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I. Regulations, Standards and Guidance

The following summarizes applicable regulations and guidance relating to the use of biological materials. It is NCCU policy that all laboratories adhere to these regulations and guidelines.

The General Duty Clause from the OSHA Act of 1970 requires that, in addition to compliance with hazard-specific standards, all employers provide a work environment free from recognized hazards that are causing or are likely to cause death, serious physical harm or illnesses.

The Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH) published guidelines entitled **Biosafety in Microbiological and Biomedical Laboratories (BMBL)**. This document contains guidelines for microbiological practices, safety equipment, and facilities that constitute the four established biosafety levels. The BMBL is generally considered the standard for biosafety and is the basis for this manual.

Research projects that utilize human blood, blood products or potentially infectious body fluids are governed by the **Occupational Safety and Health Administration (OSHA)** Bloodborne Pathogens (BBP) Standard (29 CFR 1910.1030). This federal regulation mandates a combination of engineering and work practice controls, training, Hepatitis B vaccination, and other provisions to help reduce occupational exposure to human blood and other potentially infectious materials (OPIM), such as Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV) and other bloodborne pathogens. Under the OSHA BBP Standard, employers are required to (i) develop a written Exposure Control Plan, (ii) offer employees the hepatitis B vaccination, and (iii) provide initial and annual Bloodborne Pathogens training. The OSHA BBP Standard (29 CFR 1910.1030(g)) and OSHA Specifications for accident prevention signs and tags (29 CFR 1910.145(e)(4)) require universal biohazard warning 

to be posted on all access doors used to signify the actual or potential presence of a biohazard and to identify equipment, containers, rooms, materials, experimental animals, or combinations thereof, which contain, or are contaminated with, viable hazardous agents.

The NIH document Research Involving Recombinant or Synthetic Nucleic Acid Molecules guides construction and handling of recombinant or synthetic nucleic acid molecules and organisms containing such molecules. Institutions that receive NIH funding for research involving recombinant or synthetic nucleic acid molecules are required to comply with these guidelines as a condition of funding. This document requires that each institution establish an Institutional Biosafety Committee (IBC) with the authority to approve proposed research involving recombinant or synthetic nucleic acid molecules using the NIH guidelines as the minimum standard.

Medical and biological waste products in NC fall under the purview of 15A NCAC 13B.1200 North Carolina Medical Waste Management standard.

The Department of Health and Human Services (DHHS) 42 CFR Part 73; Select Agents and Toxins

and **United States Department of Agriculture (USDA)** 7 CFR Part 331 and 9 CFR Part 121; Possession, Use, and Transfer of Select Agents and Toxins. These regulations require institutions that possess, use, or transfer certain biological agents and toxins (select agents) to be registered and approved by DHHS and/or USDA Animal and Plant Health Inspection Service (USDA APHIS).

American Society for Microbiology (ASM) provides guidelines for Biosafety in Teaching Laboratories. These guidelines provide educators with a consistent way to safely work with microorganisms in the classroom to prevent their spread.

U.S. Department of Transportation and the International Air Transportation Authority regulate transport of hazardous materials, including biological agents.

The CDC established specific regulatory requirements for international and interstate importation or transportation of etiologic materials, which include a permit application that must be submitted and approved prior to any such importations. The federal regulation governing the importation of etiologic agents is USPHS 42 CFR – Part 71 Foreign Quarantine. Part 71.54, Infectious biological agents, infectious substances, and vectors.

The U.S. Department of Commerce (DOC) has specific regulatory requirements for exportation of biological materials. These regulations are both agent and country specific and must be followed strictly.

II. Purpose

North Carolina Central University (NCCU) is committed to providing a safe and healthful environment for all persons including staff, students, visitors and the surrounding community and environment. This Biosafety Manual complements the Laboratory Safety Manual and provides a guidance to common practices related to work with biological material in teaching and research laboratories at the NCCU.

The purpose of this document is to define policies and procedures pertaining to use of biological materials in all research laboratories at NCCU. Implementation of biosafety measures reduces the likelihood that a biological incident will occur and minimize the severity if one were to occur. In addition, this manual fulfills required biosafety regulations. Laboratory work may involve exposure to biological hazards, as well as to chemical and radiological hazards. Consequently, this manual should be used in conjunction with the Chemical Safety Plan and Radiation Safety Plan, respectively.

III. Scope

This manual covers all faculty, staff, students, visitors participating in research or teaching activities that utilize biological agents.

For purposes of this manual the term biological agents include the following:

a. Recombinant or synthetic nucleic acid molecules, including their use in animals and plants.

b. Human and other primate-derived substances (blood, body fluids, cell lines or tissues).
c. 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.
d. Select agents or toxins (human, animal, or plant).
e. Biologically active agents (e.g., venoms, toxins produced by living organisms).

IV. Emergency Numbers

Agency	Phone	Hours
Emergency	911	24 hours
Environmental Health & Safety	919-530-7125 or 919-530-6925	8:00 a.m. – 5:00 p.m.
University Police	919-530-6106 or 911	24 hours
Work related injuries	919-530-6106 or 911	24 hours
Biological spill	919-530-7125 919-530-6106 or 911	8:00 a.m. – 5:00 p.m. 24 hours
NC Poison Control Center	1-800-6946	24 hours

V. Definitions and Abbreviations

ARC – The Animal Resources Complex at NCCU.

Biological Agent - any 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); microorganisms (including, but not limited to, parasites, bacteria, viruses, fungi, prions, or rickettsia), or material that may contain these microorganisms; biologically active agents produced by living organisms.

Biosafety - promotes safe laboratory practices, procedures and proper use of containment equipment and facilities among University staff and visitors.

Biosafety Level (BSL) – a specific combination of work practices, safety equipment, and facilities, intended to minimize exposure of workers and environment to certain classes of pathogens. The biosafety levels range from the lowest BSL-1 (minimal risk; agents that usually pose a minimal potential threat to laboratory workers and the environment and do not consistently cause disease in healthy adults) to the highest level BSL-4 (high risk; agents that are extremely dangerous and pose a high risk of life-threatening disease).

Blood - human blood, human blood components, and products made from human blood.

Contaminated – presence or the reasonably anticipated presence of hazardous biological material on an item or surface.

Decontamination - use of physical or chemical means to remove, inactivate, or destroy hazardous biological material on a surface or item to the point where they are no longer capable of transmitting

infectious particles and the surface or item is rendered safe for handling, use, or disposal.

Disinfection – is a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects.

Engineering Controls – strategies design to protect workers from hazardous conditions by placing a barrier between the worker and the hazard or by removing hazardous substances through air ventilation. (e.g., biosafety cabinets).

Exposure – a direct contact with biological agents or material contaminated with biological agents known to be associated with health problems, including infectious diseases, cancer, and allergies. The exposure may result from needle sticks or cuts with contaminated material, splash to unprotected face, direct contact of biological agents with mucous membranes (i.e., eye, mouth, nose, non-intact skin), or an aerosol involving human pathogen generated outside of biosafety cabinet.

HBV – a hepatitis B virus causing hepatitis (inflammation of the liver).

HIV – a human immunodeficiency virus that attacks the body's immune system.

IBC - Institutional Biosafety Committee. It provides local review and oversight for research involving recombinant or synthetic nucleic acid molecules and other biological hazards.

LAIs - Laboratory-associated infections, are all infections acquired through laboratory activities, regardless of whether they are symptomatic or asymptomatic in nature, as a result from occupational exposure to infectious agents.

Personal Protective Equipment (PPE) - is equipment worn to minimize exposure to hazards that cause serious workplace injuries and illnesses.

Potential Exposure – a failure of PPE or engineering control with no known direct contact or exposure to biological agents, aerosol, spills, needle stick or cut with object not know to be contaminated at workplace utilizing biological agents.

Recombinant DNA (rDNA) - DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) that bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome.

Regulated Waste - liquid or semi-liquid blood or OPIM; contaminated items that would release blood or OPIM in a liquid or semi-liquid state if compressed, items that are caked with dried blood or OPIM and are capable of releasing these materials during handling, contaminated sharps, and pathological and microbiological wastes containing blood or OPIM.

Standard Operating Procedures (SOPs) - a set of written instructions that describes the step-by-step process that must be taken to properly and safely perform a routine activity.

Sterilization - a process using physical or chemical methods to destroys or eliminates all forms of microbial

life, including highly resistant bacterial endospores.

Toxin - the toxic material or product of plants, animals, microorganisms (including, but not limited to, bacteria, viruses, fungi, rickettsia, or protozoa), or infectious substances, or a recombinant or synthesized molecule, whatever their origin and method of production, and includes any poisonous substance or biological product that may be engineered as a result of biotechnology, produced by a living organism; or any poisonous isomer or biological product, homolog, or derivative of such a substance.

VI. Responsibilities

The responsibility for biosafety at University is a team effort requiring the direct involvement of the University Institutional Biosafety Committee (IBC), the Environmental Health and Safety Department (EHS), Principal Investigators (PIs), NCCU Laboratory Staff, Students, and Teaching Lab Instructors.

A. Institutional Biosafety Committee

The Institutional Biosafety Committee (IBC) approves biological agent use protocols, develops biosafety policies, and provides administrative oversight of the University biosafety program, with the goal of reducing laboratory biosafety risks to the University community. Responsibilities of the IBC include:

- 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. Generally, the biosafety levels (BSLs) established by the CDC and NIH will be used as the level of containment; however, the IBC can increase or decrease the level of containment according to the specific circumstances of the project.
- 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 will recommend 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.

B. Environmental Health and Safety

The University EHS is responsible for providing guidance on safe handling of biological agents and overall management of the Biosafety program. The EHS Biosafety Specialist is a member of the IBC. Specific responsibilities of the EHS include:

- Serve as the biosafety subject matter expert for the IBC.
- Develop, implement, and enforce biosafety policies and practices.

- Develop and train emergency response plans for spills, personnel contamination, and incidents involving biological agents.
- Provide for periodic inspections of laboratories to assess safety issues.
- Provide biosafety training for university personnel as required.
- Provide information and consultation on for occupational health issues related to biohazards.

C. Principal Investigator

Principal Investigators (PIs) are responsible for the health and safety of all personnel in their laboratory. Specific responsibilities of the PIs include:

- Registering the biological materials with IBC by completing the IBC Registration form to obtain approval for work involving biohazardous material as specified in this 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). The LSP template is available on the EHS website.
- 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 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. Trainings are conducted by EHS and ARC. 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 (based on current recommendations of the CDC and the NCCU IBC).
- 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 (including laboratory spills).

- Ensuring that an accurate inventory of biological agents is maintained.
- Ensuring that biological agents are disposed of as outlined in this 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.
- Ensuring that all maintenance work in, on or around contaminated equipment is conducted only after that equipment is properly decontaminated by the laboratory staff or PI. Make sure to fill and post the Equipment Safety Clearance Form (Appendix A).
- Encouraging personnel to seek medical consultation services if they are immune compromised or otherwise have health concerns without asking laboratorians to disclose personal health information. Requests for medical evaluation services can be made by employees through EHS. Students can obtain these services directly via Student Health.
- Ensuring that periodic self-inspections of the laboratory are conducted to identify and correct health and safety deficiencies. The Laboratory Audit Checklist with the instructions (Appendix A, a part of the Laboratory Safety Manual) is available to ensure laboratories meet at least the minimum requirements dictated by regulations and relevant guidance.
- As part of vacating a laboratory space, ensuring that all biohazardous materials are inactivated, moved, or transferred to a new under an approved LSP.

D. NCCU Laboratory Staff/Laboratorians

Laboratory workers are the most important element in developing and maintaining a safe laboratory environment. Laboratory workers are responsible for their own health and safety, as well as that of their coworkers. An incident caused by one laboratory worker can have a widespread effect on others. Specific responsibilities include:

- Following all established procedures and practices.
- Knowing how to access the Biological Safety Manual (this document) and being knowledgeable of requirements and procedures contained in the manual.
- Using practices and procedures specified in this 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.

E. Teaching Laboratory Instructors/Educators

The NCCU IBC and EHS together uphold the standard of practice as described in the ASM Guidelines for

Biosafety in Teaching Laboratories to maintain consistency among university instructors/educators in biological safety levels 1 and 2 (BSL-1 and BSL-2). Specific responsibilities include:

- 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.

VII. Training

At NCCU, Principal Investigators communicate biological hazards to laboratory workers at the time of initial assignment, and whenever new exposure risks are identified.

Principal Investigators are responsible for training and retraining new staff in practices to the point where aseptic techniques and safety precautions become second nature. An evaluation of a person's training, experience in handling infectious agents, proficiency in the use of sterile techniques and biosafety cabinets, ability to respond to emergencies, and willingness to accept responsibility for protecting oneself and others is important insurance that a laboratory worker is capable of working safely. For more information on training lab workers in biosafety techniques, review the CDC Guidelines for Laboratory Biosafety Competency. In general, training should include:

- General safety practices and safety theory.
- Medical surveillance program.
- Specific safety practices and Standard Operating Procedures.
- The location and availability of information on biohazardous material, safe handling, storage, disinfection/decontamination procedures, and proper disposal of biohazard material.
- Hazards associated with biological materials including signs and symptoms associated with exposure.
- Laboratory and other research space entry and exit procedures.
- Proper use of Personal Protective Equipment and all safety equipment.
- Proper and safe handling of research animals.
- Incident and accident reporting.

Training components include training under normal operating conditions, during emergencies, system

failures, and in the event of a suspect or known exposure.

In addition to the New Employee Orientation for the Laboratory Environment, a Laboratory Biosafety Training is required to be completed initially and every 3 years afterwards.

Bloodborne pathogen (BBP) training is required initially and annually thereafter.

Training on the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules is required of all Principal Investigators with labs working with recombinant or synthetic nucleic molecules. While the online NCCU Laboratory Biosafety Training meets the minimum requirement, additional content is available online from the NIH website.

VIII. Required Documentation and Record Keeping

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 Forms
- Hepatitis B Vaccination and Declination Forms
- Reports and Investigations of Accidents

The following documents must be available in the place of work to all laboratorians and research staff. Many of these documents require initial and annual review

1. NCCU Lab Safety Manual
2. NCCU Biological Safety Manual
3. Laboratory-Specific Safety Plan
4. Access to risk assessment information for all biohazardous materials.

IX. 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 as they related to each laboratory or research space. Follow-ups are conducted after the initial inspection to ensure that corrective actions have taken place. For more details on Laboratory Safety Surveys, please see Laboratory Safety Manual. The following outlines the biological safety laboratory survey criteria and references the specific guidelines.

- Biological agent inventory
- Current IBC/EHS approval of all research.

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- Laboratory signage (OSHA 29 CFR 1910.1030(e)(2)(ii)(D), OSHA 29 CFR 1910.1030(g), and OSHA 29 CFR 1910.145(e)(4)).
- Availability of Biological Safety Manual, Exposure Control Plan, and Laboratory-Specific Safety Plan.
- Personal protective equipment availability (OSHA 29 CFR 1910.1030(d)(3)(i)).
- Hand washing sinks, eye wash, safety shower, and Class A first aid kit in lab.
- Aerosol minimizing techniques in place.
- Current biosafety cabinets certification (CDC/BMBL 6th ed., NSF/ANSI 49-2019, Annex 1).
- Biological waste storage, decontamination, and disposal practices (CDC/BMBL 6th ed., OSHA 29 CFR 1910-1030(d)(4)(iii)).
- Appropriate sharps container use (OSHA 29 CFR 1910.1030(d)(2)(viii)).

Non-compliance with applicable measures outlined in the NCCU Biological Safety Manual can result in severe repercussions for NCCU workers (e.g. disease, injury, death), the PI, and University as a whole (e.g. loss of funding, litigation, etc.). Noncompliance includes, but is not limited to:

- Failure to register biohazardous agents, including non-exempt recombinant or synthetic nucleic acid molecules.
- Failure to provide updates and/or other required documentation of the specified due date.
- Unsafe biological safety/biological containment practices as documented through lab inspections, routine or otherwise.
- Failure to correct a documented (confirmed) biological safety complaint or concern.

Noncompliance will be reported to the IBC which may result in suspension or termination of all approved registrations. The PI's Department Head, Dean, and/or other applicable administrators will be notified of the noncompliance, while granting agencies or regulatory authorities may be notified as required by their respective reporting standards.

A. Request for Hazard Investigation

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 encouraged to report such conditions to EHS (ehs@nccu.edu, by calling 919-530-7125, or on-line Health and Safety Reporting) and to request an investigation into the need for corrective action.

Persons requesting an inspection by EHS may request confidentiality, and by law, their name will not appear on any record published, released, or made available to the public, their immediate supervisor, or department head.

After EHS has concluded its investigation, results are communicated, in writing, to the party requesting the investigation and other appropriate University personnel, with due consideration to anonymity requests.

EHS will initiate corrective action if there are reasonable grounds to believe that a violation or danger exists. If EHS cannot implement corrective action within a reasonable period, EHS may terminate laboratory operations pending corrective action.

X. Laboratory-Associated Infections and Prevention

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.

A. Route of infection

Microorganisms can enter the body through various routes including mucous membranes (mouth, respiratory tract, etc.), broken or intact skin, and the eyes. Common routes of LAI include:

- **Inoculation** of skin with contaminated sharps or from animal bites or scratches.
- **Ingestion** of infectious agents to the mouth from mouth-pipetting, eating, drinking, smoking, and applying cosmetics; these practices are not permitted in the lab. Additionally, touching your face with contaminated hands or gloves can introduce infectious agents into the mouth.
- **Mucous membrane exposure** can result from splashes/spills of infectious materials or touching face with contaminated hands or gloves, membranes.
- **Inhalation of infectious aerosols or droplets.**

B. Preventive measures of LAIs

Using proper work practices that block routes of exposure protect you and others from workplace infections.

- Label all equipment and items used to store and process infectious materials with biohazard warning.
- Infectious materials must be clearly labeled and properly stored.
- Keep workspaces clean and 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 a 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. Use safety rotors or cups when centrifuging and unload them in a biosafety cabinet.
- No mouth pipette.
- Utilize safe handling and disposal techniques when working with sharps, such as needles, scalpels, pipettes, and broken glassware.
- 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.

XI. Medical Surveillance

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. In addition, medical care is provided at no cost to the employee in the event of an exposure or potential exposure.

If you are immunocompromised, you may be at a higher risk for acquiring infections and you should consult with a medical professional through the medical surveillance program. 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.

Employees and students are not required to discuss their personal medical information with their PI or anyone in the lab. Rather they are encouraged to contact EHS who can arrange for occupational medical services to be paid for by the University. Students are encouraged to reach out to NCCU Student Health Clinic for no-cost occupational health services if required.

XII. Emergency Procedures

A. Biosafety Incident and Emergency Plans

A **biosafety incident or "release"** is either an exposure to personnel or an event that results in the discharge of a biohazard agent outside primary containment. The event can be the result of a splash or spill of infectious material, needle sticks or cuts with contaminated material, aerosol generating event outside of biosafety cabinet involving a human pathogen, or potential exposure resulting from a failure of the containment system (tearing of gloves or other failure of PPE, improper biosafety cabinet performance, etc.).

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It is responsibility of each laboratory to include in their Laboratory-Specific Safety Plan information on how to respond to and report laboratory emergencies. 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 15 minutes. Use soap on skin for biological/blood exposure. Hold eyes open to ensure effective rinsing behind both eyelids.
3. Immediately after rinsing, notify the PI or lab manager who will assist in obtaining immediate medical attention and notifying EHS.
4. Report incident as described under Incident Reporting Instructions.

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, notify the PI or lab manager who will assist in obtaining immediate medical attention and notifying EHS. Report incident as described under Incident Reporting Instructions.

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, notify the PI or lab manager who will assist in obtaining immediate medical attention and notifying EHS. Report incident as described under Incident Reporting Instructions.

B. Obtaining Medical Attention

NCCU Employee

If the incident occurs during normal business hours and requires more than simple first-aid measures (i.e. cleaning and a band aid), 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.

1. If the incident occurs after normal business hours and or 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

1. If an NCCU student becomes injured or experiences an exposure event, the incident occurs during normal business hours and care is necessary, the student should contact Student Health Services at 919-370-0901 for information on how to proceed.

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2. 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.

C. 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 to EHS, even if there was no known exposure event. If the known or potential exposure occurs outside of normal work hours, laboratorians should contact their PI by phone immediately.

It is the responsibility of the PI to ensure that employees and students receive prompt treatment of any injuries or LAIs. If treatment requires more than first aid, campus police (919-530-6106) or 911 should be called to request medical care and/or transport. Never send an injured employee to seek medical attention for an injury on their own or transport them yourself.

Any employee who experiences an occupational illness or injury including exposure is required to complete the Workers' Compensation Employee Statement Form and each Supervisor must complete the Supervisor's Accident/Incident Investigation Report Form. The Supervisor's form must be submitted to the Workers' Compensation Administrator within 24 hours of a workplace incident. Visit the following link Worker's Compensation: What You Need to Know for detailed Accident Reporting Guide.

D. Incident Review

The Principal Investigator and the EHS/IBC will review the circumstances of all incidents to determine:

- Engineering controls in use at the time.
- Work practices followed.
- Protective equipment or clothing that was used at the time of the incident (gloves, eye shields, etc.).
- Location of the incident.
- Procedure being performed when the incident occurred.
- Personnel training.

If revisions to the Biosafety Manual or Lab-Specific Safety Plan are necessary, those changes will be made by the appropriate owner of the document and approved by EHS/IBC. Changes may include evaluation of the risk assessment, safer devices, additional training, etc.

E. Biohazard Spill Procedures

It is extremely important to the safety of those working in the laboratory to contain biological spill and to take all needed precautions to avoid spreading the contamination while performing biological spill cleanup. All waste produced from the cleanup must be disposed of as biological hazardous waste.

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Spills must be cleaned up as soon as practical by properly trained personnel and in accordance with the spill procedures outlined in the Laboratory-Specific Safety Plan.

F. Spill Kit

Spill kits are readily available in each laboratory and contain:

- Spill SOP and sign (see below an example)
- EPA-registered disinfectant
- Absorbent materials (pads, towels, etc.)
- Personal Protective Equipment (gloves, lab coat, booties, lab safety glasses)
- Biohazard bags
- 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)

G. Spill Inside a Biosafety Cabinet

If a spill occurs inside the Biosafety Cabinet (BSC), the focus is to keep the spill inside the BSC as the directional airflow and HEPA filter is protecting you and the environment.

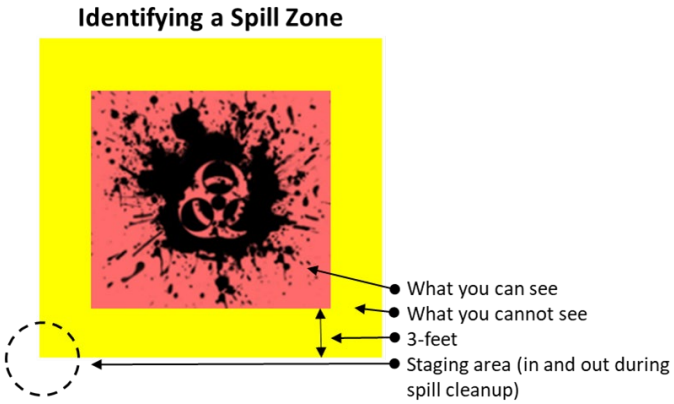
- Keep the BSC blower running and alert others in the laboratory.
- Do not remove your hands from the BSC unless you disinfect your gloves first.
- Do not place your head in the cabinet to clean the spill, keep your face behind the view screen.
- Remove any solid/sharp objects using tongs and small pieces 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.

H. Spill Outside of the Biosafety Cabinet

If a spill occurs outside the BSC, the focus is to protect you and those in the laboratory while you control the spill zone.

- 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 and spreading the contamination. 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, and leave the PPE in the staging area.
- Remove any contaminated clothing or personal protective equipment 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 and bring 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.



I. Spill in Centrifuge

To centrifuge BSL-2 agents, always use sealed safety-caps (left), sealed buckets (middle) or sealed rotors (right) as shown in the Figure below.

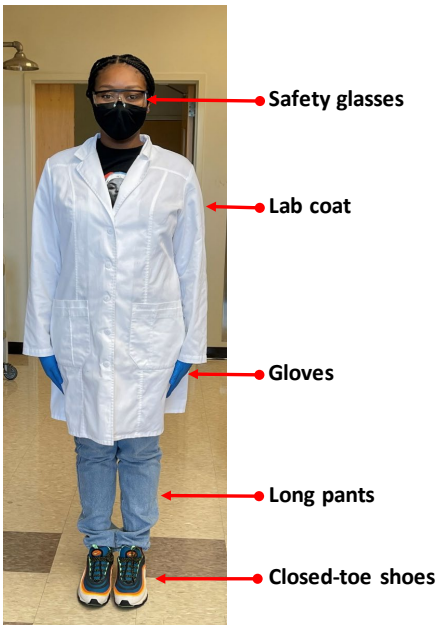
- Wait 5 minutes before opening the centrifuge following the end of a run with potentially hazardous biological material. If a spill is identified after the centrifuge lid is opened, carefully close the lid. Let aerosols settle for 30 minutes.
- Remove any contaminated protective clothing and place into 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 sharps 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 report the incident to Principal Investigator or Lab Safety Manager. If at any time you feel uncomfortable or unprepared to respond to a spill, stop what you are doing, take actions to protect yourself and others by containing the spill, and contact EHS for further assistance.

XIII. Personal Protective Equipment and Clothing

Personal protective equipment (PPE) is specialized clothing or equipment worn by laboratory personnel for protection against hazards when engineering and administrative controls are not feasible or effective. 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. The PPE must be appropriate for the task and users must be trained to understand the use and limitations of the protective gear. The PPE used in research laboratories includes laboratory coats and gowns, eye protection, face shields and the appropriate gloves. Street clothes are not PPE. PPE should be worn while working in the laboratory and must not be taken home or worn outside the laboratory in non-laboratory areas.



Under OSHA's Personal Protective Equipment standards, 29 CFR 1910.132 and 29 CFR 1910.1030(d)(3)(i), the employer must ensure that appropriate personal protective equipment (PPE) is accessible at the worksite or issued to workers.

For assistance in selecting PPE, contact the EHS.

A. Clothing and Protective Apparel

The laboratory coats/gowns/coverall suits/scrubs/smocks/foot covers are used to protect street clothing against biological or chemical spills as well as to provide some additional body protection. The specific hazard and the degree of protection required must be known before selecting coats for lab personnel. Aprons are not appropriate for the lab as long sleeves are necessary for arm protection. The CDC/NIH guidelines for bio-containment practices recommend the use of a lab coat, wrap-around gown, smock, or scrub suits

while working in research labs. Lab coats should be provided for visitors, maintenance and service workers as needed. The lab coat can be disposable or be sent for washing to a commercial laundry service. Never wear a contaminated lab coat outside of the laboratory or take them home.

B. Gloves

Skin contact is a potential source of exposure to infectious materials; it is important that the gloves are worn whenever working with biohazards, toxic substances or hazardous chemicals. Gloves are selected by the PI and lab personnel based on the hazards involved and the type of work being done. The guidance on gloves selection can be find in the OSHA publication.

General gloves considerations:

- 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 EHS to assess and determine exposure potential.
- For disposable gloves check expiration date and do not use gloves 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. Adopt The Scoop Method for proper glove removal technique.
- Consider latex-free or nitrile gloves to avoid latex-associated allergic reactions.
- Gloves do NOT replace the need for hand washing. Always wash your hands after removing gloves and before exiting lab.
- Never use gloves outside the laboratory.
- Contaminated gloves are disposed of and decontaminated as biohazard waste.
- Gloves should also be worn whenever it is necessary to handle rough or sharp-edged objects, and very hot or very cold materials. The type of glove materials to be used in these situations includes, leather, aluminum-backed gloves, and other types of insulated glove materials.

C. 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. Face protection is required in situations where chemical/biological splashes or aerosol exposure to infectious material are possible. These include necropsy of infected animals, harvesting of tissues, and manipulations of infectious materials. Goggles or safety glasses with side shields should be used in combination with masks, face shields or other splatter guards for optimal protection. The PI has the responsibility to assess the potential for eye/face injuries, to train employees on the uses and

limitations of PPE, to provide the type of protection required, and to ensure that the appropriate eye/face PPE is available and used by laboratory personnel. All eye/face protection devices must meet the requirements set forth in the ANSI Z87 standard. This standard specifies a minimum lens thickness of 3 mm, impact resistance requirements, passage of a flammability test, and lens-retaining frames. Prescription safety spectacles are recommended for employees wearing glasses.

The following is required for eye and face protection:

- **Safety glasses:** If the work involves an impact biohazard, with low probability of splashes and chemicals that are of a low hazard, safety glasses are an appropriate choice.
- **Biohazard safety goggles:** These are required whenever there is any probability, no matter how low, of solvent or infectious material splash occurring.
- **Full face protection (such as face shield):** Required whenever there is an anticipated splash or spray of biohazardous materials or a high potential for aerosol generation. These are not a replacement for eye protection; thus splash goggles should also be worn.

Contact EHS for additional information on the assessment, selection, and use of eye/face protection equipment.

D. 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.

E. 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 or chemicals, due to the potential exposure to infectious agents or toxic materials, as well as physical injuries associated with the work.

F. PPE Cleaning and Maintenance

It is important that you properly maintain and keep all PPE clean. Cleaning is particularly important for eye and face protection where dirty or fogged lenses could impair vision. 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.

XIV. Biosafety Risk Assessment

A **Biosafety Risk Assessment** is the foundation of safe laboratory operations. It needs to be conducted prior proposed experiment or project to determine the appropriate containment, i.e., work practices, PPE, equipment, training, and workspace designed to protect laboratory employees and students, maintenance and service workers, the public, agriculture, and the environment. Identifying the **Risk Group** (RG) is usually the first step in determining the appropriate **Biosafety Level** (BSL) for working with the biological agent. It is the PI's responsibility to propose a biosafety level that the IBC evaluate at the time of registration. There are four commonly recognized Biosafety Levels (BSL1, 2, 3 and 4). The proposed biosafety level should be based on a thorough risk assessment that, at a minimum, includes a review of the following resources:

- The NIH Guidelines (Appendix B) provides common biological agents used in research listed by Risk Group.
- Agent Summary Statements for some infectious agents are provided in the BMBL and indicate the appropriate biosafety level for some infectious agents. Section II of the BMBL describes the process of Biological Risk Assessment.
- The American Biological Safety Association (ABSA) website provides a searchable database of many biological agents and their assigned biosafety levels by country.
- The Pathogen Safety Data Sheets are produced by the Public Health Agency of Canada as educational and informational resources for laboratory personnel working with certain infectious substances.

When performing a risk assessment, it is advisable to take a conservative approach if there is an incomplete information available. Factors to consider when evaluating risk include the following:

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

A. Risk Groups

The NIH and WHO recommend four risk groups (RG1-4):

Risk Group	Description
Risk group 1 (RG1)	Agents that are not associated with disease in healthy adult humans; e.g., *B. subtillis, E. coli K-12*, Adeno-associated virus, ecotropic avian sarcoma virus.
Risk group 2 (RG2)	Agents that are associated with human disease, which is rarely serious and for which preventive or therapeutic interventions are often available; e.g., Human adenoviruses, human herpesviruses (except herpes B), *Staphylococcus aureus*, amphotropic murine leukemia virus, influenza virus A, B, and C (except highly pathogenic avian influenza virus strains).
Risk group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk); e.g., *Mycobacterium tuberculosis*, Venezuelan equine encephalitis virus, *Francisella tularensis.*
Risk group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk); e.g., Ebola, Marburg, Lassa, and Herpes B virus.

The risk groups designation along with the risk assessment are the steps used to determine the appropriate procedures and containment to use while working with a particular agent in the lab. Containment describes a combination of primary and secondary barriers, with the purpose to reduce the risk of exposure to staff and the unintentional release of hazardous biological agents or toxins into the surrounding community and environment.

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 biological safety 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.

B. Biosafety Levels

The BMBL, outlines safe lab practices, lab facilities, and safety equipment for four biosafety levels (BSL-1-4) that provide appropriate containment based on a proper risk assessment for manipulations of various risk group agents designated by the NIH (infectious microorganisms, toxins, and laboratory animals). The four levels are organized in ascending order by the degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

Biosafety levels and requirements for laboratory work are summarized in the Table below. Note: Currently BSL-3 and BSL-4 work is not approved at NCCU.

BSL	Agents	Special Practices[a]	Primary Barriers and PPE[a]	Facilities (Secondary Barriers)[a]
1	Well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment.	Standard microbiological practices	No primary barriers required; protective laboratory clothing; protective face, eyewear, as needed	Laboratory doors; sink for handwashing; laboratory bench; windows fitted with screens; lighting adequate for all activities
2	Agents associated with human disease and pose moderate hazards to personnel and environment	Limited access; occupational medical services including medical evaluation, surveillance, and treatment, as appropriate; all procedures that may generate an aerosol or splash conducted in a BSC; decontamination process needed for laboratory equipment	BSC or other primary containment device used for manipulations of agents that may cause splashes or aerosols; protective laboratory clothing; other PPE, including respiratory protection, as needed.	Self-closing doors; sink located near exit; windows sealed or fitted with screens; autoclave available
3	Indigenous or exotic agents; may cause serious or potentially lethal disease through the inhalation route of exposure	Access limited to those with need to enter; viable material removed from laboratory in primary and secondary containers; opened only in BSL-3 or ABSL-3 laboratories; all procedures with infectious materials performed in a BSC	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls; two pairs of gloves, when appropriate; protective eyewear, respiratory protection, as needed	Physical separation from access corridors; access through two consecutive self-closing doors; hands-free sink near exit; windows are sealed; ducted air ventilation system with negative airflow into laboratory; autoclave available, preferably in

				laboratory
4	Dangerous and exotic agents that pose high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that are frequently fatal, for which there are no vaccines or treatments; and related agents with unknown risk of transmission	Clothing change before entry; daily inspections of essential containment and life support systems; all wastes decontaminated prior to removal from laboratory; shower on exit	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls;[b] gloves;[b] full-body, air-supplied, positive pressure suit[c]	Entry sequence; entry through airlock with airtight doors;[c] walls, floors, ceilings form sealed internal shell; dedicated, non-recirculating ventilation system required; double-door, pass-through autoclave required

*PPE – Personal Protective Equipment; a - each successive BSL contains the recommendations of the preceding level(s) and the criteria in the cell; b - applies to cabinet laboratory; c - applies to suit laboratory.

1. Biosafety Level 1 (BSL-1)

BSL-1 labs are used to study infectious agents or toxins not known to consistently cause disease in healthy adult humans or animals. Workers follow basic safety procedures, called standard microbiological practices, and require no special equipment (primary barriers) or design features (secondary barriers) except for a hand washing sink and safety shower/eyewash station. Agents can be used safely on the open bench. Standard engineering controls in BSL-1 laboratories include easily cleaned surfaces that are able to withstand the basic chemicals used in the laboratory.

The standard microbiological practices are as followed:

- Access to the laboratory is limited or restricted at the discretion of the PI when experiments or work with cultures and specimens are in progress.
- Persons wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
- Mouth pipetting is prohibited; mechanical pipetting devices are used.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak-proof container, closed and placed into secondary

container for transport from the laboratory. Materials to be decontaminated off-site are packaged in accordance with applicable state and federal regulations before removal from the facility.

2. Biosafety Level 2 (BSL-2)

BSL-2 laboratories are used for work with a broad spectrum of moderate-risk infectious agents or toxins that are associated with human diseases of varying severity. Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures or ingestion of infectious materials. Procedures with high aerosol or splash potential must be conducted in primary containment equipment such as biosafety cabinets. Primary barriers (e.g., splash shields, face protection, gowns, and gloves) should be used as appropriate. Secondary barriers (e.g., hand washing and waste decontamination facilities) must be available.

In addition to BSL-1 procedures, level 2 also requires the following special practices:

- Access to the laboratory is limited or restricted by the PI when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. The PI has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.
- The PI establishes policies and procedures where only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.
- A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.
- Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine, etc.).
- Biosafety procedures are incorporated into standard operating procedures (SOPs) or in a Lab-Specific Safety Plan adopted or prepared specifically for the laboratory by PI. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
- The PI ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
- A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels (see Appendix C).

- Cultures, tissues, specimens of body fluids, or other potentially infectious materials are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
- Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.
- Spills and accidents that result in overt exposures to infectious materials are immediately reported to the PI and EHS. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
- Animals not involved in the work being performed are not permitted in the laboratory.
- Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
    - Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonic eggs.
    - High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety buckets are used, and if these rotors or safety buckets are opened only in a biological safety cabinet.
- Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.
- Protective laboratory coats, gowns, aprons, or uniforms designated for laboratory use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, and administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; personnel should never take it home.
- Gloves must be worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn

outside the laboratory. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

- Provide lockable doors for facilities that house select agents.
- Each laboratory contains a sink for hand washing.
- The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
- Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
- Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
- Install BSCs in such a manner that fluctuations of the room supply and exhaust air do not cause the BSCs to operate outside their parameters for containment. Locate BSCs away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the BSCs' air flow parameters for containment.
- An eyewash station is readily available.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without re-circulation to spaces outside of the laboratory. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed. The PI is responsible for selecting additional safety practices, which must be in keeping with the hazard associated with the agent or procedure. Laboratory personnel safety practices and techniques must be supplemented by appropriate facility design and engineering features, safety equipment and management practices.

XV. Biohazardous Materials

Both the BMBL and the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules establish best work practices for pathogenic agents divided into one of four risk groups. Specifically, the NIH establishes safe work practices for research with recombinant and synthetic nucleic acid molecules.

A. Microorganisms

Common microorganisms causing disease have been classified by the CDC and NIH according to their risk

associated with severity of disease. New or unknown pathogens that have not been assessed by CDC/NIH must go through a risk assessment to determine their biosafety containment level. It is PIs responsibility to register any project involving a pathogenic agent with the IBC and receive their approval before work is initiated. Following receipt of the completed IBC registration, the laboratory will be surveyed by the EHS Specialist to ascertain that it meets the containment requirements for the agent listed in BMBL. The EHS will report the findings to the IBC as part of the review and approval process.

B. Recombinant DNA and Synthetic Nucleic Acid Molecules

The NIH reviews all Recombinant DNA and Synthetic Nucleic Acid Molecules research proposals that fall under their scope of approval. The NCCU requires all biological research that involves genetic modifications to be filed using the IBC Registration form, regardless of whether it is exempt from NIH review. As a condition for NIH funding of Recombinant or Synthetic Nucleic Acid Molecules research, institutions shall ensure that such research conducted at or sponsored by institution, irrespective of the source of funding, shall comply with the NIH Guidelines (Section I-D, NIH, 2019). Consequences of noncompliance include suspension, limitation, or termination of NIH funds for recombinant or synthetic nucleic acid molecules research at the institution, or a requirement for prior NIH approval of recombinant or synthetic nucleic acid molecules projects at the institution.

Classification of Recombinant and Synthetic Nucleic Acid Molecules

The purpose of the NIH Guidelines is to specify institutional oversight for the safe handling and containment of:

- Recombinant nucleic acid molecules,
- Synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and
- Cells, organisms, and viruses containing such molecules including transgenic animals and plants.

At NCCU, the IBC reviews all research covered under the NIH Guidelines. To determine whether your research is subject to IBC review at NCCU see below:

Research that Requires NIH and IBC Approval before Initiation

Deliberate Transfer of Drug Resistance Trait to Microorganisms (Section III-A, NIH 2019). Experiments that fall in this category include those that transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally from their environment, specifically if this could compromise the ability to control the disease agent.

Cloning of Toxin Molecules (Section III-B, NIH 2019). Experiments in this category include experiments involving the cloning of toxin molecules with LD_{50} of less than 100 ng/kg body weight, including botulinum toxins, tetanus toxin, diphtheria toxin, and *Shigella dysenteriae* neurotoxin.

Research that Requires IBC Approval before Initiation

Use of Human Subjects for rDNA or Synthetic Nucleic Acid Transfer (Section III-C, NIH 2019). These include all experiments that involve the deliberate transfer of rDNA or synthetic nucleic acid molecules, or DNA/RNA derived from rDNA or synthetic nucleic acid molecules to one or more human research subjects. In addition to having IBC approval, these experiments require Institutional Review Board (IRB) approval and NIH Office of Biotechnology Activities (NIH/OBA) registration approval.

RG2/3/4 Pathogens, Infectious viruses, Helper Viruses in Tissue Culture, and Cultures >10 L (Section III-D, NIH 2019). This section covers whole animal or plant experiments, experiments involving the use of infectious DNA or RNA viruses, or use of defective DNA or RNA viruses in the presence of a helper virus in tissue culture, experiments involving DNA from Risk Group 2, 3 or 4 agents, experiments involving greater than 10 liters of culture, and experiments involving Influenza viruses. Prior to the commencing an experiment in this section, the PI must submit a Registration Form to the IBC. The IBC reviews and approves all experiments in this category prior to initiation. Additionally, IACUC will require filing of appropriate documentation for approval for animal experiments prior the initiation of these experiments.

Research that Requires IBC Notice

Requires IBC Notice Simultaneous with Initiation (Section III-E, NIH 2019). Experiments that involve forming rDNA or synthetic nucleic acids containing no more than two-thirds of the genome of any eukaryotic virus, genetically modified plants, transgenic rodents (ABSL-1 only), breeding of transgenic rats (ABSL-1 only). The IBC reviews and approves all such proposals, but IBC review and approval prior to initiation of the experiment is not required. When the PI is going to begin this experiment, a registration form should be submitted for approval.

Research Exempt from the NIH Guidelines

Does not Require Institutional Registration (Section III-F, NIH 2019), however, experiments in this category although exempt from the NIH Guidelines must still be registered with the EHS using the IBC Registration form. EHS will verify the exempt status of the registration. It is the responsibility of the PI to file the paperwork in a timely manner in accordance with NIH Guidelines.

C. Genetically Modified Organisms

The in-vitro incorporation of segments of genetic material from one cell into another known as recombinant DNA (rDNA) technology has resulted in altered organisms which are used to manufacture products such as vaccines, hormones, interferons, and enzymes. However, rDNA technology carries with it the potential for harm. A genetically altered organism may be directly pathogenic or toxic and if released into the environment, might crowd out beneficial organisms, transfer undesirable genetic traits to wild species or mutate into a pathogenic form. The risks associated with rDNA technology are to be assessed by the PI when registering their projects with the IBC.

1. Transgenic plants

The use of plants in biological research only necessitates IBC approval when plants are being inoculated with plant pathogens or when transgenic plants are being researched. Plants have a system for containment unique to only plants (BL1-P through BL4-P) developed by the NIH, and can be found in Appendix L, of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.

When using transgenic plants, identification on the door of the facility housing these plants should be posted to indicate need for preventing accidental release from the facility. The development of transgenic plants must be reported to the NIH and requires a full approval of the IBC before commencing. Please complete the IBC Registration form for approval.

Containment practices should be developed for the greenhouse and should be approved by the IBC. If an inadvertent release of plants or spill of microorganisms must be reported to the EHS and treated immediately. Complete a Hazard and Incident Report Form for accidental release records. Contact the EHS for guidance, training, discussion of facilities and greenhouse, and rules and regulations involving plants and plant biocontainment. All plant policies and procedures should be made available to all working on experiments in the greenhouse in their laboratory specific safety manual.

2. Transgenic Animals

The use of animals in research requires compliance with the Public Health Service Policy on the Humane Care and Use of Laboratory Animals (PHS) Policy and any state or local regulations covering the care or use of animals. All animal protocols involving the use of Recombinant or Synthetic Nucleic Acid Molecules, infectious agents, human blood, and toxic chemicals must be submitted to IBC for review and approval prior to final approval by the Institutional Animal Care and Use Committee (IACUC); iacuc@nccu.edu).

Like plants, for recombinant or synthetic nucleic acid molecule research involving animals a system for containment unique to only animals (BL1-N through BL4-N) have been developed by the NIH, and can be found in Appendix M, of the NIH Guidelines.

In the event an animal handler is bitten or scratched by an animal infected with a pathogen, a Hazard and Incident Report Form must be submitted to EHS and filed with the IBC, in addition to the accident forms that are filed with animal care (ARC Facility Manager and Attending Veterinarian) and the University. Handling bedding and animal waste must also take additional precautions and must follow the policies set forth by the **NCCU Animal Care and Use Program**, as well as policies in place for BSL-2 laboratories. All bedding from ABSL-2 animal research labs must be autoclaved prior to disposal. Contact the Biosafety Specialist (919-530-6925) and ARC Facility Manager (919-530-7002) for assistance developing a protocol for handling the animals and pathogen(s) used in the laboratory.

Factors that need to be considered when working in animal facilities and utilizing biohazardous material:

- Routes of transmission
- Volumes/concentrations of agent(s) being used
- Route of inoculation

Approved: 3/2/2022
Approved by: K. Long-Witter, Director

- Route of excretion of agents (if any)
- Zoonotic diseases to which the animals are susceptible, and humans are susceptible
- Natural parasites that could be a problem for the animals used
- Nature of the animals (do they bite, scratch, spit, etc.)
- Possible allergen considerations
- Design features required for safety and containment

For additional information refer to Laboratory Safety Manual (Chapter XIX – Laboratory Animal Safety) and to **NCCU Animal Care and Use Program**.

D. Bloodborne Pathogens

Research projects that utilize human blood, blood products or Other Potentially Infectious Materials (OPIM; e.g., body fluids, samples visibly contaminated with blood) are governed by the OSHA Bloodborne Pathogens (BBP) Standard (29 CFR 1910.1030). This federal regulation mandates a combination of engineering and work practice controls, training, Hepatitis B vaccination, and other provisions to help reduce occupational exposure to human blood and OPIM which may contain Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV) and other bloodborne pathogens. Under the OSHA BBP Standard, each employer is required to (i) establish a written Exposure Control Plan and make it available to employees with occupational exposure to BBP, (ii) offer employees the hepatitis B vaccination, and (iii) provide initial and annual Bloodborne Pathogen Training. PIs using human blood, blood products, body fluids or tissues must register with the IBC to get approval. Refer to the Section XVI and XVII in this manual to review Decontamination, disinfection, sterilization and Biohazard Waste Management requirements for materials covered under the OSHA Bloodborne Pathogens Standard.

E. Cell and Tissue Cultures

Cultures derived from humans or animals known to be infected with a pathogen, as well as cultures known or suspected to contain infectious microorganisms (e.g., herpesvirus or EBV-transformed cultures) should be assigned to the risk group appropriate for the suspected or known pathogen and handled using the relevant containment level and work practices. Repositories such as the American Type Culture Collection (ATCC) can provide information on some of the cell lines used in the lab. Cell lines that are not human or other primate cells and which do not contain known human or zoonotic pathogens are often designated for work at Biosafety Level 1 (BSL-1). These may require permits through NCDACS or USDA; Principal Investigators are responsible for obtaining all necessary permits.

In addition, mammalian cell cultures may carry unsuspected oncogenic, allergenic or infectious particles. It is impractical, if not impossible, to screen such cultures for all potentially harmful microorganisms; even well-characterized lines with a history of safe use can become contaminated by adventitious, possibly infectious, microorganisms. For this reason, it is prudent to treat all mammalian cultures as potentially infectious and to use Biosafety Level 2 (BSL-2) facilities and work practices whenever working with them.

The following cells and tissue must be listed on the Laboratory Safety Plan and handled at BSL-2:

- Human and non-human primate primary cells, established cell lines, and unfixed tissue
- Cell lines exposed to or transformed by a human or primate oncogenic virus
- Cells, cell lines or tissue infected with pathogens requiring BSL-2 containment

F. Viral Vectors

These vectors provide a broad spectrum of uses in basic and clinical sciences allowing both the transient and long-term expression of almost any gene of interest in specific tissues either in vitro (cell cultures, primary cells, organoids, microorganisms) or in vivo (humans, animals, plants). Special care should be given to the design, risk assessment (Section II of NIH Guidelines), and handling of virus vectors containing genes that make growth-regulating products, products released into the circulation, or products that may have a general effect on the host-immune system.

Baculovirus: Baculoviruses are known to infect insects. Although baculoviruses are capable of entering mammalian cells in culture they are not known to be capable of replication in mammalian or other vertebrate animal cells. Baculoviruses are commonly used in laboratory experimental work and handled at biosafety level 2 (NIH, 2019; Appendix B-V).

Adenovirus: Adenoviruses are infectious human viruses, which often cause mild respiratory illness. Rare cases of severe disease can occur, and its use as a genetic vector therefore requires the use of adequate containment equipment and practices. Biosafety Level 2 (BSL-2) is appropriate for many constructs (NIH, 2019; Appendix B-II-D). Particular care should be given to vectors containing genes that make products that may be similar to products made by the deleted adenovirus genes.

Adeno-associated virus (AAV): These are infectious human viruses with no known disease association. Some AAV types are common in the general population, and these viruses have the ability to integrate into the host chromosome. The NIH Guidelines (Appendix B-I) state that "adeno-associated virus (AAV – all serotypes), and recombinant AAV constructs, in which the transgene does not encode either a potentially tumor gene product or a toxin molecule and are produced in the absence of a helper virus" can in most cases be handled at lower biosafety level (BSL-1).

Herpesvirus: Herpesviruses include infectious human viruses such as herpes simplex virus type-1 (HSV-1), which is the most used vector system. HSV-1 is common in the general population but can cause encephalitis in rare cases; its utility as a vector system stems from its broad host cell range, ability to transduce neurons, and its large insert capacity. Generally, HSV is classified as a Biosafety Level 2 (BSL-2) organism requiring BSL-2 practices and procedures for all virus and Animal Biosafety Level 2 (ABSL-2) for all animal manipulation as well as animal housing.

Retrovirus: These are infectious viruses which can integrate into the genome of transduced cells with high frequency, and which may have oncogenic potential in their natural hosts. Retrovirus vector systems are typically based on murine viruses. These systems include ecotropic viruses (infect only murine cells),

amphotropic viruses (infect human cells) or pseudotyped viruses (replication defective; also infect human cells). Containment for vectors with the ability to infect human cells will usually be recommended at Biosafety Level 2 (BSL-2), as per the NIH Guidelines (Appendix B-V).

Lentivirus: Lentiviruses are a subset of retroviruses, with the ability to integrate into host genome, and to infect non-dividing cells. These viruses can cause severe immunologic and neurologic disease in their natural hosts. Lentivirus vector systems can include viruses of non-human origin (feline immunodeficiency virus, equine infectious anemia virus) as well as simian viruses (simian immunodeficiency virus, SIV) and human viruses (HIV). Typical lentivirus vectors take the form of virus pseudotypes bearing envelope proteins from vesicular stomatitis virus (VSV). Activities, such as producing research-laboratory-scale quantities of retroviruses, including HIV, SIV or SHIV, manipulating concentrated virus preparations, and conducting procedures that may produce droplets or aerosols, can be performed in a BSL-2 facility using BSL-3 practices.

XVI. Select Agents and Toxins

The U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) published final rules, which implement the provisions of the USA PATRIOT Act and Public Health Security and Bioterrorism Preparedness and Response Act of 2002 setting forth the requirements for possession, use, and transfer of select agents and toxins. The Select Agents and Toxins identified in the final rules (7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73,) have the potential to pose a severe threat to public health and safety, to animal and plant health, or to animal and plant products. The regulations state that anyone involved with the possession, use or transfer of select agents must be registered with the CDC or USDA. This registration involves extensive paperwork, background checks, security plans, record keeping and inspections. PIs must contact Office of Sponsored Research and Programs to register with the IBC and the appropriate federal agency prior to sending, receiving or working with any select agents. An attenuated strain of a select agent or an inactive form of a select toxin may be excluded from the requirements of the Select Agent Regulations. The following link contains a list of excluded agents and toxins. The Federal Select Agent Program does not regulate certain Select Agent and Toxins if the amount under the control of a PI does not exceed, at any time, the amounts indicated in the table below. NCCU requires that PIs maintain an accurate inventory of Select Agent and Toxins and secure the toxins in their laboratory. Inventory reports should be submitted semi-annually (every 6 months) to the EHS (ehs@nccu.edu).

Permissible toxin amounts

HHS Toxins [§73.3(d)(7)]	Amount
Abrin	1,000 mg
Botulinum neurotoxins	1 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1,000 mg

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Saxitoxin	500 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

XVII. Biological Safety Cabinets

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 (99.97% for particles of 0.3 µm diameter or more). BSCs are divided into three classes (Class I-III) based both on design and protection. **Personnel protection** is provided by the directional airflow into the cabinet, **product protection** with HEPA filtered laminar airflow, and **environment protection** with HEPA filtered exhaust. Class I and II cabinets provide a protective air barrier that separates the laboratorian from the work area. Class II cabinets also provide a HEPA-filtered laminar flow to work surface to protect sterility of products from contamination in the room. Characteristics of the different classes and types of BSCs are summarized in the table below. For additional information on BSCs refer to the NCCU Laboratory Safety Manual (Chapter XI – Engineering Controls) and to the BMBL (Appendix A, section Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets).

BSC Class,Type	Face Velocity (fpm)	Airflow Pattern	Application: Nonvolatile Toxic Chemical & Radionuclides	Application: Volatile Toxic Chemical & Radionuclides
I	75	In at front through HEPA to the outside or into the room through HEPA.	Yes	When exhausted outdoors[a,b]
II, A1	75	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to outside through a canopy unit.[c]	Yes (small amounts)[a]	Yes (small amounts)[a,b]
II, A2	100	Similar to II, A1, but has 100 fpm intake air velocity exhaust air can be ducted to the outside through a canopy unit.	Yes	When exhausted outdoors (formally B3), (small amounts)[a,b]
II, B1	100	30% recirculated, 70% exhausted. Exhaust cabinet air must pass through a dedicated, internal cabinet duct to the outside through a HEPA filter.	Yes	Yes (small amounts)[a,b]
II, B2	100	No recirculation; total exhaust to the outside through a HEPA filter	Yes	Yes (small amounts)[a,b]
II, C1	105	Can operate as either a Type A cabinet when in recirculating mode or a Type B cabinet when exhausting. Exhaust cabinet air must	Yes	Yes (small amounts, when in Type B mode)[a,b]

		pass through a dedicated, internal cabinet duct to the outside through a blower and HEPA filter		
III	N/A	Supply air is HEPA-filtered. Exhaust air passes through two HEPA filters in series and is exhausted to the outside via a hard connection	Yes	Yes (small amounts)[a,b]

a - Installation requires a special duct to the outside, and may require an in-line charcoal filter, and/or a spark-proof (explosion-proof) motor and other electrical components in the cabinet. Discharge of a Class I or Class II, Type A2 cabinet into a room should not occur if volatile chemicals are used.

b - A risk assessment should be completed by laboratory and safety facility personnel to determine amounts to be used. In all cases, only the smallest amounts of the chemical(s) required for the work to be performed should be used in the BSC. In no instance should the chemical concentration approach the lower explosion limits of the compounds.

c - Class II A1 cabinets built prior to 2010 were allowed to have potentially contaminated, positively pressurized plenums. After 2010, all Class II cabinets must have potentially contaminated plenums under negative pressure or surrounded by negatively pressurized plenums.

The **Class II BSC** is the most used BSC at NCCU. Class II cabinets are built to meet NSF/ANSI 49-2019 (NSF 49) standard. This standard provides specific construction and testing requirements. The operational integrity of a BSC must be inspected and certified before it is placed into service, after it has been repaired or relocated, and annually thereafter. EHS will ensure annual on-site certification performed by qualified professional. **Do not use BSC that (i) does not have calibration sticker on it, (ii) is overdue for annual inspection/certification, or (iii) is in alarm.** If your biosafety cabinet goes into alarm, it is an indication that worker, product, and/or environmental protection are compromised. Post the sign "DO NOT USE BSC Protection is Compromised" on biosafety cabinet and immediately contact your building liaison or the EHS.

A. Working in the Biosafety Cabinet

A properly certified and operational BSC is an effective engineering control that must be used in concert with the appropriate PPE, practices, procedures, and other administrative controls to further reduce the risk of exposure to potentially infectious microorganisms.

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Before beginning work

1. Monitor alarms, pressure gauges, or flow indicators for any changes.
2. Shut off the UV light (use of UV light is not recommended by CDC and NIH; see Appendix B).
3. Turn the cabinet on and let it run for at least 5 minutes before beginning the work to allow cabinet to purge to remove any suspended particulates in the cabinet.
4. Wipe work surface, interior walls, and the interior surface of the window with the appropriate disinfectant listed in your Lab-Specific Safety Plan. If bleach is used, wipe again with water or 70% ethanol to remove the bleach residues.
5. 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.
6. 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

7. Keep the BSC free of clutter, e.g., extra equipment and supplies.
8. Don't place objects over the front air intake grille.
9. Don't block the rear air intake grille.
10. Limit traffic in the area when the BSC is in use.
11. Make sure lab door is closed and avoid opening and closing door if located near the BSC.
12. Move arms slowly when removing or introducing items to or from the BSC.
13. Keep all materials at least 4 inches inside the sash.
14. 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.
15. Don't operate a Bunsen burner in the cabinet. Open flame in BSC creates turbulence that disrupt the pattern of HEPA-filtered airflow; gas is not allowed in BSC. Use alternatives, such as Bact-Cinerator (right), the Electric Bunsen Burner (middle), or Glass Bead Sterilizer (left).



While working

16. Work as far to the back of the BSC workspace as possible.
17. Segregate contaminated and clean items. Work from "clean to dirty."

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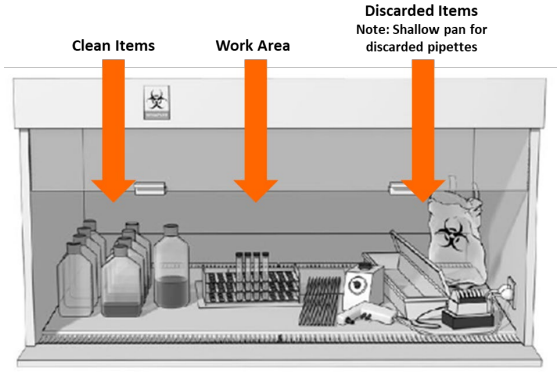


A typical layout for working from the clean to the dirty side within a Class II biosafety cabinet. This arrangement is reversed for left-handed persons.

18. Clean up all spills in the cabinet immediately; follow the procedure described under Spill Inside Biological Safety Cabinet. After the spill cleanup, allow cabinet to run for 5 minutes before resuming work.

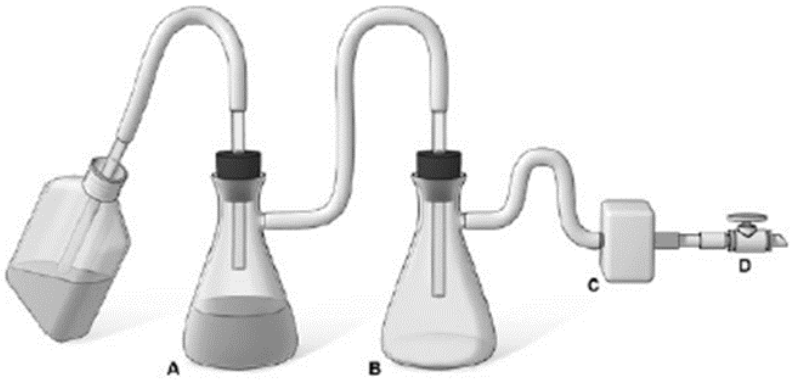
After completing work

19. Wipe down all items with an appropriate disinfectant before removing from BSC. Remove all materials and wipe all interior surfaces with an appropriate disinfectant. Note that 70% alcohol (ethanol or isopropanol) is not an EPA registered disinfectant under the OSHA Bloodborne Pathogens Standard (see section Decontamination, Disinfection, Sterilization).
20. Periodically decontaminate under work grilles.
21. Allow cabinet fan to run for 5 minutes after decontamination before turning off the blower.

XVIII. Laboratory Equipment

A. 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. Such combination provides protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing a volume of a chemical decontamination solution with a concentration sufficient to decontaminate microorganisms when the flask is filled to its maximum capacity. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste. The flask material should be resistant to the decontamination solution used. See schema below for the proper suction unit assembly.



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.

B. Centrifuges

Aerosols may be created during centrifugation from poorly sealed or capped tubes and from tubes splitting or breaking. Safety cups, caps, sealed rotors, or buckets are recommended when centrifuging biological materials (see section Spill in Centrifuge).

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:

1. Use aerosol-proof (sealed) rotors or buckets with safety caps that seal with O-rings.
2. Before use, inspect O-rings and safety caps for cracks, chips, and erosion.
3. Use tubes with threaded caps and safe-lock microcentrifuge tubes. Avoid overfilling the tube and getting caps/closures wet.
4. Wipe tubes down with disinfectant after filling.
5. Load and unload rotors and buckets inside the BSC.
6. Balance buckets, tubes, and rotors before centrifuging.
7. Disinfect the centrifuge after use.
8. 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 on Laboratory Safety Centrifuges.

C. Flow Cytometers

Teaching and research laboratories utilizing flow cytometers should operate under the same containment conditions in which the cells would normally be handled. For example, if human cells are being sorted in a flow cytometer, they need to be handled at a BSL-2 containment. If the cells being sorted are potentially infectious unfixed cells, potentially infectious aerosols will be generated when using a flow cytometer, particularly if the cell sorter fails to operate in a normal manner. The higher speed, 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 your laboratory/facility needs to be available to all users.

D. Multi-Channel Liquid Handler

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, which isolates hazardous/infectious material from lab personnel and environment. Two other features, HEPA filter and UV lamp, enables liquid handler setup to mirror their BSC enclosure. However, whether using liquid handler in BSC or as self-contained bench hood, an appropriate PPE needs to be worn, waste decontaminated and disposed as biohazard, and liquid handler decontaminated with EPA and manufacturer approved disinfectant. It is important that the laminar airflow in the BSC is evaluated so that liquid handler positioned in the BSC does not interfere with the BSC's laminar airflow.

E. Pipettes and Pipetting Aids

Pipetting must be done by mechanical means, never by mouth. Pipetting is an aerosol generating procedures and must be conducted in a BSC for all BSL2 work. Store used pipettes for disposal in approved sharps container that fits the pipette in its entirety. Use plastic over glass whenever possible. Use autoclave plastic bag with biohazard symbol for collection of pipettes and pipet tips. When the waste container of pipettes become full, it needs to be autoclaved and handled as sharps waste.

F. Loop Sterilizer and Bunsen Burners

The sterilization of a loop or needle in an open flame generates aerosols that can contain viable microbiological agents. It is strongly encouraged that laboratories use a shielded electric incinerator or a hot bead sterilize to minimize the risk of aerosol production while sterilizing a loop or needle. Another recommended option is to use disposable (one-time use) loops and needles for culture work and collecting the waste loops and plastic needles in a sharps container that fits them in their entirety. They can be autoclaved and disposed of after autoclaving in general waste in orange autoclave bag. The use of a continuous flame gas burner such as a Bunsen burner, in a BSC is prohibited, as they can produce turbulence that interferes with the airflow of the cabinet and can damage the HEPA filter.

G. Aerosol-Producing Devices

Certain devices such as blenders, homogenizers and sonicators (ultrasonic disrupters) can produce aerosols. To reduce exposure to aerosols, these devices should be used in a biosafety cabinet whenever possible.

XIX. Decontamination, Disinfection, Sterilization

The decontamination or disinfection of laboratory surfaces and items with an appropriate disinfectant are one way to mitigate the inadvertent transmission of pathogens. The PI is responsible for selecting an appropriate EPA-registered antimicrobial product and use it according to the manufacturer's instructions on the product label to ensure the product's performance against the target microorganism.

A. Cleaning

Cleaning removes gross contamination (e.g., blood, tissues, culture media) from a surface so that the surface is exposed to appropriate surface disinfectant.

B. Decontamination

Decontamination process (physical or chemical) removes or neutralizes hazardous biological material accumulated on the personnel and/or equipment. Decontamination protects workers from transfer of hazard material into clean area and making surface of items safe for handling, use, or disposal.

C. Disinfection

Disinfection with proper agent and contact time eliminates nearly all infectious agents. Factors that affect disinfection include:

1. Type and amount of contamination
2. Type and condition of surfaces, instruments, devices, and materials to be disinfected
3. Temperature
4. Contact (exposure) time

D. Sterilization

Sterilization is reducing the probability of a microorganism surviving to less than one in one million (10^{-6}). It can be accomplished by dry or moist heat, gases and vapors (e.g., chlorine dioxide, ethylene oxide, formaldehyde, hydrogen peroxide, etc.,), plasma sterilization technology, and gamma radiation.

E. Incineration

The ultimate means of sterilization of medical and microbiological waste is incineration. Animal carcasses treated with preservatives such as formalin or medical sharps from healthcare facilities are examples of materials that are shipped for incineration. However, sharps collected in research labs should be autoclaved and disposed to regular waste. Contact the EHS waste management specialist to determine the needs for your laboratory.

F. List of Disinfectants

OSHA requires use of appropriate disinfectant under the Bloodborne Pathogens Standard (29 CFR Part 1910.1030(d)(4)(ii)(A)). To locate information on proprietary disinfectants, refer to the EPA-registered disinfectants to review efficacy claims against microbes of interest. The table below provide a starting point for identifying appropriate chemicals for disinfection depending on the circumstances and type of biohazard.

	Quaternary Ammonium Cmpds.	Phenolic Cmpds.	Chlorine Cmpds.	Iodophor Cmpds.	Alcohol (ethyl or isopropyl)	Formal-dehyde	Glutaral-dehyde
Use Parameters							
Conc. of active ingredient	0.1-2%	0.2-3%	0.01-5%	0.47%	70-85%	4-8%	2%
Temp (°C)							
Relative humidity (%)							
Contact time (min)	10-30	10-30	10-30	10-30	10-30	10-30	10-600
Effective Against							
Vegetative Bacteria	+	+	+	+	+	+	+
Bacterial Spores			±			±	+
Lipophilic Viruses	+	+	+	+	+	+	+
Hydrophilic Viruses		±	+	±	±	+	+
Tubercle Bacilli		+	+	+		+	+
HIV	+	+	+	+	+	+	+
HBV		±	+	±	±	+	+
Application							
Contaminated Liquid Discard			+			±	
Contaminated glassware	+	+	+		+	±	+
Contaminated Instruments		+				±	+

*These chemical disinfection methods are recognized by the NIH, CDC, and ABSA. "+" denotes very positive response; "-" denotes a less positive response; blank denotes a negative response or not applicable.

Note that 70% alcohol (ethanol or isopropanol) evaporates too quickly to be an effective disinfectant. 70% alcohol can be used as a rinse, for example, to remove excess bleach or other EPA-registered disinfectant but is not considered as a disinfectant. Appropriate disinfectants include Amphyl, Sporicidin, and certain Clorox and Lysol formulations.

At NCCU liquid biohazard waste may be autoclaved with a test indicator (see Liquid Biohazard Waste) and disposed down the sanitary sewer. Hazardous chemicals (except for diluted bleach) may NOT be directly poured down the drain. If chemicals other than bleach are to be used to disinfect liquid media, etc., the final

waste product must adhere to all chemical waste disposal regulations. For suction flasks, make sure the approved chemical disinfectant is in the flask before suctioning off the media.

NEVER autoclave liquid that has been treated with bleach and/or alcohols.

XX. Biohazard Waste Management

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).

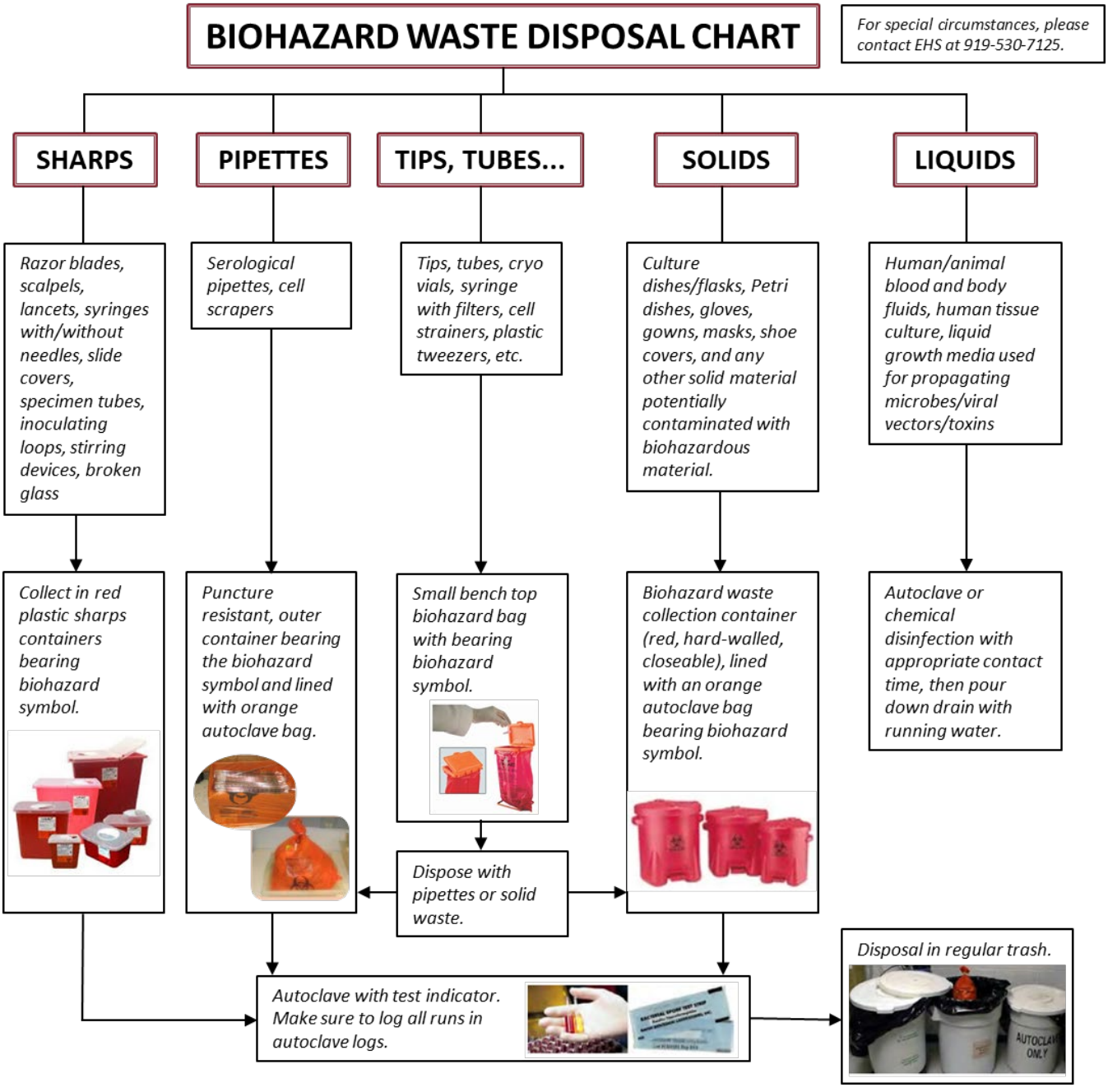
All biohazard waste generated in NCCU research and/or teaching laboratories must be properly treated prior to disposal. If treatment of waste is not an option complete an EHS Hazardous Waste Pick-up Request.

Biohazard waste handling and treatment should only be performed by workers trained under the Laboratory Safety Plan and BBP Exposure Control Plan as appropriate for their work environment.

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 and 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

Refer to this quick reference chart regarding general disposal practices of biohazard waste:



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A. Solid Biohazard Waste

Solid biological waste is typically deactivated by autoclaving.

1. Collect solid biohazard waste in red, hard-walled biohazard waste collection containers not to exceed 15 gallons and lined with an orange autoclavable bag with "Biohazard" symbol on it. The lid must remain on the container when not in use. The lid and container each must bear the biohazard symbol and the word "Biohazard".
2. Bags must be removed from containers prior to being 2/3 to ¾ full to allow headspace to seal the bag for transport to the autoclave. Place bags directly into 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 autoclave tape.
3. A log detailing autoclave performance verification must be completed on every load and maintained at the autoclave. See below for Monitoring Sterilization Process details.
4. Place all items in heat-resistant secondary containers to secure and contain spills. Bags should be opened (at least 2 inches opening) before autoclaving to ensure sterilization.

B. 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 must be listed on your Laboratory-Specific Safety Plan.

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 must follow all applicable chemical safety rules and regulations.

C. Sharps Waste

Biohazard sharps waste is material used with recombinant and synthetic nucleic acid molecules, BSL-1, or BSL-2 material that have sharp edges capable of causing punctures or cuts, including, but not limited to the following: needles, syringes, scalpels, razor blades, slides, coverslips, Pasteur pipettes, capillary tubes, and broken glass and plastic. Plastic serological pipettes are considered "sharps waste" if they are broken and have a sharp edge. See Appendix C and the NCCU Exposure Control Plan for a safe work with sharp material.

NCCU labs collect biohazard sharps waste in red plastic sharps containers labeled with "Biohazard" symbol on it. When the container is ¾ full, close it tightly, put into orange autoclavable 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.

D. 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 collected in small bags, placed in the freezer and shipped for incineration. See **Animal Resources Complex User's Guide** or contact the EHS to determine the needs for your laboratory.

E. Mixed Waste

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

F. 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.

1. Hazard and Safety Practices

Autoclaves operate with a combination of steam, temperature, pressure, and time in order to destroy microorganisms and spores. As such, autoclaves pose many hazards including burns, explosions, muscle strains, and biohazards. Using an autoclave improperly can cause serious injuries or contamination and can ruin the mechanical components. Before using the autoclave ensure that you completed the NCCU Autoclave Safety Training and Quiz, receive hands-on training from your supervisor or designee for specific autoclave unit and that autoclave is operating properly. Appendix D, Autoclave Standard Operating Procedures has a SOP that can be placed near the autoclave.

Appropriate ventilation systems should be operating in areas where autoclaves are located. Do not place hazardous chemicals in the autoclave.

Never autoclave:

- Flammable, volatile, 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

2. Autoclave Log

Everyone using the autoclave needs to complete the Autoclave Use Log (Appendix E) and when Biological Indicators are used. It is very important to complete the log so there is a record of people using the autoclave in case it malfunctions or items are left in the unit and to monitor autoclave performance.

3. Container Selection

Polypropylene bags. Commonly called biohazard or autoclave bags, these bags are tear resistant, but can be punctured or burst in the autoclave. Therefore, place bags in a rigid container during autoclaving. Polypropylene bags are impermeable to steam, and for this reason should not be twisted and taped shut but gathered loosely at the top and secured with a large rubber band or autoclave tape. This will create an opening (1-2 inch in diameter) through which steam can penetrate.

Sharps containers. Sharps disposal containers are made from rigid plastic and come marked with a line that indicates when the container should be considered full, which means it's time to decontaminate and dispose of the container. Sharp containers with biohazardous material must be labeled with universal biohazard symbol and the word "Biohazard" or be color-coded re

Polypropylene containers and trays. Polypropylene is a plastic capable of withstanding autoclaving, but resistant to heat transfer. Therefore, materials contained in a polypropylene tray will take longer to autoclave than the same materials in a stainless-steel pan.


Stainless steel containers and pans. Stainless steel is a good conductor of heat and is less likely to increase sterilizing time, though it is more expensive than polypropylene.


Autoclave biohazard bags and sharps containers are purchased either by the PI or the department.

Biohazard waste is collected into orange color autoclave bags with universal symbol for biohazard and the word "Biohazard" on it. Heat sensitive tapes may be used to indicate that the material has been decontaminated. Containers of sharps contaminated with biohazardous materials should be closed tightly and placed in an orange autoclave bag with heat sensitive tape to indicate that the material has been decontaminated after autoclaving it. After autoclaving, the bags with the waste and containers of sharps can be disposed of with the regular trash. Non-hazardous sharps should be placed in the clear/white plastic sharps containers. Once the containers are 2/3 full, close tightly and dispose of in regular trash.

4. Preparation and Loading of Material

- Only designated individuals should be allowed to set and/or change parameters for the autoclaves.
- Before using the autoclave, check inside for any items left by the previous user that could pose a hazard.
- Clean the drain strainer before loading the autoclave.
- Always place items in a secondary container.
- Do not overload or package bags too tightly. Leave sufficient room for steam circulation. If necessary, place container on its side to maximize steam penetration and avoid entrapment of air.
- Use only autoclave bags to package waste.

- Do not allow bags to touch the interior walls of the autoclave to avoid melting of plastic.
- Ensure sufficient liquid is packed with contents of autoclave bags if dry (~200 mL).
- Place soiled glassware and lab ware in secondary containers and autoclave them in the solids cycle.
- Do not fill containers more than 2/3 full of liquids. Loosen caps or use vented closures.
- In case of clean glassware and wrapped instruments, lay them in a secondary container before autoclaving.
- For secondary containment, use autoclave trays made from polypropylene or stainless steel. The trays should have a solid bottom and sides to contain the contents and catch spills.
- Choose appropriate cycle for the material. Incorrect selection of cycle may damage the autoclave, cause liquid to boil over or bottles to break.
- Close and lock door.
- Start your cycle and fill out the Autoclave Use Log.
- Check chamber/jacket pressure gauge for desirable pressure and temperature for every load.
- Do not attempt to open the door while autoclave is operating.

5. 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, special consideration should be given to the size of the load, insulating capacity of autoclaved material and nature of the article to be autoclaved. Table below summarizes the most commonly used autoclave waste decontamination settings.

Material	Temperature	Time
Laundry	121 °C (250 °F)	30 minutes
Biohazard bags containing infectious waste.	121 °C (250 °F)	1 hour
Glassware	121 °C (250 °F)	1 hour
Liquids	121 °C (250 °F)	30 minutes

In autoclave, temperature increases as pressure increases. The relationship between temperature and pressure based on complete replacement of air by steam is as below.

Pressure (psi)	Temperature (°C)	Temperature (°F)	Pressure (psi)	Temperature (°C)	Temperature (°F)
5	109	228	20	126	259
10	115	240	25	130	267
15	121	250	30	135	275

Some autoclaves use a pre-cycle vacuum to remove air prior to steam introduction. Others utilize a steam activated exhaust valve that remains open during the replacement of air by live steam until the steam triggers the valve to close. There are, in general, four standard sterilization cycles: gravity, pre-vacuum, liquids, and flash (also known as immediate use). The chart below explains these cycles and their application in greater detail.

Basic Cycles	Description	Typical Application or Load Type
Gravity	The most common sterilization cycle. Steam displaces air in the chamber by gravity (i.e., without mechanical assistance) through a drain port.	Glassware, unwrapped goods, waste, utensils, bags.
Pre-vacuum and/or Post-vacuum	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.	Wrapped goods, packs, animal cages, porous materials, bags.
Liquids	A gravity cycle with a slower exhaust rate to minimize boil-over.	Media, LB broth, water, saline, etc.
Immediate use/Flash (Healthcare sterilizers only)	High temperature cycle (over 270°F) for a shorter period of time.	Unwrapped goods.

6. Removing the Material from Autoclave

The greatest risk of personal injury occurs during the process of unloading the autoclave. When the pressure gauge reaches zero, wait one to two minutes before opening the autoclave. It is dangerous to begin opening the autoclave before the pressure gauge reaches zero.

- For unloading an autoclave wear lab coat, long-sleeved heat-insulating gloves (these gloves are compromised if wet or have holes), eye protection, and closed-toe shoes. A rubber apron and face shield may also be worn.
- Ensure cycle has completed and both temperature and pressure have returned to a safe range.
- Wearing the above PPE, stand back from the door as a precaution and carefully open the door no more than 1 inch. This will release residual steam and allow pressure within liquids and containers to normalize.
- Allow the autoclaved load to stand for 10 minutes in the chamber. This will allow steam to clear and trapped air to escape from hot liquids, reducing risk to operator.
- Do not agitate containers of super-heated liquids or remove caps before unloading.

- Remove items from the autoclave and place them in an area which clearly indicates the items are "hot" until the items cool to room temp.
- Shut the autoclave door.
- Allow autoclaved materials to cool to room temperature before transporting. Never transport superheated materials.
- Any breakage of bags or leakage of contaminated materials should be reported to the laboratory manager or supervisor at once for instructions on procedures for safe cleanup.
- Reseal the bags with tape and remove from the building. Autoclaved bags are transported within a leak-proof secondary container to the building's outside trash dumpster.

7. 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.

Heat sensitive tape is NOT an indicator of sterilization. It is a process indicator designed to change color or develop a marking once critical temperature is reached. Operators shall use heat sensitive sterilization indicator tape for each load to differentiate between autoclaved and non-autoclaved materials. Ensure that the heat sensitive tape used does not contain a lead based indicator, as this type of tape must be collected and managed as hazardous waste. Lead-free autoclave tape can be purchased from Fisher Scientific, 3M, or VWR.

Biological Indicator. The NC Medical Waste Rules require that autoclaves be monitored under conditions of full loading for effectiveness weekly through the use of Biological Indicators. *Geobacillus stearothermophilus* indicators must be used with average spore populations of 10^4 to 10^6 organisms. There are many commercially available biological indicators with a choice of spore ampoules or spore strips with growth media.

1. Follow the instructions provided by the manufacturer of the biological indicators. Most require refrigeration when kept in storage.
2. Place the indicator in the middle of the waste bag or material to be autoclaved. It is best to put the indicator in the waste bag before it is filled completely. To aid recovery of the indicator after sterilization, tape it to a brightly colored sheet of paper or to a long string allowed to protrude from the bag. Indicators can also be placed in test waste bags filled with materials that simulate full loading for the test.
3. Autoclave the waste following normal procedures. Once the cycle is complete and contents have cooled, remove the indicator from the waste bags wearing appropriate protective equipment. Prepare and incubate the indicator and a control indicator that was not autoclaved as recommended by the manufacturer.
4. A record of each test is required (Appendix E).

5. Follow manufacturers recommendations for incubation periods. There should be signs of growth on the control indicator that was not autoclaved or the test is invalid. If there are signs of growth on the indicator placed in the waste, the waste was not sterilized properly. The time, temperature and autoclave procedures should be re-evaluated. If an autoclave problem is suspected, EHS must be notified immediately and the vendor contacted for repair. The autoclave should be marked "DO NOT USE" until the issue is resolved.
6. Waste does not have to be held until the results of the testing confirm effectiveness.
7. Following autoclave repairs, the first load run in the autoclave should be tested with a biological indicator to insure proper functioning.

Chemical indicators are 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. Chemical indicators use one or more chemicals that undergo either a physical or chemical change, that is visible to the human eye, after exposure to predetermined critical parameters such as time and temperature. All bags autoclaved with a failed chemical integrator will be autoclaved again. 3M SteriGage Test Packs #41360 is currently the system accepted for this test.

8. Autoclave Preventive Maintenance

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

1. 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.
2. Clean the interior surfaces of residues collected from the steam or materials being sterilized as needed.
3. Visually inspect the gaskets, doors, shelves and walls for residue buildup or wear regularly.

All autoclaves mechanical and monitoring systems should be serviced and checked annually by a service provider to ensure that the unit is running correctly. Check with the autoclave manufacture for service providers in the area.

9. Autoclave Failure

Discontinue use immediately if an autoclave is not working properly. Post a sign alerting others not to use the autoclave.

Mechanical failures need to be attended to by a trained technician. Contact the service company responsible for the maintenance of your autoclave or EHS for further guidance.

All failures and repairs must be documented and provided to inspectors upon request.

XXI. Shipping and Transporting Biological Material

A. Training Requirement

Most biological materials require specific packaging, labeling, and documentation. Transport of infectious materials 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 package and/or ship infectious biological materials.

B. Import and Transfer Permits

Some biological materials require a permit to be imported or transferred to another institution outside of NCCU. The importation or interstate transfer of an etiological agent and hosts or vectors of human disease require an import permit from the Center for Disease Control (CDC). This permit applies to the etiological agents themselves, unsterilized biological material (e.g., patient samples) containing an etiological agent, and animals that could be a host or vector of disease in humans.

- CDC Import Permit Program

The United States Department of Agriculture (USDA) requires a permit for import or interstate transfer of infectious materials affecting livestock and biological materials containing animal material. Tissue culture materials and suspensions of cell culture grown viruses or other etiological agents containing growth stimulants of bovine or other livestock origins are controlled by the USDA due to the potential risk of introducing exotic animal diseases into the US.

- USDA Import and Export: Animal and Animal Products

- USDA Plant Health Import and Export

The U.S. Fish and Wildlife Service requires an import permit for certain live animals.

- U.S. Fish and Wildlife Services ePermits

Food (excluding most meat and poultry), drugs, biologics, cosmetics, medical devices, and electronic products that emit radiation, may be subject to examination by the Food and Drug Administration (FDA) when they are being imported or offered for import into the United States. These items must meet the same standards as items available in the US.

- Import Program - FDA

Contact EHS to assist you with import and transfer permit applications. Once the permit is granted you will receive the permit and a set of labels which must accompany the shipment upon its arrival in the US. You will have to send these labels to the senders of your materials.

If you are sending a material that requires an import or transfer permit it is your responsibility to ensure the recipient has the proper permits to receive the material before shipping the materials.

C. Export Licenses

Some pathogens, toxins, and genetically modified organisms require government licenses in order to be legally exported. The Department of Commerce and Department of State regulate the export of some biological materials, chemicals, and equipment. Do not assume that you will not need an export license based on the item's availability in the US. Failure to obtain an export license when one is needed can result in significant fines, loss of export privileges, or jail time.

If you are not certain that the item, you are shipping does not need an export license contact EHS to determine if one is required. EHS will file export license applications when they are needed. This process can take several weeks so identify any possible licenses you will need well in advance of your planned shipping date.

D. Transporting on Campus

Potentially infectious materials must be placed in a durable leak-proof secondary container for transport out of the laboratory. Containers should be lidded and sealable with the biohazard label on the outermost surface.

XXII. Appendix A Equipment Safety Clearance Form

Faculty/Staff name: Click or tap here to enter text.

Department: Click or tap here to enter text. Building/Room Number: Click or tap here to enter text.

Equipment (manufacturer model): Click or tap here to enter text.

Serial Number: Click or tap here to enter text. NCCU Property ID: Click or tap here to enter text.

This is to certify that the laboratory equipment and/or room listed above is considered safe for maintenance work and/or occupancy. All hazardous materials have been removed. All potentially contaminated surfaces have been decontaminated in accordance with Environmental Health & Safety requirements.

Hazardous materials removed	Yes ☐	No ☐	N/A ☐
Cleaned	Yes ☐	No ☐	N/A ☐
Decontaminated	Yes ☐	No ☐	N/A ☐
Rad safety survey conducted	Yes ☐	No ☐	N/A ☐
<600 dpm/100 cm^2 ☐	OR	<0.05 mR/hr or 500 cpm ☐	
Additional info: Click or tap here to enter text.			
Hazard/warning signage removed/defaced	Yes ☐	No ☐	N/A ☐

Date completed: Click or tap here to enter text.

Name of Principle Investigator or Authorized Designee: _____

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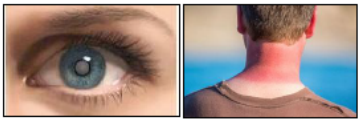
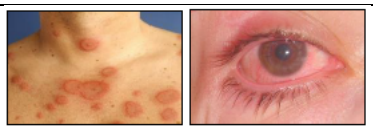
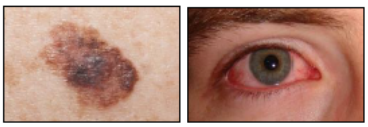
XXIII. Appendix B Ultra Violet (UV) Safety

Ultraviolet (UV) light is an electromagnetic radiation with wavelength between 100 nm and 400 nm. UV radiation is invisible to naked eye and are broken down into three main bands: UV-A, UV-B, and UV-C. These three regions of UV rays can be found in research laboratory (e.g., biosafety cabinets, laminar flow cabinets; UV-transilluminators). The UV light levels found in UV equipment greatly exceed the levels found in nature. The critical organs which are affected by the UV radiation are the skin and the eye.

Band	Wavelength	Hazard Potential	Description	Biological Effects
UV-A (near UV)	315-400 nm	Lowest	Accounts for up to 95% of UV radiation. Penetrates deeper into skin layers.	 Cataract Sunburn
UV-B (middle UV)	280-315 nm	Mid to High	Biologically active but cannot penetrate beyond the superficial skin layers. Most solar UV-B is filtered by the atmosphere.	 Erythema Photokeratitis
UV-C (far UV)	100-280 nm	Highest	Most damaging. Completely filtered by the atmosphere and does not reach the earth's surface.	 Skin cancer Conjunctivitis

Biosafety cabinets (BSC) equipped with germicidal lamps emit radiation almost exclusively in the far-UV range of 254 nm. It is usually used in laminar air flow hoods, in biosafety cabinets, and PCR workstations for sterilization/decontamination purposes.

The CDC and the NIH agree that UV lamps are not recommended nor required in BSC. The National Sanitation Foundation (NSF) Standard 49, the industry testing standard for all biohazard cabinetry, does not provide any performance criteria for UV lighting and specifically states in section I-1.3.3.3 that "UV lighting is not recommended in BSCs." as it is possible to produce ozone levels from UV wavelengths below 250 nm sufficient to affect rubber or other polymer made materials; low or no ozone UV light bulbs are commercially available. NCCU EHS does not recommend the use of UV lights as a method of disinfection and they should never be used as the sole method of disinfection.

Contact EHS's Radiation Safety Officer at ehs@nccu.edu or at 919-530-7125 for assistance with shielding, PPE, hazard evaluation and training.

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To prevent eye and skin damage from UV rays:

- Avoid working in or around the safety cabinet when the UV light is on or avoid using the room when UV light is on. Ensure the UV light is off prior working at the cabinet.
- Always close the sash completely when the UV light is on. Even small opening of the sash can cause skin damage and other biological effects.
- Always wear appropriate protective clothing and PPE including fully buttoned, long sleeve lab coat gloves, and UV rated eye/face protection.
- Warning signs must be posted on any equipment that emits UV radiation.

Eye/Face protection must be marked with ANSI Z87.1 UV certification (Z87U)

- A polycarbonate face-shield and/or wrap around safety glasses
- Ordinary prescription eyeglasses or contacts do not provide protection against UV rays.

Gloves

- Wear nitrile gloves to protect exposed skin on the hands. Ensure exposed skin (wrist and forearms) are covered.

XXIV. Appendix C Sharps Precautions

Items are generally considered "sharps" if the item was designed to cut or puncture skin or if the item has the potential to penetrate skin when not properly handled. Non-contaminated sharps can pose puncture and laceration hazards. Sharps contaminated with chemical, biological, and radioactive hazards pose additional exposure and disposal concerns.

General guidance for the safe handling of sharps.

- Identify and reduce sharps in the workplace. Determine whether an alternative is available. One example is replacing the use of a glass item with plastic.
- Consider safer sharps devices for items that are designed to cut or puncture skin such as needles and scalpels. Use the resources from the CDC Stop Sticks Campaign to help you review your sharps devices: listing of safety devices and manufacturers.
- Be properly trained by senior personnel on new techniques and equipment in a controlled setting before employing these in a procedure involving chemical, biological, or radioactive hazards.
- Do not leave sharp devices out any longer than necessary. For reusable sharps devices (i.e., knives, scissors), have a storage container that will enclose the sharp end (i.e., a bucket or enclosed tray) readily available at the point of use.
- Never recap, bend, shear, break, or remove a needle from a disposable syringe, or otherwise manipulate a needle by hand before disposal. This avoids the generation of aerosols and eliminates unnecessary handling that could cause sharps injury.
- Do not handle broken glassware directly. Instead, use a brush and dustpan, tongs, or forceps to remove broken glassware. Substitute plasticware for glassware whenever possible.
- Transport non-disposable sharps in a hard walled container to a processing area for decontamination.

Safely dispose of sharps

- For disposable sharps, have a puncture-resistant container designed for sharps disposal readily available, preferably within arm's reach for disposal of sharps immediately after use if your procedures permit you to do so.
- Non-contaminated sharps may be disposed of in broken glass waste boxes lined with a plastic bag (including empty glass chemical bottles since they may break during transportation).

For further information about sharps contaminated with:

- human blood or other potentially infectious material, refer to the NCCU Exposure Control Plan.
- radioactive materials, refer to the EHS website for the Radiation Safety Plan.
- chemicals, submit material for characterization and proper disposal to EHS.

Injury response

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- For major injuries (item lodged in skin or eye, profuse bleeding, etc.) dial 911 and seek immediate care from Authorized Medical Providers.
- For minor injuries with potentially contaminated sharps, see Needle Stick or Cut with Contaminated Sharp Item.
- For other minor injuries notify your supervisor immediately and follow the Incident Reporting Instructions.

XXV. Appendix D Autoclave Standard Operating Procedures

General Information

1. Most important, wear personal protective equipment (PPE) before using the autoclave: eye or face protection, heat resistant gloves, closed toe shoes, lab coat or rubber apron.
2. Materials that are to be decontaminated/sterilized should be carried to the autoclave in closed and leak proof containers.
3. Containers: Stainless steel containers are durable and a good conductor of heat. Polypropylene containers are durable heat resistant plastic containers. (Other type of plastics will melt.)
4. Always clean up spills prior to running the autoclave and after the autoclave cycle is completed.

Packaging and Loading

1. Use approved autoclave bags for decontaminating biohazardous waste.
2. Use secondary containers to protect autoclave and contain spills.
3. Prepare and load material to ensure steam penetration. Add ~200 mL of water to bags containing solids. Close bag loosely with 1-inch diameter opening to allow steam to enter.
4. Do not overfill containers (prevent spill and boil over) or use sealed containers (pressure buildup and lack of steam penetration).
5. Select appropriate cycle for the load. Determine the appropriate exposure time for the load. Consider agents or amounts of material that affect exposure time.
6. Affix temperature sensitive tape to bags or other materials.

Operating the Autoclave

1. Before use, check log and previous readings to ensure the autoclave is operating properly.
2. Autoclave door clamps and seals should be inspected for wear and damage. Also, remove debris from the autoclave chamber floor drain.
3. Ensure the autoclave cycle attains the desired temperature (normally 121 °C) and pressure (at least 15 psi) for the desired time (≥30 min) and is appropriate for the material to be autoclaved.
4. Most importantly, check steam pressure valve and make sure it is on the appropriate setting. Otherwise, autoclave will not work properly, and alarm will sound.
5. Record information in the Autoclave Use Log.

Unloading the Autoclave

1. Verify temperature (tape) and duration of exposure (print out/gauge) has been met.
2. Wait until the chamber pressure gauge reads zero before opening.
3. Open slightly to allow steam to escape (protect yourself from the steam).
4. Wait at least 10 minutes for the contents of the autoclave to cool.
5. Remove the material carefully to reduce the risk of spillage or injury (use a cart and gloves).

Verifying Autoclave Efficacy

1. Perform weekly testing using a Biological indicator (*Geobacillus stearothermophilus*)

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2. Record results on Autoclave Use Log form.

Completed Autoclave Log should be maintained by the Department for 3 years.

XXVI. Appendix E Autoclave Use Log

Year: Click or tap here to enter text.

Location: Click or tap here to enter text. **PI/Lab:** Click or tap here to enter text.

Indicator Manufacturer/Model: Click or tap here to enter text.

Date Autoclaved	Type of Autoclaved Material	Biological Indicator Lot Number Exp. Date	Cycle	Time (min)	Initials	Comments/ Results (+/-)

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