and gloves required; Face and eye protection as needed	BSL-1 plus: <ul style="list-style-type: none"> • Eyewash readily available • Autoclave available • Windows that open are fitted with screens • Doors should be self-closing
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 plus: <ul style="list-style-type: none"> • Controlled access • Decontamination of all waste • Decontamination of lab clothing before laundering • Baseline serum 	Primary barriers: Class I or II BSC or other physical containment device used for all open manipulations of agents, vacuum lines protected with disinfectant trap and HEPA filter; PPE: Lab coat and gloves required; face, eye, and respiratory protection as needed	BSL-2 plus: <ul style="list-style-type: none"> • Physical separation from access corridors • Self-closing double-door access • Exhaust air not recirculated • Negative airflow into laboratory

Table 1. Summary of defining characteristics and lab requirements for BSL 1-4.

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. Examples of BSL-1 materials are non-pathogenic strains of bacteria and fungi.

Biosafety Level 2 is appropriate for handling materials potentially containing moderate-risk agents that cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure. BSL-2 is appropriate when work is done with

- Any infectious pathogenic microorganisms.
- Any human primary cells or cell lines.
- Other Potentially Infectious Materials (OPIM), defined as any human-derived blood, body fluids, or tissues, where the presence of an infectious agent may be unknown.
- Cells or other materials from animals known to have been infected with human pathogens.

Biosafety Level 3 is appropriate for agents of indigenous or exotic origin with a known potential for aerosol transmission and for agents that may cause serious and potentially lethal infections. Research with BSL-3 agents is CURRENTLY NOT APPROVED AT UNCG.

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. Research with BSL-4 agents is CURRENTLY NOT APPROVED AT UNCG.

Microorganisms listed by Biosafety Level can be found [HERE](#), and a list of organisms and toxins classified as “Select Agents” (having the potential to pose a severe threat to public, animal or plant health or to animal or plant products) can be found [HERE](#).

Biosafety Controls

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.
- 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 furniture shall be capable of supporting anticipated loads and uses.
- Laboratory windows that open to the exterior should be fitted with screens.

Biosafety Level 2

All BSL-1 precautions shall apply, plus:

Safety Equipment (Primary Barriers And Personal Protective Equipment)

- Properly maintained BSCs (preferably Class II) 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 potentially infectious 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. Clothing known to be contaminated shall be autoclaved prior to laundering or disposal.
- Wear two pairs of gloves when appropriate.
- 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. Gloves shall be worn to protect hands from exposure to hazardous materials.
- Potentially infectious materials shall be stored or transported in durable, leak proof containers.

Laboratory Facilities (Secondary Barriers)

- Laboratory doors should be self-closing and shall have locks for access control.
- Infectious materials shall be secured by lock when not immediately attended by authorized personnel.
- 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.

- 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). See the UNCG Policy on Regulated Medical Waste for more information.

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.

All BSL-2 precautions shall apply, plus:

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 by a validated method 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.
- Eye, face, and respiratory protection shall be used in rooms containing infected animals.

Laboratory Facilities (Secondary Barriers)

- 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. 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.
- All windows in the laboratory shall be sealed.
- 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.

- 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 BSL-4 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 BSL-4 requirements can be found in the BMBL handbook and other online sources.

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 follow the UNCG Bloodborne Pathogen Exposure Control Plan.
- 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.

Recombinant and Synthetic DNA

All work with rDNA must be registered with the [Institutional Biosafety Committee](#) and comply with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*.

Select Agents and Toxins

Certain biological agents or toxins which may pose a severe threat to human, animal, or plant health and safety are classified as Select Agents and require federal registration and approval. A list of Select Agents and Toxins can be found [HERE](#). The US Department of Health and Human Services (DHHS) regulates the possession, use, and transfer of agents or toxins that could pose a severe threat to public health and safety. The US Department of Agriculture (USDA) regulates the possession, use, and transfer of agents or toxins that could pose a severe threat to animal or plant health, or animal or plant products. As such, entities must register with CDC or APHIS if they possess, use, or transfer select agents or toxins.

The procurement and use of any agent designated by the Federal Government as a Select Agent is currently prohibited at UNCG. Any UNCG investigator desiring to initiate work with BSL3 or Select Agents must contact the Vice Chancellor for Research and Economic Development. Registration of Select Agents with the Federal Government must be done through the university's Responsible Official (Vice Chancellor for Research and Economic Development) or their designee.

Bloodborne Pathogens

The Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard (BBP, 29 CFR 1020.1030) requires specific training and inoculation for individuals with an occupational exposure to bloodborne pathogens. Bloodborne pathogens are disease-causing microbes present in blood, unfixed tissue, and other potentially infectious material. Pathogens include, but are not limited to Human Immunodeficiency Virus (HIV) and Hepatitis B and C (HBV, HBC), other infectious viruses, bacteria and protozoa. Occupational exposure is reasonably anticipated skin, eye, mucous membrane, or parenteral (subcutaneous) contact with blood or other potentially infectious materials that may result from the performance of an employee's duties.

Laboratory workers with an occupational exposure must complete Laboratory Biosafety Training within 10 days of their work assignment or commencement of activities presenting an occupational exposure, and annually thereafter. Once initial training is completed, workers must complete and submit the Bloodborne Pathogens Exposure Control Plan Enrollment Form and complete or decline the HBV vaccine series, at no cost to them. The vaccination is not required if the individual has completed the vaccine series previously or tests positive, but the declination form must be completed. Gove Student Health Center administers the vaccine to students and employees as a series of three injections, the second and third administered one and six months after the first.

The principal investigator or laboratory director is responsible for identifying workers with an occupational exposure and ensuring that required training and vaccinations (or declinations) are completed. The laboratory department is responsible for the cost of the HBV vaccination.

Training

All laboratory and support personnel working in the laboratory where biohazards are present, shall be trained on the potential hazards, 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 understand the controls necessary to ensure safe operation.

The Principal Investigator shall ensure that affected lab members complete initial training prior to commencement of work duties with potential exposure to biohazards, and annually thereafter. The training shall include, at a minimum:

- Risk factors of specific biohazardous agents used in the laboratory
- Routes of exposure

- Appropriate containment barriers per Biosafety Level
- Hand washing and other hygiene procedures
- Personal Protective Equipment
- Use of Biological Safety Cabinet (if applicable)
- Decontamination and sterilization procedures
- Emergency procedures and handling of spills or releases
- Disposal procedures for biohazardous waste

Sharps

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. Plastic ware 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.

Signs & Labels

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 EH&S Department for each entry door to the laboratory. Entry signs shall incorporate the universal biohazard symbol, appropriate biosafety level, and name and contact information for the PI and safety supervisor.

Biohazard warning labels incorporating the universal biohazard shall be placed on all stored material and equipment (BSC, incubator, centrifuge, freezer, etc.) used with potentially infectious material. Individual vials do not require labeling if stored in a labeled secondary container (rack, box) when not in use. A refrigerator or freezer does not qualify as a secondary container for this purpose.

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 is the responsibility of the Principal Investigator or their designee.

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):

- 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, & 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:

- 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.

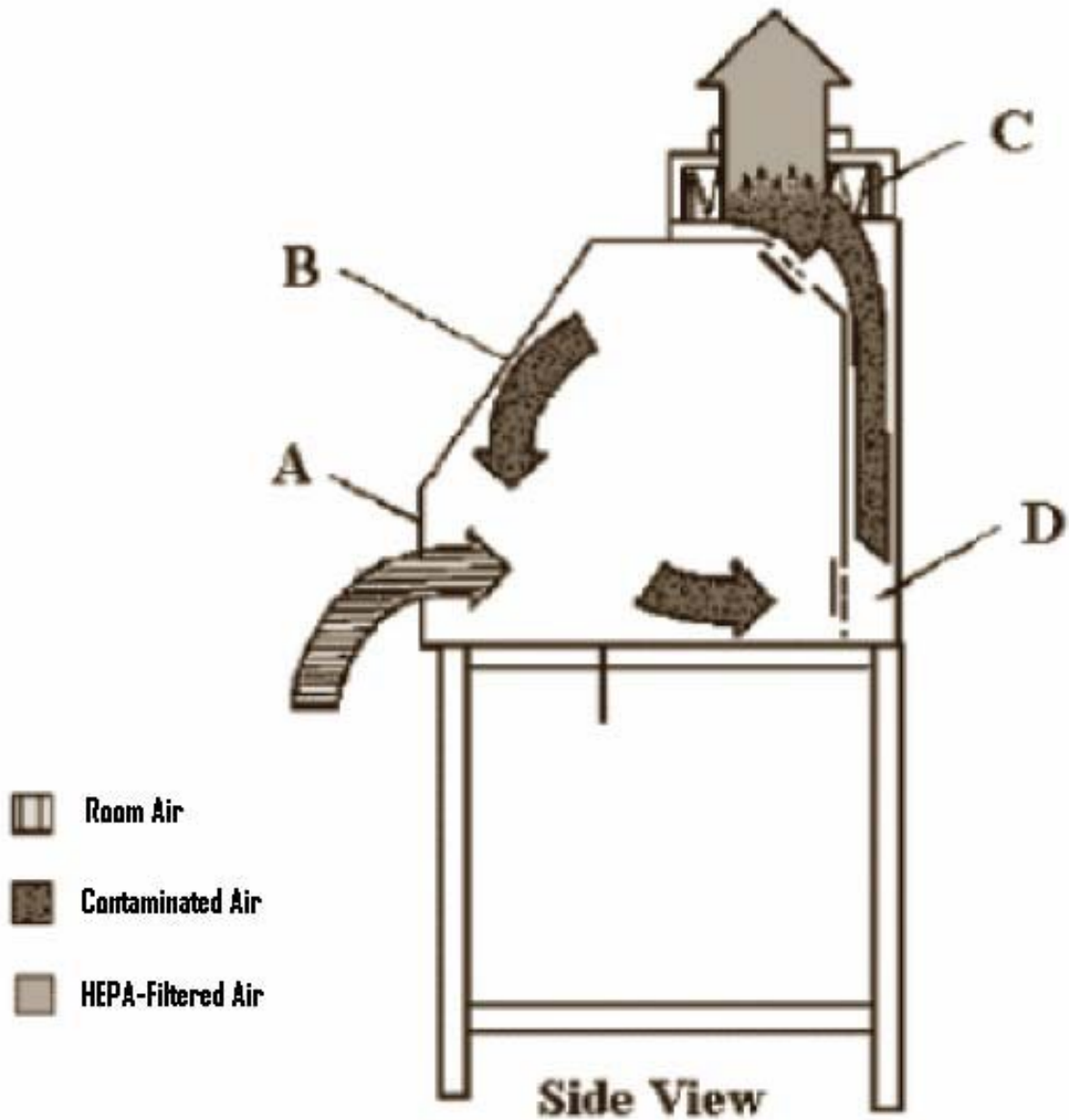


Figure 1. Class I BSC. A. front opening, B. sash, C. exhaust HEPA filter, D. exhaust plenum. Note: The cabinet needs to be hard connected to the building exhaust system if toxic vapors are to be used.

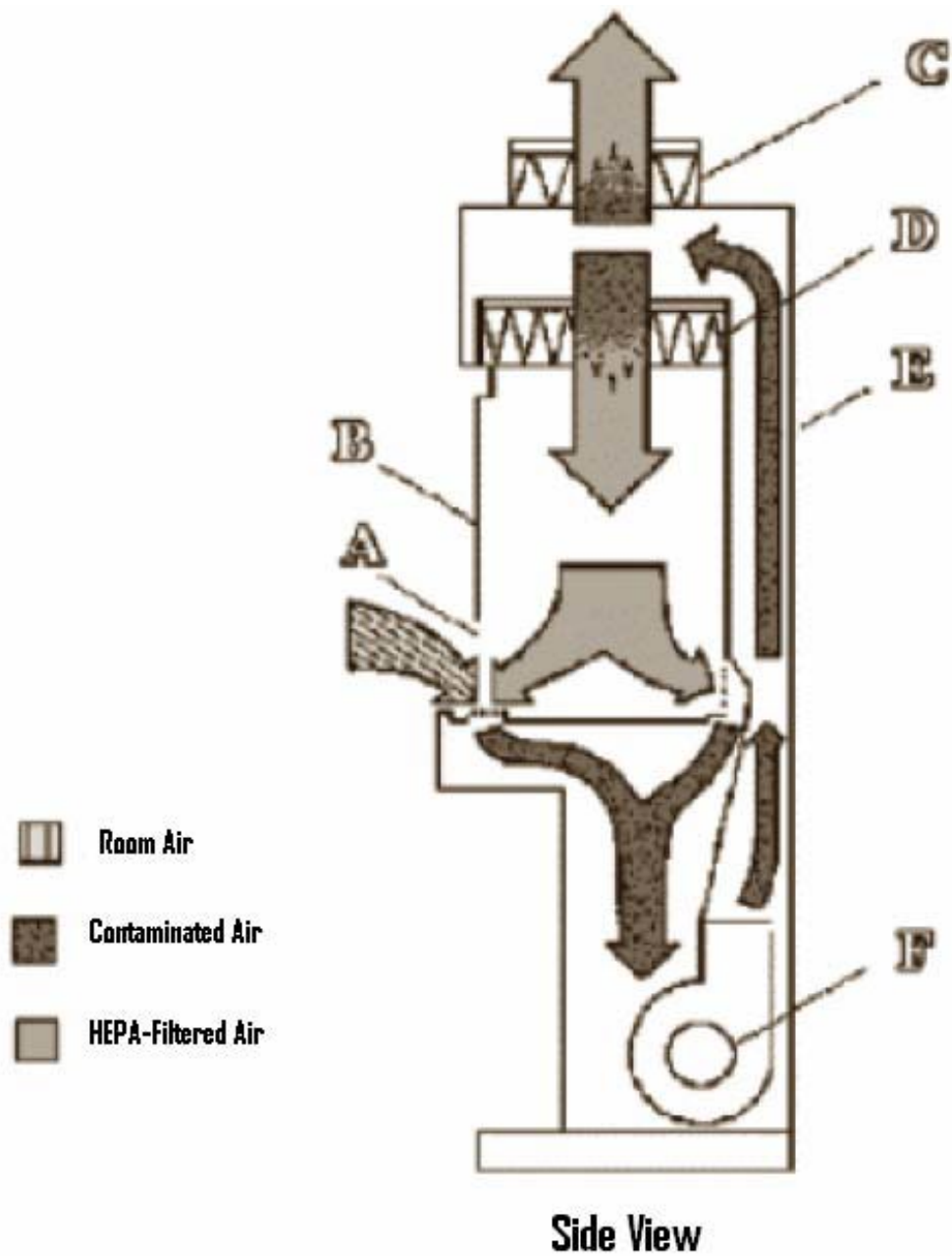


Figure 2. Class II, Type A1 BSC. A. front opening, B. sash, C. exhaust HEPA filter, D. supply HEPA filter, E. common plenum, F. blower.

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. And remember for international delivery, there may be restrictions and duty or VAT costs on shipments once they reach their destination country. A summary of current requirements is presented here. The investigator must 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 EH&S Department to ensure compliance with Department of Transportation requirements.

Export Control Regulations

Due to heightened concerns about national and homeland security, any shipments sent outside of the country must be thoroughly screened by the University Export Control Officer (ECO) to ensure the university is in compliance with Federal Export Control Regulations. Contact the ECO as soon as you know you will need to ship something out of the country. If it is determined an export license is required, it can take several weeks to obtain. These regulations are administered by the Department of Commerce, the US Department of State, the Office of Foreign Assets Control under the US Department of the Treasury, and the US Customs Service. Failure to operate within these regulations, even inadvertently, may result in significant fines, confiscation of shipped items and jail time for the shipper.

The screening process will ask the following questions:

- What is the item being shipped? (classification)
- Where is it going? (country of ultimate destination)
- Who will receive it? (ultimate end user)
- What will they do with it? (ultimate end use)
- What else do they do? (conduct of end user)
- What is the value of the item being shipped?

More information on these Export Control Regulations and how to get in touch with the university ECO can be found on the [UNCG Export Control website](http://exportcontrol.uncg.edu) at exportcontrol.uncg.edu. Or you can send an email directly to exportcontrol@uncg.edu.

Permits

To obtain the appropriate permits after the Export Control screening, 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 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/eaipp/>.

- 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 EH&S Department 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 (November 2013) 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 by the Public Health Service or as animal pathogens or plant pests under the US Department of Agriculture, Animal and Plant Health Inspection Service.
- Host organisms should be shipped as etiologic agents if they contain 1) rDNA that includes the complete genome of an organism or virus that is regulated as a human or animal pathogen, or plant pest; 2) rDNA that codes for a toxin involved in eliciting human, animal, or plant disease or inhibiting plant growth and is carried on an expression vector or within the host chromosome and/or when the host organism contains a conjugation proficient plasmid or a generalized transducing phage; or 3) rDNA from an organism regulated as a human or animal pathogen, or a plant pest that has not been adequately characterized to demonstrate that it does not code for a factor involved in eliciting human, animal, or plant disease.

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.

Biosecurity

The objective of biosecurity is to prevent loss, theft or misuse of microorganisms, biological materials, and research-related information. This is accomplished by limiting access to facilities, research materials and information. At a minimum, access to infectious materials shall be limited to authorized personnel, both administratively and by lock. Additional security measures shall be determined, based upon risk assessment, personnel expertise and responsibility, control and accountability for research materials, and existing access control elements. Additional security measures may include periodic inventories, personnel background checks, multi factor access controls, and information security policies.

Waste

University Policy

All potentially infectious materials shall be treated prior to disposal to the general waste stream. All cultures, stocks, and specimens of microorganisms or other potentially infectious materials and any lab equipment or waste coming in contact with these materials must be treated prior to disposal.

Infectious agents (RG2/BSL-2) and some bodily fluids fall under the NCDENR Regulated Medical Waste Rules and require specific treatment procedures, including weekly validation of the autoclave cycle parameters or pre-approval of chemical disinfection method, as described in the [UNCG Regulated Medical Waste Policy](#). Exceptions to this policy are items contaminated with blood or bodily fluids contained on bandages and personal hygiene products, and cleanup materials not related to research activities. These items are not required to be treated prior to disposal.

The following table identifies the appropriate treatment method for specific types of biological waste, which includes any other materials or equipment, including PPE, contaminated with the biological material.

Biological Material	Treatment Method
Stocks and cultures of nonpathogenic materials and microorganisms (RG/BSL-1)	Autoclave or chemical treatment
Pathogenic microorganisms (RG/BSL \geq 2)	³ Validated autoclave or approved chemical treatment
Human blood, blood components and products, and OPIM ² , in individual containers less than 20mL	Autoclave or chemical treatment
Human blood, blood components and products, and OPIM ² , in individual containers greater than 20mL	³ Validated autoclave or approved chemical treatment
Animal or Human primary cells, cell lines, and culture media	Autoclave or chemical treatment
rDNA waste, transgenic flies and plants	Autoclave or chemical treatment
¹ BSL1 rDNA waste which is not in organisms or viruses	None required
Unfixed tissue, cells (and culture media), and fluids from humans or animals known to have been infected with human pathogens.	³ Validated autoclave or approved chemical treatment
Animal tissues, organs, parts	Autoclave or incineration
Animal carcasses	Incineration by commercial vendor

¹BSL1 rDNA waste which is not in organisms or viruses (e.g., DNA, RNA, oligos waste, PCR waste, Microarrays, etc.). rDNA waste from experiments which use Escherichia coli K-12 host-vector systems that use only non-conjugative plasmids as vectors (e.g., pBR322, pBR313) and does not contain conjugation proficient plasmids or generalized transducing phages. rDNA waste from experiments involving Saccharomyces cerevisiae and Saccharomyces uvarum host-vector systems.

²Other Potentially Infectious Materials (OPIM) are defined as human semen; vaginal secretions; cerebrospinal, synovial, pleural, pericardial, peritoneal, and amniotic fluids; and body fluids visibly contaminated with blood or in situations where it is difficult to differentiate between body fluids.

³See UNCG Regulated Medical Waste Policy for specific treatment requirements.

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 EH&S Department.

Autoclave Waste Decontamination

Autoclaving, or steam sterilization, is a dependable method for the destruction of all forms of microbial life and is therefore the preferred method of treatment for solid and liquid biological waste. Successful treatment is dependent upon adequate temperature, pressure, and time for steam to penetrate all parts of the waste. Therefore, the autoclave user must be mindful of the cycle parameters, as well as

the volume and packaging of waste to prevent the entrapment of air. If all the air is not allowed to escape from the waste during the cycle, steam will not replace the air. Individual trials should be done to determine the proper loading and cycle parameters to for adequate sterilization. NC Medical Waste Rules state that autoclaves are to be provided with a chart recorder which accurately records time and temperature for each cycle.

Parameters

The autoclave is to be operated at a minimum of 121°C (250°F) and 15 psi for a minimum of 45 minutes, or other combination of parameters shown to effectively treat the waste. Suggested parameters are shown in the table below. Some autoclaves are equipped to operate at higher temperatures, which may allow for shorter exposure times. The parameters used for each type of waste in the laboratory must be validated weekly using biological indicators to ensure effective sterilization (see procedure below).

Autoclaves may have settings for "LIQUIDS" or "DRY GOODS". "LIQUID" settings run for longer periods at lower temperatures to minimize liquid evaporation and spills. For solid materials, the "DRY GOODS WITH VACUUM" should be used for infectious waste as it is the most effective at moving steam and heat into the deepest parts of large bags producing the best conditions for killing persistent organisms. "DRY GOODS WITHOUT VACUUM" should only be used for clean items that need to be sterilized. Exhaust settings should also be appropriate for the type of waste being autoclaved. FAST exhaust should be used for solid items and SLOW exhaust should be used for liquids.

Material	Temperature	Time
Laundry	121° C (250° F)	30 minutes
Trash (biohazard bags containing infectious waste)	121° C (250° F)	1 hour
Glassware	121° C (250° F)	1 hour
Liquids	121° C (250° F)	1 hour
Animals	121° C (250° F)	8 hours

Solid waste

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. Do not overfill waste bags or the autoclave. This will interfere with steam penetration. Add about 50-100 ml of water to each bag of solid waste to facilitate steam penetration in the bag. If there is naturally occurring water in the load, adding additional water is not necessary. Keep the waste bags slightly open to allow for steam penetration. Bags must be placed into stainless steel or polypropylene trays prior to autoclaving.

Liquid waste

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. The containers should not be filled to more than 75% capacity. The caps or stoppers on the containers should be loosened. Never autoclave sealed containers of liquid. This could result in an explosion of superheated liquid. Liquid containers should be placed in a stainless steel or polypropylene tray with 1/4 to 1/2 inch of water in the bottom of the tray. The tray should be placed on a shelf in the autoclave and not on the bottom of the chamber.

Autoclaved liquid wastes may be discharged directly to the sanitary sewer.

Liquid waste containing sodium hypochlorite (bleach) should not be autoclaved because of the potential to produce toxic gases and corrosion of autoclave equipment. Liquid waste treated chemically does not need to be autoclaved prior to disposal, see the **Treatment of Liquid Biohazardous Waste** section for more details on *Chemical Disinfection*.

Training

Owners and authorized users of autoclaves shall read and understand the manufacturer's owner manual and be thoroughly familiar with the safe operation of their autoclave. It is the responsibility of the department head that all users are familiar with the safe operation of their departmental autoclaves.

Autoclave Precautions

The hazards associated with autoclaves include extreme heat and high pressure and large, heavy doors and loading carriage. When operating an autoclave the following safety procedures must be followed:

- Become familiar with the autoclave's owner's manual. Though the principle is the same for each, manufacturer recommendations for use can vary widely.
- Firmly lock autoclave doors and gaskets in place before you run the autoclave to prevent a sudden release of high-pressure steam. Some autoclaves do not have safety interlocks that prevent the autoclave from running if the door isn't closed properly. If your autoclave does not have safety interlocks, you will need to take additional precautions to ensure that the doors are secured.
- If you have an older autoclave that has little or no heat shielding around the outside, attach signs warning of "Hot Surfaces, Keep Away" on or next to the autoclave to remind people of the hazard. Do not stack or store combustible materials (cardboard, plastic, volatile or flammable liquids, compressed gas cylinders) next to an autoclave.

- When a cycle is complete, wait until the pressure has normalized before opening the door of the autoclave.
- Wait at least 30 seconds after opening the door before reaching or looking into the autoclave.
- Open the door slowly, keeping head, face, and hands away from the opening.
- Allow contents to cool before removing them from the autoclave.
- Remove solutions from the autoclave slowly and gently; some solutions can boil over when moved or when exposed to room temperature. Thick, heat-resistant gloves, safety goggles or face shield and a rubber apron must be worn when removing hot liquids from the autoclave. Liquids should stand for over 1 hour before being handled without heat-resistant gloves.
- Never put solvents, toxic, volatile or corrosive chemicals (e.g., phenol chloroform, bleach, formalin, fixed tissues, etc.), or radioactive materials in an autoclave. Call the EH&S Department at 334-4357 if you have questions about proper disposal of these materials.
- Clean up any spills immediately.
- Report any malfunctions or accidents immediately to your supervisor.

Treatment of Liquid Biohazard Waste

Even though the rules and definitions for liquid biohazard waste vary somewhat from solid waste procedures, autoclaving is the method of choice for disinfection of the following:

- Liquid human blood
- Animal blood/body fluids
- Human tissue culture, human cell lines (primary or established)
- Human body fluids as defined under the UNC Laboratory Exposure Control Plan
- Liquid growth media removed from human tissue cultures

Chemical disinfection

Chemical disinfection, such as bleach treatment, may be an acceptable alternative to autoclaving biohazard waste generated in research laboratories at UNCG. The NC Medical Waste Rules do not allow chemical disinfection of regulated liquids followed by disposal to the sanitary sewer unless approval has been obtained from the NC Division of Waste Management. Regulated liquids include the following:

- Liquid waste media from cells/tissue used for propagating Risk Group 2 or 3 pathogens or toxins, including those produced in recombinant DNA procedures
- "Microbiological waste" as defined by the NC Regulated Waste rules: e.g. cultures and stocks of **infectious agents** (RG/BSL \geq 2)
- Fluids from animals known to be infected with infectious agents

For more information on regulated liquid waste treatment, see the UNCG Regulated Medical Waste Manual.

Choosing a Chemical Disinfectant

When choosing a disinfectant, 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.)?
- Does organic matter inactivate the disinfectant (Proteins in organic matter can inactivate or slow down the activity of certain disinfectants, such as bleach.)?
- Is the disinfectant compatible with work surfaces and equipment (e.g., metal, tile, plastic, wood, concrete)
- Disposal of chemical waste
- 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 compatible with 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 of 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.
- Strong oxidizers are very corrosive to metal surfaces, as well as to the skin, eyes, and the respiratory tract.
- Formalin – Requires initial monitoring prior to use. Contact the EH&S Department 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 EH&S Department 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 EH&S Department 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 (*Biosafety in Microbiological and Biomedical Laboratories*, CDC) for Guidelines for Working with Toxins of Biological Origin.

Treatment Off-Site

UNCG uses Ozone Waste Solutions for all off-site disposal needs.

Contact Information:

Telephone: (336) 550-4037

Each package of RMW shall be marked on with the following information:

- The generator's name, address, and telephone number
- The transporter's name, address, and telephone number
- Storage facility name, address, and telephone number, when applicable
- Treatment facility name, address, and telephone number
- The words "INFECTIOUS WASTE" or "MEDICAL WASTE"

Spill / Release

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

Spills within the 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 as outlined below.

Spills 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 EH&S Department 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 as follows.

Spill Cleanup Procedures:

- Everyone not needed for spill cleanup shall be cautioned to stay away from the spill area until cleanup has occurred. Signs may be posted if necessary.
- Any potentially contaminated clothing shall be removed and placed in a biohazard waste bag for decontamination.
- Personnel decontamination takes priority. Hands and any other contaminated skin shall be washed thoroughly with soap and water.
- 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 UNCG Respiratory Protection Manual on the EH&S Department webpage).
- Any sharp, contaminated objects shall be removed from the spill area using mechanical means, never with hands.
- 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.
- *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.
- 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.
- 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.
- After initial clean up, paper towels shall again be placed on the spill area, flooded with disinfectant, and left to soak for at least 20 minutes or according to manufacturers' instruction. Adequate contact time is important to ensure complete decontamination.
- 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.
- If radioactive material is involved in the spill, also wash the surface with detergent and follow the guidelines for radioactive spill cleanup and disposal.
- Autoclave any contaminated waste, cleanup materials, PPE, or clothing prior to disposal or removal from the lab area.
- Wash hands and exposed skin thoroughly with soap and water.
- Complete spill report and forward a copy to the EH&S Department.

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 for Biohazard Spill Cleanup 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 quarternary 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

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

Appendices

A. Weekly Autoclave Validation Log

B. Bloodborne Pathogens Exposure Control Plan Enrollment Form

