Laboratory Biosafety Training

This training provides an overview on biosafety for all personnel working in labs with biological agents and is required to be completed initially and every 3 years afterwards.

Principal Investigators/Supervisors are responsible for ensuring lab-specific policies are trained and that training is documented.
Regulatory Requirements and Policies

- **OSHA:** 29 CFR 1910.1030 - Bloodborne Pathogens Standard
- **OSHA:** 29 CFR 1910.1030(g) and 29 CFR 1910.145(e)(4) - Specifications for accident prevention signs and tags
- **15A NCAC 13B.1200** – North Carolina Medical Waste Management
- **DHHS:** 42 CFR Part 73 - Select Agents and Toxins
- **USDA:** 7 CFR Part 331 and 9 CFR Part 121 - Possession, Use, and Transfer of Select Agents and Toxins
- **CDC:** 42 CFR – Part 71 – regulatory requirements for importation or transportation of infectious biological agents, substances, and vectors
Guidelines

• NIH/CDC:  Biosafety in Microbiological and Biomedical Laboratories

• NIH:  Research Involving Recombinant or Synthetic Nucleic Acid Molecules

• American Society for Microbiology (ASM):  Biosafety in Teaching Laboratories
Scope

NCCU Biological Safety Manual applies to all University activities involving biological agents:

• Recombinant or synthetic nucleic acid molecules, including their use in animals and plants;

• Human and other primate-derived substances (blood, body fluids, cell lines or tissues);

• Organisms or viruses infectious to humans, animals or plants (e.g., parasites, viruses, bacteria, fungi, prions, rickettsia) or biological materials that may contain these microorganisms;

• Select agents or toxins (human, animal, or plant);

• Biologically active agents (e.g., venoms, toxins produced by living organisms) that may cause disease in humans or cause significant impact if released into the environment.
Responsibilities

It is a team effort requiring the direct involvement of the:

- Institutional Biosafety Committee (IBC)
- Environmental Health and Safety (EHS)
- Principal Investigators (PIs)
- All NCCU Laboratorians (Scientist, Students, Staff)
- Teaching Laboratory Instructors/Educators
Institutional Biosafety Committee Responsibilities

• Developing biosafety policies applicable to University activities, including work practices, biohazardous waste, and medical surveillance of personnel.

• Reviewing and approving new research proposals in accordance with CDC/NIH guidelines.

• Setting required containment levels for research projects.

• Developing design specifications and criteria for containment facilities.

• Investigating significant violations of University biosafety procedures or policies, and significant accidents or illnesses involving biological agents. If appropriate, the IBC recommends disciplinary action to the proper University officials.

• Notifying the NIH Office of Biotechnology Activities of reportable incidents as specified in the latest edition of the NIH Guidelines.

Environmental Health and Safety Responsibilities

• Serving as the biosafety subject matter expert for the IBC.

• Developing, implementing, and enforcing biosafety policies and practices.

• Developing and training in emergency response plans for spills, personnel contamination, and incidents involving biological agents.

• Providing for periodic inspections of laboratories to assess safety issues.

• Providing biosafety training for University personnel as required.

• Providing information and consultation on for occupational health issues related to biohazards.
**Principle Investigator Responsibilities**

- Registering the biological materials with IBC by completing the IBC Registration form to obtain approval for work involving biohazardous materials specified in the NCCU Biosafety Manual.

- Submitting amendments in writing using [IBC Registration form](#) when the change may have safety consequences.

- Ensuring that laboratory hazards are effectively communicated to laboratory personnel.

- Developing laboratory-specific standard operating procedures (SOPs) that cover the hazards and activities (both routine activities and specific events) relevant to the laboratory and make available copies of the specific biosafety procedures in each laboratory/facility. The PI shall ensure that all laboratory personnel, understand and comply with these laboratory-specific biosafety procedures.

- Ensuring that all laboratory personnel, maintenance personnel and visitors who may be exposed to any biohazardous agents are informed in advance of their potential risk and of the precautions required to minimize that risk.

- Ensure that laboratory door signs are accurate and posted at the initial completion or update of the Laboratory-Specific Safety Plan (LSP).

- Ensuring that proper engineering controls are available, in good working order, and used appropriately to minimize exposure to biohazardous agents.

- Ensuring that appropriate personal protective equipment (PPE) is available and used by laboratory personnel.

- Ensuring that all laboratory personnel receive general biosafety training as well as laboratory-specific training on the hazards, procedures, and practices relevant to the laboratory they are working in. All training must be documented, and records maintained.

- Ensuring that laboratory workers are provided immunizations and medical surveillance prior to exposure to biohazardous agents as appropriate.

- Notifying the EHS of any spills or incidents involving biological agents that result in exposure to laboratory personnel or the public, or release to the environment.

- Ensuring that an accurate inventory of biological agents is maintained.

- Ensuring that biological agents are disposed of as outlined in the [NCCU Biosafety Manual](#).

- Ensuring that biohazardous materials to be transported are packaged and shipped in accordance with regulations, and that persons performing these duties have appropriate and current training.

- Ensure that all maintenance work in, on or around contaminated equipment is conducted only after that equipment is properly decontaminated.

- Ensuring that periodic self-inspections of the laboratory are conducted to identify and correct health and safety deficiencies.

- Upon expiration or close-out of a Laboratory Safety Plan, ensuring that all materials indicated in the submission are inactivated, moved, or transferred to a new under an approved LSP.
Laboratory Staff/Laboratorians Responsibilities

Laboratory workers are the most important element in developing and maintaining a safe laboratory environment. Specific responsibilities include:

- Following all established procedures and practices.
- Knowing how to access the NCCU Biosafety Manual and being knowledgeable of requirements and procedures contained in the manual.
- Using practices and procedures specified in Biosafety Manual, presented in training, and other accepted good laboratory practices to minimize exposure to biological agents, and to avoid other incidents (such as fire, explosion, etc.).
- Complete biosafety and laboratory safety trainings as required.
- Report unsafe laboratory conditions, incidents or near incidents involving personnel exposure, releases outside of containment, or other biosafety issues to the PI and EHS.
- Utilize control measures such as biological safety cabinets and personal protective equipment to prevent exposure to biological agents, and contamination of personnel and facilities.
Teaching Laboratory Instructors/Educators Responsibilities

- **Register with the IBC** if coursework involves biological agents.
- Prior to beginning lab-work, provide all students with documented lab-specific training for the course.
- Require students to sign safety agreements explaining that they have been informed about safety precautions and the hazardous nature of any biohazards they will handle throughout the course.
- Advise students to contact Student Health for a consultation if they have health concerns due to an immunocompromising condition, including pregnancy.
- Ensure all students wear appropriate personal protective equipment.
- Ensure, students are aware of emergency procedures and how to report spills, exposure and injuries/accidents.
Trainings

Principal Investigators

• Must communicate biological hazards to laboratory workers at the time of initial assignment, and whenever new exposure risks are identified

• Are responsible for training and retraining new staff in practices to the point where techniques and safety precautions become second nature

• Review the CDC Guidelines for Laboratory Biosafety Competency

• Include training under normal operating conditions, during emergencies, system failures, and in the event of a suspect or known exposure.
Trainings

1. **Orientation for Laboratory Environment**
2. **Laboratory Biosafety Training** - complete initially and every 3 years afterwards.
3. **Bloodborne Pathogen (BBP) Training** - complete initially and annually thereafter.
4. **Training on the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules** - is required by the NIH of all Principal Investigators with labs working with recombinant or synthetic nucleic molecules.
OSHA regulations require maintenance of monitoring and medical records for a period of 30 years following termination of employment. The records that EHS maintain include:

- Copies of Laboratory Specific Safety Plans
- NCCU Laboratory Worker Registration Form
- Hepatitis B Vaccination and Information Form
- Reports and Investigations of Accidents
Biological Safety Survey

Laboratory safety surveys are conducted by EHS on annual basis in all NCCU research laboratories. The focus of the survey is to ensure compliance with general safety, biological safety, and chemical safety.

- Biological agent inventory
- Current IBC/EHS approval of all research
- Laboratory signage
- Personal protective equipment availability
- Presence and maintenance of hand washing sinks, eye washing, safety shower, first aid kit
- Aerosol minimizing techniques in place
- Current biosafety cabinets certification
- Biological waste storage, decontamination, and disposal practices
- Appropriate sharps container use

The Occupational Safety and Health Act of North Carolina makes provisions for employees to request an inspection or evaluation of conditions that they believe may constitute a health or safety hazard. University employees are encourage to report such conditions to EHS (ehs@nccu.edu, or by calling 919-530-7125, or on-line Health and Safety Reporting) and to request an investigation into the need for corrective action.
A laboratory-associated infection (LAI) is an infection that results from laboratory work, whether it occurred in a laboratory worker or in another person who happened to be exposed as a result of laboratory/animal research or clinical work with infectious agents.

If you are immunocompromised, you may be at a higher risk for acquiring infections and you should meet with occupational health physician or your personal physician for a medical consultation to determine your risk of infection. Also, if you are pregnant, you should discuss the kind of work and materials that you are exposed to with your physician to determine the risk to you and/or your fetus.
Route of Infection and Prevention

- Label all equipment used to store and process infectious materials with biohazard warning.
- Infectious materials must be clearly labeled and properly stored.
- Keep workspaces uncluttered.
- Plan work with safety in mind.
- Do not eat, drink, smoke, apply cosmetics, handle personal electronic device or phones or handle contact lenses in the lab.
- Cover any exposed broken skin with waterproof bandage.
- Change gloves often and as soon as possible when visibly contaminated.
- Wash your hands frequently with soap and water. Hands should be washed immediately after gloves are removed and before leaving the lab.
- Minimize aerosol production by working carefully. Perform procedures that may result in aerosol or splashes in a biosafety cabinet.
- No mouth pipette.
- Utilize safe handling and disposal techniques when working with sharps.
- Employ proper animal restraint and handling to prevent scratches or bites.
- Decontaminate work surfaces and equipment immediately after using biohazardous materials and after a spill occurs.
- Always wear appropriate PPE in the lab.
A medical surveillance program is provided through NCCU for personnel who are occupationally at risk of exposure to BBP, have direct contact with research animals, require use of a respirator, and/or receive vaccines for infectious agents used in the laboratory.

Components of medical surveillance program:

1. Pre-screening and clearance for work with biohazards
2. Ongoing evaluation of health status for individuals after initiation of biohazardous work
3. Prompt evaluation and follow-up of any signs or symptoms of potential infection, and after any known or potential exposure to biohazards.

- Principal Investigators are required to perform a risk-based assessment of the BSL-2 agents with respect to the individual health status of their personnel.
- All faculty, staff and students should consult with their laboratory supervisor to determine which agents or activities require occupational medical services (e.g., vaccination, clearance to wear a respirator, etc.).
- Lab workers that have an autoimmune or chronic disease, heart disease, are taking immune suppressing medications or are otherwise immunocompromised, should self-identify to the occupational healthcare provider for appropriate counseling and guidance.
Emergency Procedures

• It is responsibility of each laboratory/PI to develop an Emergency Plans that includes documentation how to cease, terminate, and secure laboratory in case of a lab or campus emergency.

• Spill response plan specific to the agents used in the lab and reporting requirements to PI and EHS in case of incident should be posted in the lab and reviewed by all lab personnel on an annual basis.

• The PI should keep written records to document that all lab personnel have been trained in the Emergency Plan.
Biohazard Exposure Procedures

Hazardous Material on Skin or Splashed in Eye
1. Remove contaminated clothing, shoes, jewelry, etc.
2. Immediately flood exposed areas with water from safety shower, eyewash, or faucet for at least 10-15 minutes. Use soap on skin for biological/blood exposure. Hold eyes open to ensure effective rinsing behind both eyelids.
3. Immediately after rinsing, obtain medical attention.
4. Notify the PI and EHS.

Needle Stick or Cut with Contaminated Sharp Item
1. Remove gloves and force wound to bleed.
2. Immediately wash the area with soap and water for at least 5 minutes.
3. Utilize First Aid kit if necessary.
4. Immediately after rinsing, obtain medical attention.
5. Notify the PI and EHS.

Injury Involving Research Animal
1. Bite/Scratch/Cut: Remove gloves and force wound to bleed.
2. Wash the area with soap and water for at least 15 minutes.
3. Utilize First Aid kit if necessary.
4. Immediately after rinsing, obtain medical attention.
5. Notify the animal facility manager, PI, and EHS.
Obtaining Medical Attention

NCCU Employee

- If the incident occurs during normal business hours and requires more than simple first-aid measures, the employee should immediately contact the NCCU Worker’s Compensation Administrator at 919-530-7943 or callsbrook@nccu.edu for instructions on how to obtain medical care and complete Worker’s Compensation paperwork.

- If the incident occurs after normal business hours and requires emergency care, employees should call 911. It is not recommended that the injured or another NCCU employee or student transport an injured person for care.

NCCU Students

- If the incident occurs during normal business hours, the student should contact Student Health Services at 919-370-0901 for information on how to proceed.

- If the incident occurs outside of normal business hours or requires immediate emergency care, 911 should be called for emergency care and if necessary transport. It is not recommended that the injured or another NCCU employee or student transport an injured person for care. Students should report all lab-associated incidents to the Office of Student Affairs.

Incident Reporting Instructions

- All incidents, exposures (direct or potential), spills, or any symptoms associated with exposure to an agent are to be immediately reported to the PI and EHS using Hazard and Incident Report Form.

PI’s responsibility

- Ensure that employees and students receive prompt treatment of any injuries or LAIs.
- Never send an injured employee to seek medical attention for an injury on their own or transport them yourself.
- Completes the Supervisor Statement Form.
- Provides employees with the Workers’ Compensation Employee Statement Form.
- Forms must be submitted within 24 hours.
Biohazard Spill Procedures

- Spills must be cleaned up as soon as practical and in accordance with the spill procedures outlined in the LSP.
- All employees participating in cleanup must be properly trained prior to attempting remediation.
- All waste produced from the cleanup must be disposed of as biological hazardous waste.

**Spill kit** shall be readily available in each laboratory and contain:
- Spill SOP and sign
- EPA-registered disinfectant(s)
- Absorbing materials (pads, towels, etc.)
- Personal Protective Equipment (gloves, lab coat, booties, lab safety glasses)
- Biohazard bags and zip ties
- Disposal container (container for sharps management and container for biohazard waste)
- Mechanical device for removing sharps (forceps, tongs, scoops, pans)
- Swiffer sweeper (brooms are not recommended for biological hazard)
Spill Inside of Biosafety Cabinet

- Keep the BSC blower running and alert others in the laboratory.
- Do not remove your hands from the BSC unless you disinfect your gloves first.
- Do not place your head in the cabinet to clean the spill, keep your face behind the view screen.
- Remove any solid/sharp objects using tongs and small pieces with tweezers. Place sharp objects into container designated for biohazard sharps.
- Cover the spill with absorbent and pour disinfectant onto the absorbent from outside rim to the center of the spill.
- If necessary, flood the work surface as well as the drain pans with disinfectant; be sure the drain valve is closed before flooding the area under the work surface.
- Allow sufficient contact time (based on disinfectant and as described under SOP).
- Wipe cabinet walls, work surfaces, and inside the view screen with disinfectant.
- Allow disinfectant to stand for appropriate contact time.
- If necessary, place a container under the drain valve and drain the disinfectant under the work surface into the container.
- Disinfect the spill again with clean absorbents.
- If you used bleach as a disinfectant, rinse the area well with sterile water or wipe down with 70% ethanol to remove any corrosive residues.
- Dispose of absorbent materials as biohazard waste.
- Disinfect and remove gloves before leaving the BSC.
- Remove your laboratory coat and wash your hands with soap and water for at least 30 seconds.
- Report incident to supervisor.
Spill Outside of the Biosafety Cabinet

- Evacuate all personnel from the room and close the door if aerosols are a concern. Wait 30 minutes to allow aerosol to settle before attempting to clean up the spill.
- Alert others to avoid exposure. Post spill sign from the spill kit.
- Identify the spill zone (3-feet from what is visible) and secure the area.
- Establish a staging area, remove contaminated PPE.
- Remove any contaminated clothing or PPE and place in a biohazard bag for decontamination and/or disposal. Wash your hands.
- Put on clean gloves, lab coat, and eye/face protection.
- Get spill supplies needed to effectively and safely clean the spill to the staging area.
- Remove any solid/sharp objects using tongs and small pieces with tweezers. Place sharp objects into container designated for biohazard sharps.
- Place absorbent materials over spill zone and soak with disinfectant from the outer rim of the spill inwards until absorbent material is completely saturated.
- Allow disinfectant to stand for appropriate contact time.
- Push absorbent materials from outside towards the center of the spill and dispose in biohazard bag.
- Clean area again with disinfectant.
- Rinse area with 70% ethanol or water if bleach is used to remove any corrosive residues.
- Remove PPE and wash hands with soap and water for at least 30 seconds.
- Report incident to supervisor.
Spill in Centrifuge

To centrifuge BSL-2 agents, always use sealed safety-caps, sealed buckets, or sealed rotors.

- Wait 5 minutes before opening the centrifuge following the end of a run with potentially hazardous biological material. If a spill is identified after the centrifuge lid is opened, carefully close the lid. Let aerosols settle for 30 minutes.
- Remove any contaminated protective clothing and place into a biohazard bag. Wash hands and any exposed skin surfaces with soap and water.
- Put on clean gloves, lab coat, and eye/face protection.
- Clean up spill.
- Keep rotors and buckets closed and transfer them to a biological safety cabinet.
- Carefully retrieve any broken glass from inside the centrifuge and/or rotor or bucket using forceps and discard into a sharp's container. Smaller pieces of glass may be collected with forceps.
- Immerse rotor/buckets in 70% ethanol or a non-corrosive disinfectant for appropriate contact time. Allow to completely air dry.
- Intact tubes may be wiped down with disinfectant and placed into a new container.
- Wipe the inside of the centrifuge with disinfectant.
- If bleach is used, follow with 70% ethanol to remove any corrosive residues.
- Dispose of absorbent materials as biohazard waste.
- Wash hands with soap and water.
- Report incident to supervisor.

ALWAYS LOG AND REPORT THE INCIDENT TO SUPERVISOR AND EHS.
Personal Protective Equipment

PIs are required to determine all exposures to hazards in their workplace and determine what type of PPE should be used to protect their lab workers.

Clothing and Protective Apparel

- Lab coat, wrap-around gown, smock, or scrub suits are recommended for biocontainment practices.
- Disposable or washed regularly; DO NOT take lab coats home or wear outside the lab.
- Lab coats should be also provided for visitors, maintenance and service workers as needed.

Eye/Face Protection Equipment

- The eyes and mucous membranes are two potential routes of transmission of pathogens.
- Eye protection should always be worn in the laboratory.
- Goggles or safety glasses with side shields should be used in combination with masks, face shields or other splatter guards for optimal protection.
- All eye/face protection devices must meet the requirements set forth in the ANSI Z87 standard.
- Prescription safety spectacles are recommended for employees wearing glasses.
Personal Protective Equipment

Gloves

- Wear gloves whenever working with biohazards, toxic substances or hazardous chemicals.
- **Gloves selection** is based on the hazards involved and the type of work being done.
- Before use, inspect gloves for discoloration, puncture, and tears. If you find that a glove has been torn or punctured while working with an infectious or potentially infectious material, report to the EHS to assess and determine exposure potential.
- For disposable gloves, do not use past the expiration date.
- Replace gloves periodically, depending on frequency of use and permeability to the material handled.
- Consider double gloving when handling highly infectious material or spill cleanup.
- Gloves should overlap the sleeve of the lab garment.
- Never reuse disposal gloves.
- Take gloves off carefully to avoid contaminating hands.
- Consider latex-free or nitrile gloves to avoid latex-associated allergic reactions.
- Glove use should never replace the need for hand washing. Always wash your hands with soap and water for at least 30 seconds after removing gloves and before exiting lab.
- Never use gloves outside the laboratory.
- Gloves contaminated with biohazard material need to be disposed with other biohazard material and decontaminated before final disposal.
- Gloves should also be worn whenever it is necessary to handle rough or sharp-edged objects, and very hot or very cold materials.

Contaminated Glove Removal

1. Grasp the outside of the glove in the wrist area
2. Peel the glove away from your body, turn it inside-out
3. Hold the inside-out glove in the other hand
4. Slide your fingers under the wrist of the other glove
5. Peel the glove away from your body, turn it inside-out, leave the first glove inside the second
6. Dispose the glove in appropriate waste receptacle
7. Wash hands with soap and running water
Personal Protective Equipment

Respirators
- Respirators can only be used when it is not possible to minimize or eliminate exposure to a contaminant through other means.
- Respirators are used in biological laboratories when there is a potential for exposure through inhalation or in some cases to animal allergies.
- The selection and use of respirators must be done in accordance with 29 CFR 1910.134 and NCCU Respiratory Protection Plan.
- All individuals issued respirators must go through training, medical screening and fit testing to be approved to wear a respirator.

Foot Protection
- For most biological lab use, comfortable shoes such as tennis shoes or nurse shoes should be worn.
- Sandals and other types of open-toed shoes are not permitted in labs using biohazards.
- Boots, shoe covers, or other protective footwear, and disinfectant footbath are required for work in animal facilities.

PPE Cleaning and Maintenance
- It is important to properly maintain and keep all PPE clean.
- PPE should be inspected, cleaned, and maintained at regular intervals so that the PPE provides the required protection.
- Employees should not share PPE until it has been properly cleaned and sanitized.
A biohazardous material is any biological material capable of causing harm to humans, animals or plants, including both biohazardous agents and non-replicating materials such as toxins, and may also be used to refer to material that harbors a biohazardous agent.

A Biosafety Risk Assessment is fundamental to safe laboratory operations. It needs to be conducted prior to the initiation of the proposed experiment or project to identify the Risk Group and to determine the appropriate set of containment/Biosafety level, i.e., work practices, PPE, equipment, training, and workspace design to protect laboratory personnel, students, maintenance and service workers, the public and environment.

The determination of Biosafety Level (BSL) for work with specific biological material should be based on a thorough risk assessment that, at a minimum, includes a review of the following resources:

- The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules - provides common biological agents used in research listed by Risk Group.

- NIH/CDC guidelines Biosafety in Microbiological and Biomedical Laboratories (BMBL) - indicates the appropriate biosafety level for some infectious agents and describes the process of Biological Risk Assessment.

- The American Biological Safety Association - provides database of many biological agents and their assigned biosafety levels by country.

- The Pathogen Safety Data Sheets produced by the Public Health Agency of Canada for organisms/infectious substances according to their risk.

New or unknown pathogens that have not been assessed by CDC/NIH must go through a risk assessment to determine their biosafety containment level.
NIH and WHO recommend four Risk Groups (RG1-4) based upon the following hazardous characteristics of an agent:

- ability to infect and cause disease in a susceptible human or animal host
- virulence as measured by the severity of the disease
- availability of preventative measures and effective treatments for the disease

Determination of the appropriate Risk Group is the first step in determining the appropriate biosafety level (BSL) for working with the agent.

Factors to consider when evaluating risk:

- Pathogenicity
- Route of transmission
- Agent stability
- Infectious dose
- Concentration
- Origin
- Availability of prophylaxis or therapeutic intervention
- Medical surveillance
- Experience and skill level of at-risk personnel
Safety Considerations

**Primary barrier**
Is the protection of personnel and the immediate laboratory environment from exposure to infectious agents. It is accomplished by:

- The use of appropriate safety equipment such as biosafety cabinets, enclosed containers, PPE, and other biosafety controls designed to protect personnel
- A strict adherence to standard microbiological practices and techniques
- The use of vaccines may provide an increased level of personal protection.

**Secondary barrier**
Is the protection of the environment external to the laboratory from exposure to infectious materials. It is accomplished by a combination of:

- Facility design (i.e., separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), specialized ventilation systems to assure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules for isolation of the laboratory), and
- Operational practices.
Biosafety Level 1

BSL-1 labs are used to study infectious agents or toxins not known to consistently cause disease in healthy adult humans or animals.

1. Laboratories have doors for access control.
2. Laboratories have a sink for handwashing.
3. An eyewash station is readily available in the laboratory.
4. The laboratory is designed so that it can be easily cleaned.
5. Carpets and rugs in laboratories are not appropriate.
6. Spaces between benches, cabinets, and equipment are accessible for cleaning.
7. Laboratory furniture can support anticipated loads and uses.
8. Benchtops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
9. Chairs used in laboratory work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
10. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method.
11. Laboratory windows that open to the exterior are fitted with screens.
12. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
Biosafety Level 2

Biosafety Level 2 (BSL-2) builds upon BSL-1. BSL-2 is suitable for work with agents associated with human disease and pose moderate hazards to personnel and the environment.

BSL-2 differs from BSL-1 primarily because:

1) Laboratory personnel receive specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures;

2) Access to the laboratory is restricted when work is being conducted; and

3) All procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.
Biosafety Level 2

1. A biohazard sign is posted on the entrance to the laboratory.
2. Laboratory doors are self-closing and have locks in accordance with the institutional policies.
3. Access to the lab is limited or restricted when work with biohazard is in progress.
4. Lab personnel receive appropriate immunizations or tests for the agents handled or present in the lab.
5. Biosafety procedures are incorporated into standard operating procedures (SOPs) or in a LSP adopted or prepared specifically for the laboratory by PI.
6. Lab personnel receive appropriate training on the potential hazards associated with the work involved, the precautions to prevent exposures, and the exposure evaluation procedures.
7. PPE are available and need to be worn when working in BSL2 laboratory. Lab personnel are trained in proper PPE donning and doffing procedures.
8. Laboratories have a sink for handwashing. It should be located near the exit door.
9. An eyewash station is readily available in the laboratory.
10. The laboratory is designed so that it can be easily cleaned.
11. Laboratory furniture can support anticipated loads and uses.
12. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they are fitted with screens.
13. Illumination is adequate for all activities and avoids reflections and glare that could impede vision.
14. Vacuum lines in use are protected with liquid disinfectant traps and in-line HEPA filters or their equivalent.
15. There are no specific requirements for ventilation systems. However, the planning of new facilities considers mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
16. BSCs and other primary containment barrier systems are installed and operated in a manner to ensure their effectiveness.
Laboratory Equipment – Biological Safety Cabinet

**Biological Safety Cabinets (BSC)** control airborne contaminants during the work with infection material via the use of laminar flow and high efficiency particulate air (HEPA) filtration.

- Personnel protection is provided by the directional airflow into the cabinet
- Product protection is provided with HEPA filtered laminar airflow
- Environment protection is provided with HEPA filtered exhaust.

Proper selection of the BSC is contingent on an accurate risk assessment of the hazards inherent to the work planned in the unit (e.g., chemical, radiological, biological hazards). Review the CDC/NIH Appendix A, Primary Containment for Biohazards Selection, Installation and Use of Biological Safety Cabinets of BMBL publication and contact the EHS with questions.

BSCs are divided into three classes (Class I-III) based on design and protection.

- **The Class II BSC** is the most used BSC at NCCU.
  - Class II cabinets are built to meet NSF/ANSI 49-2019 (NSF 49)
  - The operational integrity of a BSC must be validated before it is placed into service and after it has been repaired or relocated.
  - On-site field certification must be performed by experienced, qualified professional annually.
  - Do not work in a biosafety cabinet that is in alarm. Post the sign “DO NOT USE (protection is compromised)” on BSC and immediately contact your building liaison or EHS.
Working in Biological Safety Cabinet

Before beginning work
- Monitor alarms, pressure gauges, or flow indicators for any changes.
- Shut off the UV light (use of UV light is not recommended by CDC).
- Turn the cabinet on and let it run for at least 5 min before beginning the work to allow cabinet to purge and to remove any suspended particulates in the cabinet.
- Wipe work surface, interior walls, and the interior surface of the window with the appropriate disinfectant listed in your LSP. If bleach is used, wipe again with water or 70% ethanol to remove the bleach residues.
- Plan your work and place everything needed for the procedure, including the container for your discards, inside the BSC. Wipe all items with appropriate disinfectant before placing in BSC.
- Place a container filled with disinfectant or lined with a small biohazard bag inside the BSC to collect waste. Avoid reaching outside of the BSC during procedures to discard waste in floor containers.

Avoid airflow disruption that could affect the level of protection provided by the BSC
- Keep the BSC free of clutter, e.g., extra equipment and supplies.
- Don’t place objects over the front air intake grille.
- Don’t block the rear air intake grille.
- Limit traffic in the area when the BSC is in use.
- Make sure lab door is closed and avoid opening and closing door if located near the BSC.
- Move arms slowly when removing or introducing items to or from the BSC.
- Keep all materials at least 4 inches inside the sash.
- Place a centrifuge or blender that creates air turbulence in the back 1/3 of the cabinet and stop other work while the equipment is running.
- Don’t operate a Bunsen burner in the cabinet. Open flame in BSC disrupt the pattern of HEPA-filtered airflow; gas is not allowed in BSC. There are alternatives for need to disinfect instruments in BSC, such as Bact-Cinerator, the Electric Bunsen Burner, or Glass Bead Sterilizer.

While working
- Work as far to the back of the BSC workspace as possible.
- Segregate contaminated and clean items. Work from “clean to dirty.”
- Clean up all spills in the cabinet immediately. After the spill cleanup, allow cabinet to run for 5 minutes before resuming work.

After completing work
- Wipe down all items with an appropriate disinfectant before removing from BSC. Remove all materials and wipe all interior surfaces with an appropriate disinfectant.
- Periodically decontaminate under work grilles.
- Allow cabinet fan to run for 5 minutes after decontamination before turning off the blower.
Vacuum Systems

- Aspirator bottles or suction flasks should be connected to an overflow collection flask containing appropriate disinfectant and to an in-line HEPA or equivalent filter.
- Once decontamination occurs, liquid materials can be disposed of as noninfectious waste.
- The flask material should be resistant to the decontamination solution used.

Aspiration flask (A) – collect contaminated fluids (e.g., tissue culture media) into appropriate disinfectant (e.g., 10% bleach); Overflow flask (B) – collects overflow fluids and tube submerged on liquid minimizes aerosols; HEPA filter (C) – in-line filter prevents contamination of vacuum; Vacuum line (D) – in-house central vacuum system or vacuum pump.

Centrifuges

- Aerosols may be created during centrifugation from poorly sealed or capped tubes and from tubes splitting or breaking.
- Use of aerosol containment devices such as safety cups, caps, sealed rotors, or buckets is required with the following materials:
  - Laboratory cultured samples known to contain agents infectious to humans
  - Agents covered under the federal regulations for Select Agents and Toxins
  - Agents that are approved for work at BSL-2 and above
- Follow these procedures for the proper use of aerosol containing devices when centrifuging biological materials:
  - Use aerosol-proof (sealed) rotors or buckets with safety caps that seal with O-rings.
  - Before use, inspect O-rings and safety caps for cracks, chips, and erosion.
  - Use tubes with threaded caps and safe-lock microcentrifuge tubes. Avoid overfilling the tube and getting caps/closures wet.
  - Wipe tubes down with disinfectant after filling.
  - Load and unload rotors and buckets inside the BSC.
  - Balance buckets, tubes, and rotors before centrifuging.
  - Disinfect the centrifuge after use.
  - Place small, low-speed centrifuges in a BSC during use to contain aerosols.

One-time training Fundamentals of Centrifuge Safety is required for those using centrifuge(s) in their workplace. For brief safety information, review the [OSHA Quick Facts sheet on Laboratory Safety Centrifuges](#).
Flow Cytometers

- Flow cytometers should operate under the same containment conditions in which the cells would normally be handled, e.g., if human cells are being sorted in a flow cytometer, they need to be handled at a BSL-2 containment.
- The higher speed of sorting, the higher the number of aerosols generated.
- Wear the proper PPE when working with a flow cytometer.
- A standard operating procedure (SOP) for flow cytometer used in the laboratory/facility needs to be available to all users.

Multi-Channel Liquid Handlers

- If liquid handler is to be used with BSL-2 material, it either needs to be positioned in BSC or should be turned into self-contained benchtop hood.
- The later should be equipped with shield doors, to restrict deck access and isolate hazardous/infectious material from lab personnel and environment, HEPA filter and UV lamp, to mirror their BSC enclosure.
- Wear the appropriate PPE.
- Decontaminate and dispose waste as biohazard.
- Decontaminate liquid handler with EPA and manufacturer approved disinfectant.

Pipettes and Pipetting Aids

- Pipetting must be done by mechanical means, never by mouth.
- Pipet work involving infectious material should be done in a BSC.
- Minimize hazard by using cotton-plugged pipettes and pipette tips.
- Store used pipettes for disposal in approved sharps container that fits the pipette in its entirety.

Loop Sterilizer and Bunsen Burners

- The sterilization of a loop or needle in an open flame generates aerosols.
- To minimize the risk of aerosol production containing viable microbial agents, it is strongly encouraged that laboratories use a shielded electric incinerator, or a hot bead sterilize while sterilizing a loop or needle.
- Another option is to use disposable loops and needles for culture work and collecting the waste loops and plastic needles in a sharps container that fits them in their entirety.
- Containers with sharps can be disposed of after autoclaving in general waste.
- The use of a continuous flame gas burner in a BSC is prohibited.
Decontamination, Disinfection, Sterilization

The decontamination or disinfection of laboratory surfaces and items with antimicrobial substances and other practices are to mitigate the possibility of transmission of pathogens to laboratory workers, the public, and the environment.

The PI is responsible for selecting an appropriate EPA-registered disinfectant and use it according to the manufacturer’s instructions on the product label to ensure the product’s performance against the target microorganism.

Cleaning is the removal of gross contamination from a surface to the extent necessary for further processing for intended use.

Decontamination removes or neutralizes hazardous biological material accumulated on the personnel and/or equipment.

Disinfection eliminates nearly all recognized pathogenic microorganisms, but not necessarily all microbial forms (e.g., bacterial spores). It is generally a less-lethal process than sterilization. The factors affecting disinfection include:
- Nature and number of microorganisms
- Amount of organic matter present (e.g., soil, feces, blood)
- Type and condition of surfaces, instruments, devices, and materials to be disinfected
- Temperature
- Contact (exposure) time

Sterilization is defined as a process after which the probability of a microorganism surviving on an item subjected to treatment is less than one in one million (10⁻⁶).
It can be accomplished by dry or moist heat, gases and vapors.

Incineration is the ultimate means of sterilization of medical and microbiological waste. Animal carcasses treated with preservatives such as formalin, medical sharps, etc. are examples of materials that are shipped for incineration.
The procedures for biological waste and animal tissue disposal at NCCU are consistent with the North Carolina Medical Waste Rules (15A NCAC 13 B.1200) and the applicable sections of the OSHA Bloodborne Pathogens Standard 29 CFR 1910.1030(d)(4)(iii).

- All biohazard waste generated in NCCU research and/or teaching laboratories must be properly treated prior to its disposal.
- If treatment of waste is not an option complete an EHS Hazardous Waste Pick-up Request.

**Biohazard waste that requires treatment prior to disposal:**

- Materials contaminated or potentially contaminated during the manipulation or clean-up of material generated during research and/or teaching activities requiring BSL-1 or BSL-2 and ABSL-1 or ABSL-2.
- Liquid blood and body fluids.
- Materials contaminated with human/primate tissue or human/primate tissue cultures (primary and established) because these are handled at BSL-2.
- Animal blood, fluids and bedding from animals infected with BSL-2 agents.
- Tissue, anatomical remains, and sharps containers that require removal by EHS.
- Recombinant and synthetic nucleic acid molecules.
- Select agents or toxins (human, animal, or plant).
- Biologically active agents.
Biohazard Waste Management

Solid Biohazard Waste
- Solid biohazard waste is typically deactivated by autoclaving.
- Collect waste in red, hard-walled biohazard waste collection containers, lined with an orange autoclave bag.
- Keep lid on the container closed when not in use.
- The lid and container each must bear the biohazard symbol and the word “Biohazard”.
- Autoclave bags must have the biohazard symbol and the word “Biohazard” on the outside of the bag.
- Bags must be removed from collection containers prior to being 2/3 to 3/4 full to allow headspace to seal the bag for transport to the autoclave.
- Place bags directly into heat-resistant secondary containers to contain spills. For dense or dry loads, add 200 mL of water to the bag to ensure steam penetration. Use only lead-free heat-sensitive autoclave tape.
- Bags should be opened (1-2 inches) before autoclaving to ensure sterilization.
- A log detailing autoclave performance verification must be completed on every load and maintained at the autoclave.

Liquid Biohazard Waste
- The preferred method for disinfecting recombinant and synthetic nucleic acid molecules, BSL-1 and BSL-2 liquid waste for drain disposal is autoclaving on the liquid cycle (a minimum of 30 minutes at 121°C and 15psi).
- If the liquid waste was used for propagating microbes, viral vectors, or toxins, approved chemical disinfection followed by drain disposal is used.
- Most liquid wastes can be deactivated with a 1:10 final dilution (vol/vol) of household bleach (5.25% of an active sodium hypochlorite).
- Remember that bleach is a corrosive chemical, and its use requires the availability of an eye wash station within 10 seconds of travel from point of use.

Sharps Waste
- Needles, syringes, scalpels, razor blades, slides, coverslips, Pasteur pipettes, capillary tubes, and broken glass and plastic are considered “sharps waste”.
- Collect biohazard sharps waste in red plastic sharps containers labeled with “Biohazard” label on it.
- To avoid injury, do NOT clip, bend, shear, or separate needles from syringes and do NOT recap needles.
- When the container is ¾ full, close it tightly, put into orange autoclave bag marked with heat sensitive tape (to signal that the material has been decontaminated) and autoclave as applicable.
- Decontaminated material can be disposed of in general waste.
Biohazard Waste Management

Disposal Practices for Research Involving Whole Animals

• Animals (parts, dead neonates or carcasses) treated or not with infectious agents, preservatives such as formalin, genetically modified or treated with viable recombinant or synthetic nucleic acid molecule-modified microorganisms are shipped for incineration.

Mixed Waste

Mixed waste often requires special procedures. Please contact the EHS to establish proper disposal procedures.

• Mixed biological/chemical waste can be disinfected by using carefully selected chemical treatments only if compatible with the other chemicals in the experiment. Handle resulting waste as hazardous chemical liquid waste. Contact the EHS office for advice on avoiding adverse chemical reactions.
Biohazard Waste Management - Autoclaving

Autoclaving
- Autoclaves provide a physical method for disinfection and sterilization.
- They are used to decontaminate biological waste and sterilize liquid media, instruments, and other lab ware.
- Autoclaves operate with a combination of steam, temperature, pressure, and time. As such, autoclaves pose many hazards including burns, explosions, muscle strains, and biohazards.

Before using the autoclave ensure that you:
- Completed the NCCU Autoclave Use and Safety Training and Quiz
- Received hands-on training from your supervisor or designee for specific autoclave unit and that autoclave is operating properly.

Never autoclave:
- Flammable, reactive, corrosive, toxic or radioactive materials
- Household bleach
- Any liquid in a sealed container
- Any material contained in such a manner that it touches the interior surfaces of the autoclave
- Paraffin-embedded tissue
- Polyethylene plastics (LDPE and HDPE)

Always wear personal protective equipment:
- Lab coat
- Eye/Face protection
- Closed-toe shoes
- Heat-resistant gloves to remove items, especially hot glassware

Everyone using the autoclave needs to complete the Autoclave Use Log. When Biological Indicators are used the Weekly Autoclave Testing Log needs to be completed as well.
Biohazard Waste Management - Autoclaving

Container Selection for Autoclaving

**Polypropylene bags**, also called biohazard or autoclave bags are:
- Tear resistant but can be punctured or burst in the autoclave. Therefore, place bags in a rigid container during autoclaving.
- Impermeable to steam. Therefore, they should not be twisted and taped shut, but gathered loosely at the top and secured with a large rubber band or autoclave tap leaving 1-2 inches in diameter opening for steam to penetrate.

**Sharps containers** are made from rigid plastic and come marked with a line that indicates when the container should be considered full. Sharp containers with biohazardous material must be labeled with universal biohazard symbol and the word “Biohazard” or be color-coded red.

**Polypropylene containers and trays** are capable of withstanding autoclaving, but resistant to heat transfer unlike a stainless-steel material.

**Stainless steel containers and pans.** Stainless steel is a good conductor of heat and is less likely to increase sterilizing time.

Sterilization Cycle and Time Selection

A typical standard for steam sterilization is achieved after 30 minutes under at least 15 psi (106 kPa) of pressure once all surfaces have reached a temperature of 121 °C. However, precautions for time selection need to consider the size of the load, insulating capacity of autoclaved material and nature of the article to be autoclaved.

<table>
<thead>
<tr>
<th>Basic Cycles</th>
<th>Description</th>
<th>Typical Application or Load Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravity</td>
<td>The most common sterilization cycle. Steam displaces air in the chamber by gravity (i.e., without mechanical assistance) through a drain port.</td>
<td>Glassware, unwrapped goods, waste, utensils, bags.</td>
</tr>
<tr>
<td>Pre-vacuum and/or Post-vacuum</td>
<td>Air is mechanically removed from the chamber and load through a series of vacuum and pressure pulses. This allow the steam to penetrate porous areas of the load that couldn’t otherwise be reached with simple gravity displacement.</td>
<td>Wrapped goods, packs, animal cages, porous materials, bags.</td>
</tr>
<tr>
<td>Liquids</td>
<td>A gravity cycle with a slower exhaust rate to minimize boil-over.</td>
<td>Media, LB broth, water, saline, etc.</td>
</tr>
<tr>
<td>Immediate use/Flash (Healthcare sterilizers only)</td>
<td>High temperature cycle (over 270°F) for a shorter period of time.</td>
<td>Unwrapped goods.</td>
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</tbody>
</table>
Biohazard Waste Management - Autoclaving

Monitoring the Sterilization Process
Verification of autoclave performance is critical to ensure that hazardous materials are being fully decontaminated and critical lab reagents are being fully sterilized. The NC Medical Waste Rules require that autoclaves be monitored under conditions of full loading for effectiveness weekly through the use of Biological Indicators.

Biological Indicator, an indicator of sterilization. *Geobacillus tearothermophilus* indicators must be used with average spore populations of $10^4$ to $10^6$ organisms following the manufacturers’ instructions. A record of each test is required.

Chemical Indicator is used to monitor autoclave parameters needed to achieve sterilization of instruments or tools. These are NOT indicators of disinfection as are biological indicators and should only be used if sterilization of tools or equipment are required.

Heat sensitive tape - is a process indicator designed to change color or develop a marking once critical temperature is reached. It is NOT an indicator of sterilization.

Autoclave Preventive Maintenance
Autoclave operators should perform the following preventative maintenance on their autoclave to maintain the autoclave’s effectiveness:

- Remove the plug screen or drain strainer to make sure it is free of dirt, dust, or sediment that may collect in it, and it should be cleaned, as necessary.
- Clean the interior surfaces of residues collected from the steam or materials being sterilized as needed.
- Visually inspect the gaskets, doors, shelves and walls for residue buildup or wear regularly.

Autoclave Failure
- Discontinue use immediately if an autoclave is not working properly.
- Post a sign alerting others not to use the autoclave.
- Contact the service company responsible for the maintenance of your autoclave or EHS for further guidance.

WARNING
DO NOT USE

Autoclave is Out of Service

<table>
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<tr>
<th>Description</th>
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<tr>
<td>Contact Info</td>
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DO NOT remove this sign or use this autoclave until its performance has been tested.
Shipping and Transporting Biological Material

• Most biological materials require specific packaging, labeling, and documentation.

• Infectious materials (materials containing or expected to contain pathogens affecting humans) are regulated by the US Department of Transportation (DOT) and the International Air Transport Association (IATA).

• You must complete a hazardous materials shipping training course to be certified to ship infectious biological materials. Certification is valid for 2 years and must be renewed every 2 years. This training is also required to be able to properly identify your materials according to DOT and IATA guidelines.

• Some biological materials require a permit to be imported or transferred to another institution.

• When transporting infectious material on campus, it must be placed in a durable leak-proof secondary container for transport out of the laboratory. Containers should be lidded and sealed with the biohazard label on the outermost container.
Quiz

• You must successfully complete a post-training quiz with a score of 80% or greater to receive credit for this training.

• You must log in with your NCCU credentials

• Please, click here to complete and submit the quiz
Questions

Department of Environmental Health and Safety
(919) 530-7125
ehs@nccu.edu

EHS Website