Laboratory Biosafety Manual

North Carolina State University

Environmental Health & Safety Center
2620 Wolf Village Way
Raleigh, NC  27695

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A summary of changes from the previous version is available here.
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Procedures Governing the Use of Biohazardous Agents

This Biosafety Manual provides a guide to common practices related to working with biological materials at North Carolina State University in teaching, research, and diagnostic laboratories. This Chapter provides procedures governing the registration and procurement of biological materials at NC State. Subsequent chapters provide a review of pertinent federal and state government regulations, information about training, safe work practices, safety equipment, and personal protective equipment.

Biohazardous agents, or "biohazards" at NC State, are infectious agents or hazardous biological materials that present a risk or potential risk to the health of humans, animals or the environment. The risk can be direct through infection or indirect through damage to the environment.

Biological materials that investigators may not consider to be biohazardous may still be regulated under federal, state, or local statutes and guidelines as biohazardous materials. Therefore NC State requires the following of investigators using any of the biological materials listed below:

1. Investigators, diagnostic lab directors, and course instructors must obtain approval from the Institutional Biosafety Committee (IBC) of the following biological materials prior to the procurement of the materials necessary to initiate the project (see PRR 04.20.09):
   - recombinant or synthetic nucleic acid molecules (Refer to the NCSU Recombinant and Synthetic Nucleic Acid Molecules classification guide) including their use in animals (including arthropods) and plants,
   - human and other primate-derived substances (blood, body fluids, cell lines or tissues),
   - organisms or viruses infectious to humans, animals or plants (e.g. parasites, viruses, bacteria, fungi, prions, rickettsia) or biological materials that may contain these microorganisms;
   - Select Agents or Toxins (human, animal, or plant) – refer to the list here;
   - biologically active agents (e.g., venoms, toxins produced by living organisms) that may cause disease in humans or cause significant impact if released into the environment.

2. Investigators and course instructors must procure all of the biological materials listed above through the MarketPlace online procurement process (This site contains the procurement process for suppliers not presently listed in the MarketPlace).

Even if IBC approval is required as stated above, there may still be permits required through NCDACS, USDA, CDC, etc. to import and/or work with materials of biological origin. Principal Investigators are responsible for obtaining all necessary permits regardless of the need to register with the IBC.
Investigators, diagnostic lab directors, and course instructors register projects with the IBC by completing the Biological Use Authorization (BUA) form indicated below. It is recommended that the approved BUA be stored together in a Lab Safety Binder with this manual, the Safety Plan, and other pertinent safety documents such as safety orientation, informed consent, and training for each worker. Emeritus faculty may not be the primary contact of a BUA.

Registration Form for Use of Biological Materials at NC State

To register your biological materials, complete a Biological Use Authorization (BUA) form and submit it to the University Biosafety Officer (BSO) at Environmental Health and Safety as indicated in the instructions on the form. An approved BUA assures that an appraisal has been made of the potential impacts associated with the intended use of an organism. All submissions (e.g., new projects and renewals) must be completed using the latest version of the form. For laboratories, courses, or projects with work practices alternative to or not addressed in this Laboratory Biosafety Manual, the PI must attach SOP’s with their BUA for such practices. The BSO will forward your form to the Institutional Biosafety Committee (IBC) for review and may contact you with questions or concerns about your proposal (e.g. documentation, lab practices, containment, training, equipment, personal protective equipment, facilities, etc.).

The IBC reviews registrations at regularly scheduled meetings. The IBC will accept BUAs at any time. However, if a BUA is submitted after the two-week deadline and is subsequently recommended for discussion at the next IBC meeting, then the discussion of that BUA could be deferred until the following scheduled IBC meeting. This two-week period allows adequate time for review by the committee members. Common reasons that BUAs are recommended for discussion at the next IBC meeting include: incomplete risk assessment; containment concerns; and regulatory requirements. Therefore, investigators should expect final IBC approval to take as little as three weeks or as long as several months depending on the time and completeness of the submission and/or the type of project.

The BUA must be renewed every 3 years. A BUA that is not renewed within 3 years indicates that IBC approval has expired.

Amendments
Amendments are required to be submitted in writing when the change may have safety consequences but the basic thrust of the study stays the same. In general amendments are appropriate when the change will not involve a change in containment. Contact the Biosafety Officer to discuss your amendment.

Close-out
Upon expiration or close-out of a BUA, all materials indicated in the submission must be inactivated or, if transferred, adopted under the BUA of an appropriate Principal Investigator. Refer to the EHS Laboratory Close-out Procedures for general guidance.
The Exposure Control Plan for Bloodborne Pathogens

Any research, diagnostic, or teaching activity conducted with material that was derived from humans including blood, body fluids, tissues, primary or established cell lines requires the PI to indicate “Bloodborne Pathogens” on their Safety Plan and complete the appropriate section of the BUA. In addition, an Exposure Control Plan must also be adopted to meet OSHA regulation 1910.1030 for Bloodborne Pathogens in the workplace. The Exposure Control Plan must be updated annually and according to the instructions on the form. For more information, refer to the EH&S website for Bloodborne Pathogens.

The Safety Plan

Each BUA lists its associated Safety Plan number. Availability of biological safety cabinets and autoclaves are also to be indicated on the PI's Safety Plan. Registration documents may be uploaded to the Safety Plan but this does not constitute approval of the BUA by the IBC. The BUA is completed aside from the Safety Plan and submitted to the Biological Safety Officer as stated above.

The BSL-2 Checklist

In 2007 the CDC enhanced requirements for all research, diagnostic laboratory, and teaching activities conducted at Biosafety Level 2 (BSL-2). The BSL-2 checklist is completed per the instructions on the form to ensure laboratories meet basic requirements at the federal, state, and local levels for BSL-2 practices and containment.

Principal Investigator (PI) Responsibilities

For the purposes of this manual, the PI is defined as the faculty member or other person acting in their official capacity as a University representative in whose assigned space a research, diagnostic laboratory, or teaching activity is conducted. The PI is responsible for full compliance with the policies, practices and procedures set forth by NC State. This responsibility extends to all aspects of biosafety involving all individuals who enter or work in the PI's laboratory or collaborate in carrying out the PI's activities. Although the PI may choose to delegate aspects of the Biosafety Program in his/her laboratory to other laboratory personnel (laboratory directors or supervisors) or faculty, this does not absolve the PI of his/her ultimate responsibility. The PI remains accountable for all activities occurring in his/her laboratory. Documentation of training and compliance with appropriate biosafety practices and procedures are essential. The PI is responsible for assuring the appropriate safety training of employees and for correcting unsafe working conditions.

As part of general responsibilities the PI shall:

1. Develop and implement written laboratory-specific biosafety procedures that are consistent with the nature of current and planned activities and make available copies of the specific biosafety procedures in each laboratory facility. The PI shall ensure that all laboratory personnel, including other faculty members, understand and comply with these laboratory-specific biosafety procedures.
2. Delay initiation of the project until the protocol has been approved by the IBC.
3. Ensure 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. It is essential that everyone who may have potential exposure to biohazardous agents be informed of such hazards and appropriate safety practices before entering or working with such hazards.

4. Ensure 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.

5. Ensure that materials are properly decontaminated before disposal and that all employees are familiar with the appropriate methods of waste disposal. For standard decontamination procedures at NC State, refer to Chapter 8 Biohazard Waste Management of this manual. For specific questions contact the Biosafety Officer (BSO) at 919-515-6858.

6. Report any significant problems, violations of the policies, practices and procedures to the BSO as soon as reasonably possible.

7. Notify the BSO immediately if:
   a. A laboratory-acquired infection is known or suspected.
   b. A spill of any quantity involving an agent infectious to humans, plants, or animals occurs in a public area.

8. Receive training in standard microbiological techniques.

9. Ensure that all personnel are appropriately trained in biosafety and receive appropriate medical surveillance when needed. The PI should refer to Chapter 4 Training of this manual regarding training requirements and contact the BSO for assistance with specific biosafety training needs.

10. Coordinate with the BSO and develop emergency plans for handling accidental spills and personnel contamination.

11. Create and foster an environment in the laboratory that encourages open discussion of biosafety issues, problems and violations of procedure. The PI will not discipline or take any adverse action against any person for reporting problems or violations to the IBC, BSO, Risk Management, or State or Federal agencies.

12. Comply with shipping requirements for biohazardous agents and select agents. EHS conducts shipping training as required for all lab personnel. The PI should refer to Chapter 10 Shipping of this manual and contact EHS to ensure that all applicable transportation safety regulations have been met prior to shipping microbiological cultures, tissues (human or animal) or body fluids. These materials are often regulated for shipment and must only be shipped by personnel who have been properly trained and authorized by NC State to ship such materials on its behalf.

*In submitting proposed work to the IBC, the PI shall:*

1. Make an initial determination of the required levels of physical and biological containment in accordance with the requirements set forth by the *NIH Guidelines* (see Chapter 2 of this manual) and the CDC “Biosafety in Microbiological and Biomedical Laboratories” document (see Chapter 3 Risk Groups and Biosafety Levels) as applicable.

2. Select appropriate microbiological practices and laboratory techniques to be used for the research project, diagnostic lab, or lab course.

3. Complete and submit registrations to the IBC using the most current form(s). Registration forms for the use of infectious agents, recombinant or synthetic
nucleic acids, and acute biological toxins can be found at:
http://www.ncsu.edu/ncsu/ehs/biosafety.htm

4. Submit any significant changes in a given project to the BSO for review and recommendations.
5. Certify that the protocol has been reviewed for research of a dual use concern.

Prior to initiating the project, the PI shall coordinate with the BSO as necessary to:
1. Make available to all laboratory staff and involved facilities staff (such as animal care staff) the protocols that describe the potential biohazards and the precautions to be taken.
2. Instruct and train all personnel in:
   a. Identification of the biohazard(s) present,
   b. Practices and techniques required to ensure safety and reduce potential exposure,
   c. Procedures for dealing with accidents, spills and exposures.
3. Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).
4. Ensure that collaborators are made aware in advance of any biohazardous agents sent to them, and comply with all applicable packaging and shipping requirements. These materials are often regulated for shipment and must only be shipped by personnel who have received proper training and are authorized by NC State to ship such materials on its behalf.
5. Maintain a formal inventory of all biological material received and sent. Logs should include the approximate quantity of the materials and where it is stored in the laboratory.

During the conduct of the project the PI shall:
1. Supervise the safety performance of the laboratory staff to ensure that required safety practices are employed.
2. Investigate and report in writing to the IBC any significant problems pertaining to the operation and implementation of containment practices and procedures.
3. Immediately notify the BSO of any laboratory spills, accidents, containment failure or violations of biosafety practice which result in the release of biohazardous agents and/or the exposure of laboratory personnel (or the public) to infectious agents.
4. Correct work errors and conditions that may result in the release of biohazardous agents.
5. Ensure the integrity of all containment systems used in the project, lab, or course.
6. Restrict access as required by the laboratory-specific biosafety practices and procedures, and by the biosafety containment level approved by the IBC.
7. Immediately notify the BSO if a Select Agent (see Section V, M; Appendix A of this Charter) or other high-consequence pathogen (i.e. Risk Group 3 or 4) has been isolated and confirmed from environmental and/or diagnostic specimens.

Failure to Comply

Non-compliance with the standards outlined in this Manual can result in severe repercussions for NC State University workers (e.g. disease, injury, death) and the university as a whole (e.g. loss of funding, litigation, etc.). Noncompliance includes, but
is not necessarily limited to:

1. Failure to register biohazardous agents, including non-exempt recombinant or synthetic nucleic acid molecules (refer to NCSU Recombinant and Synthetic Nucleic Acid Molecules Classification Guide);
2. Failure to provide updates and/or other required documentation within 60 days of the specified due date;
3. Poor biological safety/biological containment practices as documented through lab inspections, routine or otherwise; or
4. 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.
Chapter 2: Recombinant and Synthetic Nucleic Acids

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid (r/sNA) Molecules (NIH Guidelines) apply to all institutions that receive NIH funding for r/sNA research. All Investigators at the institution must comply with the Guidelines even if their individual research is not funded by NIH. Consequences of noncompliance include suspension, limitation, or termination of NIH funds for r/sNA research at the institution, or a requirement for prior NIH approval of r/sNA projects at the institution.

The original guidelines were issued in 1976 due to public concern for safety, environmental impact, and ethical implications of recombinant DNA technology. The scope of the NIH Guidelines has expanded in recent years to include advances in synthetic nucleic acid synthesis. The purpose of the NIH Guidelines is to specify institutional oversight for the safe handling and containment of (1) recombinant nucleic acid molecules, (2) synthetic nucleic acid molecules, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, and (3) cells, organisms, and viruses containing such molecules including transgenic animals and plants.

At NC State, the Institutional Biosafety Committee (IBC) reviews all research covered under the NIH Guidelines. For more information regarding IBC membership, schedules, and procedures, refer to the EHS webpage for NC State University Safety Committees and click on Institutional Biosafety Committee.

Classification of research involving recombinant or synthetic nucleic acid molecules:

To determine whether your research is subject to IBC review at NC State University, refer to the NC State University guide Classification of Research Involving Recombinant or Synthetic Nucleic Acid Molecules.

Incident Reporting to NIH

Minor spills of low-risk agents, contained and properly disinfected, generally don’t need to be reported. Incidents meeting the threshold for reporting as noted below should be reported to the EHS Biosafety Officer who in turn will report to NIH Office of Biotechnology Activities (NIH OBA). Reports will include the processes implemented by the PI to mitigate the problem and preclude its reoccurrence.

The following incidents require immediate reporting to EHS and NIH OBA:

1. Spills or accidents involving rDNA requiring BSL2 containment resulting in an overt exposure, e.g. needlestick; splash in eyes, nose, mouth; or accidental aerosolization/inhalation;
2. Spills or accidents involving rDNA requiring BSL3 containment resulting in an overt exposure or potential exposure, e.g. spills of high risk recombinant materials occurring outside of a biosafety cabinet.
The following incidents must be reported immediately to EHS so that EHS can provide the proper documentation to NIH OBA within 30 days:

1. Any significant problems or violations of the NIH Guidelines, e.g. failure to adhere to the containment and biosafety practices in the Guidelines;
2. Any significant research-related accidents and illnesses, e.g. spill or accident leading to personal injury or illness or a breach in containment, e.g. escape or improper disposition of a transgenic animal.

Chapter 3: Risk Groups and Biosafety Levels

When completing the Biological Use Authorization form and whenever planning a new project using potentially hazardous materials or processes, a thorough risk assessment should precede the selection of precautions. A biosafety risk assessment is conducted to determine the appropriate containment for a proposed experiment or project. At NC State University containment consists of a documented set of practices, PPE, equipment, training, and work space designed to protect laboratory employees or students, maintenance or service workers, the public, agriculture, or the environment. This Chapter is intended to provide a starting point for the biosafety risk assessment.

The Risk Group is a comparative descriptor for a given microbe based on the inherent pathogenic nature to (typically) humans. Identifying the Risk Group is usually the first step in the biosafety risk assessment process. The PI may need to provide supporting documentation for each proposed Risk Group on the Biological Use Authorization form (BUA). The four commonly recognized Risk Groups are listed here:

Summary of Risk Groups (RG)

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<th>RG</th>
<th>Risk Description</th>
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<tr>
<td>RG1</td>
<td>Agent not associated with disease in healthy adult humans; <em>B. subtilis</em>, <em>E. coli K-12</em>, AAV, ecotropic avian sarcoma virus</td>
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<tr>
<td>RG2</td>
<td>Associated with human disease which is rarely serious and preventive or therapeutic interventions are often available; Human adenoviruses, human herpesviruses (except herpes B), <em>Staphylococcus aureus</em>, amphotropic murine leukemia virus, influenza viruses type A, B, and C</td>
</tr>
<tr>
<td>RG3</td>
<td>Serious or lethal human disease; preventive or therapeutic interventions may be available; <em>Mycobacterium tuberculosis</em>, VEE, <em>Francisella tularensis</em></td>
</tr>
<tr>
<td>RG4</td>
<td>Serious or lethal human disease; preventive or therapeutic interventions are usually not available; Ebola, Marburg, Lassa, and Herpes B virus</td>
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Resources for assigning Risk Group & Biosafety Level

How and where the investigator plans to interact with the microbe play a key role in determining containment level (i.e. “biosafety level”). Adverse consequences are more likely to occur if the risks are underestimated. By contrast, imposition of safeguards more rigorous than actually needed may result in additional expense and burden for the lab, with little safety enhancement. However, if there is insufficient information to make a clear determination of risk, it is prudent to consider the need for additional safeguards until more data are available.
The PI will propose a biosafety level on the BUA that the IBC will evaluate at the time of registration. There are four commonly recognized biosafety levels as illustrated in the following tables. The proposed biosafety level should be based on a thorough risk assessment that, at a minimum, includes a review of the following resources:

1. The NIH Guidelines Appendix B provides common biological agents used in research listed by Risk Group.
2. 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.
3. The American Biological Safety Association (ABSA) website provides a searchable database of many biological agents and their assigned biosafety levels by country.
4. 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.

Teaching Labs

The ASM Guidelines for Biosafety in Teaching Laboratories were finalized in 2013 as the standard of practice for all teaching courses and projects where students handle live microorganisms. The NC State Institutional Biosafety Committee upholds this standard of practice to maintain consistency among university instructors in biological safety. Some important items to consider include:

- Register teaching labs with the Institutional Biosafety Committee.
- Instructors may not inquire about the health status of a student.
- Prior to beginning lab-work, all students must be provided with a list of all cultures (and their sources) used in the course with a Physician Notification Form.
- Students shall not subculture isolates from the environment at BSL-1. Unknown environmental microbes can be isolated and plated in a BSL-1 laboratory, but then the plates must be sealed and only observed.
- Students should use institution-provided writing instruments.

For more information on the items above and others regarding biosafety in NC State teaching labs, visit the EHS website here.

Environmental Samples

Animal and plant pathogens in their naturally occurring environment are not subject to IBC review until intentionally introduced, cultivated, collected, extracted, etc. Unknown environmental microbes can be isolated and plated at BSL-1 but further manipulation may require enhanced precautions depending on the source, in which case a BUA should be submitted for IBC review. Samples known or suspected to contain human pathogens such as sewage are to be included on the BUA for IBC review.
Human blood, blood products, body fluids, tissues, and cells

Biosafety level 2 practices and containment must be followed when handling human materials that may contain bloodborne pathogens (e.g. HBV, HCV and HIV) in the laboratory (see BMBL, Appendix H – Working with Human, NHP and other Mammalian Cells and Tissues). Also, the OSHA Bloodborne Pathogens (BBP) Standard (29 CFR 1910.1030) applies to all work in the laboratory with human blood or other potentially infectious materials. Under the OSHA BBP Standard, Departments and/or Principal Investigators are required to (1) develop a written Exposure Control Plan, (2) offer employees the hepatitis B vaccination, and (3) provide initial and annual BBP training. For more information on the impact of the OSHA BBP standard on the laboratory setting at NC State, refer to your department's or laboratory's Exposure Control Plan and the Biosafety website for Bloodborne Pathogens.

Since the mid 1990's OSHA's position has been that workers handling human cell cultures (primary or established) fall under the purview of the Bloodborne Pathogen (BBP) Standard. For more information, review the OSHA interpretation letter on the applicability of 1910.1030 to established human cell lines (06/21/1994).

Refer to the Disinfection section of the Safe Work Practices and PPE chapter in this manual to review disinfection, treatment, and disposal requirements for materials covered under the OSHA Bloodborne Pathogens Standard.

Other cultured cells and tissue

Cultured cells which are known to contain or be contaminated with a biohazardous agent (e.g. bacteria or viral) are generally classified at the same risk group as the agent. 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. These may require permits through NCDACS or USDA (Principal Investigators are responsible for obtaining all necessary permits).

The following cells and tissue must be listed on the Biological Use Authorization form and handled at BSL2:

- 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.

General Laboratory Facility Biosafety Levels

The CDC/NIH publication Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Ed. outlines safe lab practices, lab facilities, and safety equipment for four biosafety levels that provide appropriate containment based upon a proper risk assessment for manipulations that begins with the various risk group agents (RG1-RG4) designated by the NIH. The BMBL also describes animal biosafety levels for the use of research animals. The summary tables below were adapted from BMBL (5th Edition) and include NC State University practices.
This table summarizes the biosafety levels and requirements for laboratory work at NC State University:

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<th>BSL</th>
<th>AGENT</th>
<th>PRACTICES</th>
<th>PRIMARY BARRIERS AND SAFETY EQUIPMENT</th>
<th>FACILITIES (SECONDARY BARRIERS)</th>
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<tr>
<td>1</td>
<td>Not known to cause disease in humans</td>
<td>Standard Microbiological Practices</td>
<td>Gloves, lab coat, eye protection, and proper footwear.</td>
<td>Handwashing sink, safety shower/eyewash and, autoclave required</td>
</tr>
<tr>
<td>2</td>
<td>Transmitted by percutaneous injury, ingestion, mucous membrane exposure. Consider aerosolization.</td>
<td>BSL-1 practice plus:</td>
<td>At a minimum, BSL-1 protection, plus: Physical containment devices used for all manipulations requiring BSL-2 (microbes, rDNA, toxins) that cause splashes or aerosols of infectious materials; Class I or II Biological Safety Cabinets</td>
<td>Same as BSL-1</td>
</tr>
<tr>
<td>3</td>
<td>Potential for aerosol transmission</td>
<td>BSL-2 practice plus:</td>
<td>Primary barriers:</td>
<td>BSL-2 plus:</td>
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<td></td>
<td>• Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum</td>
<td>• Class I or II BSCs or other physical containment devices used for all open manipulation of agents</td>
<td>• Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into lab</td>
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<tr>
<td>4</td>
<td>NC State does not have BSL-4 facilities</td>
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### Live Vertebrate Animal Work

All activities that involve the use of live animals must be registered, reviewed and approved by Institutional Animal Care and Use Committee (IACUC) before the work is initiated. This table summarizes the biosafety levels for activities in which vertebrate animals are experimentally or naturally infected with agents that infect humans as well as animal agents that may pose theoretical risks if inoculated into humans.

<table>
<thead>
<tr>
<th>ABSL</th>
<th>RISKS/ ROUTES of Transmission</th>
<th>PRACTICES</th>
<th>PRIMARY BARRIERS AND SAFETY EQUIPMENT</th>
<th>FACILITIES (SECONDARY BARRIERS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Well characterized agents not known to cause disease in humans</td>
<td>• Restricted access • Standard animal care and management practices, including appropriate medical surveillance programs • Specific training in facility procedures • Universal Sharps precautions</td>
<td>As required for normal care of each species plus standard laboratory safety practices. Eye protection when risk of splashes is present.</td>
<td>Standard animal facility: • No recirculation of exhaust air • Directional air flow recommended • Hand washing sink is available • eyewash and shower station are readily available</td>
</tr>
<tr>
<td>2</td>
<td>Agents associated with human</td>
<td>ABSL-1 practice plus: • Biohazard warning signs</td>
<td>ABSL-1 equipment plus primary barriers: • Containment equipment</td>
<td>ABSL-1 plus: • Autoclave available • Hand washing sink available</td>
</tr>
</tbody>
</table>
### Required Procedures for Work in ABSL2 Animal Facilities:

**The researcher is responsible for:**

1. Registering work with IBC when applicable and providing applicable sections of the BUA to the animal facility director.
2. Communicating the start date of the study and conveying the IBC approved Biological Use Authorization for the animal work to the animal facility director, as well as the manager of the rooms or procedure areas where the hazardous agent work will occur. (Refer to these links for contact information: Laboratory Animal Facility at CVM; Biological Resources Facility on central campus; or Todd See for CALS farm areas)
   - **This communication should occur at least five (5) business days prior to initiation of the work.**
3. Initiating the work **only after** obtaining confirmation that your notification has been received.
4. **Placing the proper signs on the animal room door and cages** prior to the initiation of the study.
5. Removing the signs when the study is complete.
   - **Cage Cards and Door Signs:** As soon as the animals have been dosed with the biohazardous agent, cages must be marked with the biohazard cards and the appropriate sign must be posted on the outside of the animal room door by research staff. This sign will be removed by research staff once the infected animals and biohazardous agents are no longer in the animal room.

---

<table>
<thead>
<tr>
<th><strong>Agents with potential for causing serious or lethal disease; potential for aerosol transmission</strong></th>
<th><strong>ABSL-2 practice plus:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease; percutaneous injury, ingestion, mucous membrane exposure</td>
<td>Controlled access</td>
</tr>
<tr>
<td>PPE*:</td>
<td>Decontamination of all infectious wastes and of animal cages prior to washing</td>
</tr>
<tr>
<td><strong>ABSL-2 equipment plus:</strong></td>
<td>Containment equipment for housing animals and cage dumping activities</td>
</tr>
<tr>
<td></td>
<td>Class I, II or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols.</td>
</tr>
<tr>
<td></td>
<td><strong>PPEs:</strong></td>
</tr>
<tr>
<td></td>
<td>Appropriate respiratory protection</td>
</tr>
</tbody>
</table>

*PPE – Personal Protective Equipment*
Arthropod Containment

The Arthropod Containment Guidelines are based on recommendations of the American Society of Tropical Medicine and Hygiene and the American Committee of Medical Entomology. The document describes arthropod handling practices, safety equipment and facilities for Arthropod Containment Levels 1-4. These guidelines specifically do not cover Drosophila spp. unless modified in such a manner that they would be of public health concern, however, the NIH Guidelines may still apply (see Chapter 2). If arthropods are infected with an agent, the containment level is automatically increased to at least that required for handling the agent, regardless of competence of the arthropod to transmit that agent (e.g., male mosquitoes to propagate a risk group 2 agent would require a minimum of level 2 containment). Furthermore, in recognition of the fact that escape of uninfected exotic or genetically modified arthropods is to be prevented by all reasonable means, unless the IBC decides otherwise, these are also handled at level 2 or higher. For clarification, contact the Biosafety Officer.

Guidance for design, construction, maintenance and operation of facilities for containment of nonindigenous arthropod herbivores, parasitoids and predators which may be used in biological control research is provided in the USDA APHIS – PPQ Guidelines for Containment of Nonindigenous Arthropod Herbivores, Parasitoids and Predators.

Plant Work and Plant-Associated Organisms

Since plant research usually (but not always) does not pose a human health hazard, biosafety principles are designed instead to protect the natural and agricultural environment.

Plant research involving noxious weeds, invasive plants, and certain plant pests, plant-associated microbes, and plant diseases is regulated by the North Carolina Dept. of Agriculture & Consumer Services, especially when the import, export, or transfer of these or materials is required. Contact the Division of Plant Industry for rules and regulations specific to North Carolina. Review the Guide to USDA/APHIS permits for federal regulations.

The NIH Guidelines (see Chapter 2) provide containment levels for genetically engineered plants, genetically engineered plant-associated microbes, and genetically engineered plant-associated macro organisms (arthropods and nematodes) in Appendix P. The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment.

A good resource is A Practical Guide to Containment Greenhouse Research with Transgenic Plants and Microbes -- Website.
This table summarizes the Biosafety levels for activities in which rDNA or certain plant pests are used in whole plants.

<table>
<thead>
<tr>
<th>BSL-P</th>
<th>RECORDS</th>
<th>PRACTICES</th>
<th>INACTIVATE/DECON</th>
<th>BARRIERS AND FACILITIES</th>
</tr>
</thead>
</table>
| 1     | records of rDNA experiments in progress | Standard greenhouse care and management practices, including limited access. | Inactivate organisms before disposal outside of the greenhouse facility. | • No special barrier to contain or exclude pollen, microbes, or arthropods and birds  
• Floors may be gravel  
• Windows etc. may be open for ventilation, screens recommended. |
| 2     | BSL1-P plus: Records of all organisms entering and exiting. | BSL1-P practice plus:  
• **Immediate reporting of spills or releases to IBC**  
• Arthropods contained  
• Biohazard or warning signs  
• Greenhouse practices manual | BSL1-P plus:  
• Consideration of decontamination run-off water | • Floors of impervious material in greenhouse  
• Autoclave available  
BL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macro organisms in a manner that satisfies the intent of the foregoing clauses. |
| 3     | BSL2-P plus: Records of all access, storage and use. Decontamination records for all materials removed from facility. Security access records. | BSL2-P practice plus:  
• Access to the lab is limited to individuals that have completed appropriate training and been approved by APHIS.  
• Accompanied visitors that have been approved through EHS may also enter. | BSL-2P plus:  
• All biological material, equipment, and clothing must be autoclaved or decontaminated before removal.  
• All waste water is treated.  
• Shower out. | • Floors and walls of impervious material  
• Pass through autoclave available in facility  
• Greenhouse and lab must be under negative pressure  
• Greenhouse glass is double laminate and sealed where attached to greenhouse frame.  
• Effluent collection system available  
• Shower for use when exiting the facility  
• Protective laboratory clothing based on agent in use.  
• Physical separation from access corridor.  
• Self-closing, double-door access  
• Sealed penetrations. |

NC State does not have level 4 BSL-P facilities

**Select Agents and Toxins**

Select Agents and Toxins are federally regulated because of their potential for use in biological warfare. [Click this link to access the latest Select Agents and Toxins List.](#) Materials known to contain agents or toxins on the list (samples, specimens, etc.) are subject to the Select Agent regulations.

Laboratories providing clinical or diagnostic services for humans, animals, or plants must report the identification of a Federal Select Agent or Toxin within 24 hours to (1) the appropriate federal agency (CDC/USDA) and (2) the Biosafety Officer at EHS. Within 7 calendar days of the identification of a Select Agent or Toxin, the [Form 4 Report of the Identification of a Select Agent or Toxin](#) must be submitted. These procedures, if not outlined in the Biological Use Authorization form (see Chapter 1), will be requested by the IBC during review.
Before possessing, using, sending, or receiving agents or toxins on the Federal Select Agents and Toxins List, the Principal Investigator, together with NC State University must register with CDC, and/or USDA. A high-level containment laboratory with security enhancements including individual security background checks is required. Contact the Biosafety Officer at EHS for more information on the application and security risk assessment process.

**Federally Exempt Quantities of Toxins**

The toxins listed below are exempt from CDC and USDA registration requirements if the maximum allowable exempt quantity per Principal Investigator is not exceeded. PIs must submit a Biological Use Authorization form (BUA) to the IBC, document their compliance with the Federal Select Agent Program Toxin Due Diligence Provision on their BUA, provide locked storage, and document their inventories to ensure the maximum exempted amount is not exceeded.

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Maximum Exempted Amount per PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abrin</td>
<td>100 mg</td>
</tr>
<tr>
<td>Botulinum neurotoxins</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Clostridium perfringens epsilon toxin</td>
<td>100 mg</td>
</tr>
<tr>
<td>Conotoxins</td>
<td>100 mg</td>
</tr>
<tr>
<td>Diacetoxyscirpenol (DAS)</td>
<td>1000 mg</td>
</tr>
<tr>
<td>Ricin</td>
<td>100 mg</td>
</tr>
<tr>
<td>Saxitoxin</td>
<td>100 mg</td>
</tr>
<tr>
<td>Shiga-like ribosome inactivating proteins</td>
<td>100 mg</td>
</tr>
<tr>
<td>Shigatoxin</td>
<td>100 mg</td>
</tr>
<tr>
<td>Staphylococcal enterotoxins</td>
<td>5.0 mg</td>
</tr>
<tr>
<td>Tetrodotoxin (TTX)</td>
<td>100 mg</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>1000 mg</td>
</tr>
</tbody>
</table>

**Dual Use Research and DURC**

Despite its value and benefits, certain types of life sciences research conducted for legitimate purposes can be utilized for both benevolent and harmful purposes, such research is called “dual use research.” All BUA submissions must contain an attestation from the Principal Investigator that the seven listed experimental effects of concern are evaluated. Instructions are listed on the BUA form.

If the BUA contains Select Agents, the IBC will review the BUA in compliance with the 2015 U.S. Government Dual Use Research of Concern (DURC) Policy. If one or more of the 15 listed Select Agents within the Federal DURC Policy is used, the Biosafety Officer (BSO) and/or IBC Chair will contact the PI to further determine if the DURC Policy applies. If Section 6.2 of the DURC Policy applies, the IBC will take on the role of the Institutional Review Committee (IRE) for mitigation review as described in Section 7.2.E of the federal DURC policy and the BSO will ensure the Principal Investigator notifies the VC of Sponsored Programs for analysis of funding sources. The BSO will report this analysis back to the IRE. The IRE will establish measures that mitigate the risks of DURC in a manner that minimizes, to the extent possible, adverse impact on legitimate research, is commensurate with the risk, includes flexible approaches that leverage existing processes, and endeavors to preserve and foster the benefits of research.
Chapter 4: Training

Training, experience, knowledge of the agent and procedure hazards, good habits, caution, attentiveness, and concern for the health of coworkers are prerequisites for a laboratory staff in order to reduce the inherent risks associated with work involving hazardous agents. Not all workers who join a laboratory staff will have these prerequisite traits even though they may possess excellent scientific credentials.

Training involves numerous components that include general safety practices and safety theory which progresses to task-specific safety practices and Standard Operating Procedures (SOPs), entry and exit procedures, room and suite specific procedures, use of PPE and equipment, animal handling, incident and accident reporting, etc. These components include training under normal operating conditions, during emergencies, systems failures, and in the event of a suspect or known exposure. Training is often conducted in a layered approach to include a review of manuals and SOPs, classroom training, hands-on training with a skilled and knowledgeable mentor that may start with less hazardous organisms, progress to a “watch one, do one” approach, and culminate in demonstration of competency.

Laboratory Training Requirements for Biosafety

EHS offers an introductory biosafety primer that includes much of the material in this manual and the BSL-2 Checklist. This Laboratory Biosafety Training, available from the EHS website, is required for those who use—or supervise a laboratory that uses—recombinant or synthetic nucleic acid molecules at BSL-1 or BSL-2 containment or any work requiring BSL-2 containment. This Laboratory Biosafety Training is strongly recommended for those using biological materials (other than recombinant or synthetic nucleic acid molecules) at BSL-1 containment.

The introductory Laboratory Biosafety Training primer includes an exam. After successfully completing the exam, an exam confirmation email is automatically sent to the email address entered on the exam page. Principal Investigators should print and maintain this exam confirmation email in their lab safety binder for future reference.

The BUA contains the Statement of Informed Consent to be used by Principal Investigators to communicate hazards to laboratory workers, guide discussion of worker immunocompetence, and document completion of the online Laboratory Biosafety Training. Principal Investigators are required to add the names of new workers as they join the project.

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 BSCs, ability to respond to emergencies, and willingness to accept responsibility for protecting one’s self 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.
Other EHS training sessions are available from the EHS website at http://www.ncsu.edu/ehs/training.htm

**Additional Training for PI’s working with Recombinant and Synthetic Nucleic Acid Molecules**

Training on the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules is required by the NIH of all Principal Investigators with labs working with recombinant or synthetic nucleic acid molecules. While the online EHS Laboratory Biosafety Training (above) meets the minimum requirement, additional training slides are available online from the NIH website.

**Personal Health Status**

Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women who may be or are planning to become pregnant should be provided with information regarding immune competence and conditions that may predispose them to infection such as known allergies to antibiotics or other chemoprophylactics that could be used to treat a laboratory acquired infection.

**Chapter 5: Medical Surveillance**

People with underlying health complications may be more vulnerable to complications resulting from exposure to biological agents. Lab workers that have an autoimmune or chronic disease (no matter how well managed), heart disease, are taking immune suppressing medications (e.g., chemotherapy, systemic steroids) or are otherwise immunocompromised (e.g., transplant recovery, cancer, lupus), should be encouraged to self-identify to the occupational healthcare provider for appropriate counseling and guidance. The PI’s approved Biological Use Authorization form should be shared with the medical provider. Complete and send the Exam Request Form in a sealed envelope to: Occupational Medicine Coordinator, Student Health Services, Box 7304. This form should also be sent if health status changes while working on a project.

Females that are pregnant or planning conception should review the university’s Reproductive Health Protection Program.

A medical surveillance program is provided through NC State for personnel who are occupationally at risk of exposure to bloodborne pathogens (BBP), have direct contact with research animals, require use of a respirator, and/or receive vaccines for various infectious agents, e.g. vaccinia, rabies, measles, used in the laboratory. The bloodborne pathogens program follows the department or supervisor’s Exposure Control Plan and includes hepatitis B vaccine and post-exposure evaluation and follow up at no cost to the employee.

In addition to being offered recommended vaccines, lab workers routinely exposed to pathogens may be offered tests as determined appropriate for agents handled in the lab, e.g. TB skin test. All personnel working in BSL-3 laboratories may be required to
participate in medical surveillance programs. All medical surveillance and vaccination requirements specific to laboratory research are listed on the Biological Use Authorization for review by the IBC at the time of registration.

Chapter 6: Biosafety Cabinets and Other Safety Equipment

Biological safety cabinets (BSC) control airborne contaminants during work with infectious material through the use of laminar airflow and high efficiency particulate air (HEPA) filtration. The Class II BSC is the most commonly used BSC at NC State.

The table below shows the type of protection provided by common hoods used at NC State. Both the Chemical Fume Hood (CFH) and the BSC provide worker protection by enclosing the hazardous operation. However, the CFH is rarely substituted for the BSC because the CFH does not protect the product from contaminating particles found in the surrounding laboratory. Notice, the Clean Bench does not offer worker protection.

<table>
<thead>
<tr>
<th>Types of Protection</th>
<th>Worker</th>
<th>Product</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Fume Hoods (Protection From Vapors And Gasses)</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biological Safety Cabinets (Protection From Particulates)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Clean Benches/ (Horizontal Laminar Flow Hoods) (No Worker Protection)</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>


Biosafety Cabinet Alarms

Do not work in a biosafety cabinet that is in alarm. If your biosafety cabinet goes into alarm, which is an indication that worker, product, and/or environmental protection are compromised. Post the biosafety cabinet prominently with the words “DO NOT USE (protection is compromised)” and immediately contact your building liaison or the Biosafety Officer. The EHS recommended posting is available here.

Biological Safety Cabinets at NC State

Biological safety cabinets can only protect the worker and the product if they have been properly selected for the intended containment function. Questions about BSCs at NC State should be directed to the EHS Biosafety Officer at 919-515-6858.
1. Selection

Proper selection of the BSC is contingent on an accurate risk assessment of the hazards inherent to the work planned in the unit (e.g. chemical, radiological, biological hazards). Selection should consider (1) the hazard classification of the agent; (2) the need for protection of research material or personnel; and (3) the extent to which hazardous aerosols are involved. Review the CDC/NIH Appendix A, Primary Containment for Biohazards Selection, Installation and Use of Biological Safety Cabinets of Biosafety in Microbiological and Biomedical Laboratories (5th edition) publication (BMBL) and contact the EHS Biosafety Officer with questions.

A common mistake among investigators is selecting a Laminar Flow Clean Bench instead of a Class II Biosafety Cabinet.

2. Location and Installation

Because the delicate air curtain created at the front of the cabinet can be easily disrupted, certain considerations must be made to ensure maximum effectiveness of this primary barrier. Consider the following:

- The BSC should be located away from air supply registers, entrances, windows that open, high traffic areas, and laboratory equipment that create turbulence.
- Gas lines should not be installed on BSCs at NC State and the use of gas flame burners in BSC's should be prohibited (click the link for background and alternatives).

Dimensions of the BSC:

- Will the BSC need to fit through the door?
- Will the BSC location fit the ceiling height? (may need 12-14 inches above the BSC for annual certification, lights may need to be moved; if the BSC will be hard-ducted, is there space for duct? etc.)

3. Certification

Post a "DO NOT USE (protection is compromised)" sign prominently on the BSC until the unit can be certified for the first time or if the unit is past due for annual certification. NC State follows the BMBL 5th edition guidance in Appendix A (see link in the Selection section above) that the minimum requirement for BSC certification is annually.

Before running any performance tests, the BSC shall be properly installed and leveled and airflows adjusted to the nominal set point (+/- 3.0 ft./min [+/- 0.015 m/s]).

Accredited field certifiers are used to test and certify BSCs in accordance with NSF/ANSI 49 Annex F plus Addendum #1. When identifying companies qualified to conduct the necessary field performance tests, contact the EHS Biosafety Officer.
4. Decontamination

Gaseous decontamination is mandatory when moving or surplusing a BSC. It is also required when maintenance work, filter changes, and performance tests require access to any interior portion of the cabinet. When identifying companies qualified to conduct the decontamination procedure, consult the EHS Biosafety Officer.

Because of the potential for release of toxic gas during the decontamination process, the Principal Investigator must follow this checklist closely prior to conducting gas decontamination:

- Contact EHS Biosafety Officer for **required** room air pressure differential test. A radiation survey may also be necessary.
- Disinfect and remove all items from the BSC
- Surface disinfect the interior of the BSC w/ appropriate disinfectant
- Remove any Rad. & carcinogen stickers as appropriate
- Schedule a full gas decontamination (**must** be conducted by a BSC certification vendor, typically the same professionals conducting annual certification)
- Contact building liaison to ensure no HVAC disruption is scheduled at that time because the gas decon procedure uses high concentrations of toxic gas
- Schedule for lab to be vacant during the gas decon procedure
- Post “DO NOT ENTER” signs at entryways to the gas decontamination area
- After gas decon, remove/cover biohazard stickers and
- Ensure the gas decon vendor posts a label/sign indicating the following:
  1. Vendor contact information
  2. Date and time the gas decon was performed
  3. Method used for gas decontamination
  4. If the gas decon method was successful or not
- Contact building liaison to disconnect gas lines, vacuum, etc. from BSC
- If exhaust is hard-ducted, the duct will need to be disconnected

Safe and Effective Use of the BSC

1. Before beginning work:
   a. Monitor alarms, pressure gauges, or flow indicators for any changes.
   b. Shut off the UV light.
   c. Turn the cabinet on and let it run for 3-5 minutes.
   d. Wipe work surface with an appropriate disinfectant.
   e. Place a pan filled with disinfectant or lined with a small biohazard bag inside the BSC to collect discards. Avoid reaching outside of the BSC during procedures to discard waste in floor containers.
   f. Plan your work and place everything needed for the procedure, including the pan for your discards, inside the BSC. Wipe items with disinfectant before placing in BSC.
2. Avoid airflow disruption that could affect the level of protection provided by the BSC:
   a. Keep the BSC free of clutter, e.g. extra equipment and supplies
   b. Don’t place objects over the front air intake grille.
   c. Don’t block the rear air intake grille.
   d. Limit traffic in the area when the BSC is in use
   e. Make sure lab door is closed, and avoid opening and closing door if located near the BSC.
   f. Move arms slowly when removing or introducing items.
   g. Keep all materials at least 4 inches inside the sash.
   h. 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.
   i. Don’t operate a Bunsen burner in the cabinet.

3. While working:
   a. Work as far to the back of the BSC workspace as possible.
   b. Segregate contaminated and clean items. Work from “clean to dirty.”
   c. Clean up all spills in the cabinet immediately. Allow cabinet to run for 3-5 minutes before resuming work.

4. After completing work:
   a. Wipe down all items with an appropriate disinfectant before removing. Remove all materials and wipe all interior surfaces with an appropriate disinfectant.
   b. Periodically decontaminate under work grilles.

Aerosol-proof rotors and safety cups for centrifuges

Aerosols may be created during centrifugation from poorly sealed or capped tubes and from tubes splitting or breaking. Follow the procedures below when centrifuging biohazardous materials:

1. Use aerosol-proof rotors or safety buckets with 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. Avoid overfilling the tube and getting caps/closures wet. Wipe tubes down with disinfectant after filling.
4. Load and unload rotors and buckets inside the BSC
5. Balance buckets, tubes and rotors before centrifuging.
6. Disinfect the centrifuge after use.
7. Place small, low-speed centrifuges in a BSC during use to contain aerosols.

Other safety equipment for aerosol-producing devices

The use of certain devices, e.g. blenders, homogenizers, sonicators (ultrasonic disrupters) can produce aerosols. To reduce exposure to aerosols, these devices should be used in a biosafety cabinet whenever possible.

Safety blenders and the BeadBeater homogenizer (BioSpec Products) are designed to prevent leakage of aerosols. The devices should be used in the BSC to prevent accidental release of aerosols.
Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols that may contain viable microorganisms. The use of a shielded electric incinerator minimizes aerosol production during loop sterilization. Alternatively, disposable loops and needles can be used.

**Chapter 7: Safe Work Practices and PPE**

At NC State, each individual Principal Investigator is responsible for ensuring that proper safety practices, procedures, and equipment are in place. The PI is considered the responsible “supervisor” from an OSHA point of view, regardless of whom they delegate these tasks to. Supervisors are responsible for conducting workplace assessments and to select and train employees in the proper use of PPE (e.g., lab coats, gloves, safety glasses, face shields, etc.). A workplace assessment may be conducted during protocol review, at lab meetings, or while mentoring novel techniques and practices. This assessment should be documented and, if necessary, practices written up as a safety SOP. Considerations for practices at NC State are described below.

**Personal Protective Equipment:**

Personal protective equipment (PPE) is specialized clothing or equipment worn by laboratory personnel for protection against hazards. 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. For assistance in selecting PPE, contact the Biosafety Officer or EHS.

The minimum PPE required for the BSL-2 laboratory is no different from PPE used in BSL-1: lab coats, gloves, and safety glasses (or goggles).

1. Laboratory garments, e.g. lab coats, scrubs, and gowns, should be long-sleeved in order to prevent contamination of skin and street clothes. The garment must be fluid-resistant in order to protect workers from splashes. Lab coats should be provided for visitors, maintenance and service workers as needed. EHS offers more guidance at this [lab coat selection and disposal](#) link.

2. Gloves must be worn when working with biohazards. Temperature resistant gloves must be worn when handling hot material or dry ice. If personnel develop or have latex allergies, then nitrile gloves should be used in the lab with biohazards instead of latex gloves. Gloves should overlap the sleeve of the lab garment. Double-gloving adds further protection and is recommended in some circumstances, e.g. for BSL-3 laboratories, or in situations where a spill or splash is possible. More [information on proper glove selection](#) is available at the EHS website.

3. Face protection is required in situations where chemical splashes or aerosol exposure to infectious material are possible. Goggles or safety glasses with side shields should be used in combination with masks, face shields or other splatter guards for optimal protection.

4. Respirators may be necessary in some cases. Personnel who require respiratory protection must be evaluated by the UEOHC and trained in respirator selection and usage. Personnel required to wear tight-fitting respirators must enroll in NCSU’s [Respirator Protection Program](#).
Sharps Precautions:

Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Click here for Fact Sheet on general sharps precautions. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of injuries from sharps. The list of precautions below, must always be followed when working with sharp items.

1. Use of needles and other sharps should be minimized and avoided entirely whenever possible. Many glass items such as Pasteur pipettes have plastic counterparts that are safer alternatives.
2. If the use of sharps is necessary, extra precautions should be taken. Sharps should be disposed of immediately after use in a designated puncture-resistant sharps container. When the container is 2/3 full, submit a hazardous waste collection request from EH&S for its removal. Never allow the container to overfill.
3. Needles must never be recapped, removed from the syringe, sheared, bent or broken. If a needle must be recapped, use a one-handed method or a mechanical device, e.g. forceps.
4. Use a mechanical device to remove scalpel blades, and not your fingers.
5. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Contact EH&S for help in the evaluation and selection of safe medical devices, or complete the Safety Feature Evaluation Form and submit to Box #8007.

General Biosafety Work Practices:

Proper work practices protect you and others from exposure to infectious materials, reduce the possibility of cross-contamination, and improve the quality of the work performed.

1. Label all equipment used to store infectious materials with a biohazard warning label.
2. Keep an uncluttered work space
3. Plan work procedures with safety in mind
4. Remove PPE and wash hands when leaving the lab
5. Don’t eat, drink, smoke, apply cosmetics, and handle contact lenses in the lab
6. Don’t mouth pipette
7. Decontaminate work surfaces at the end of an experiment and after a spill occurs
8. Decontaminate reusable PPE as soon as possible after it has been contaminated. Lab coats can be spot treated with 10% bleach or autoclaved before laundering. Never take lab coats home.
9. Protect house vacuum lines and vacuum pumps by using a hydrophobic HEPA filter installed between the collection flask and vacuum source
10. Change gloves often and as soon as possible when visibly contaminated
11. Minimize aerosol production by working carefully
12. Perform procedures that may result in aerosols or splashes in a BSC
13. Use aerosol-proof rotors or safety cups when centrifuging and load and unload them in a BSC
Door Signs for BSL-2 and BSL-3

Laboratory door signs are generated by EH&S at the initial completion or update of the Safety Plan. Complete the New Door Sign Request Form located on the EH&S website. BSL-2 and BSL-3 door sign information contains the universal biohazard symbol, biosafety level, and office and after-hours contact numbers for the PI and alternate who is familiar with the laboratory hazards and operations. Additional placards for BSL-2 and BSL-3 areas may be required if additional information such as PPE and enhanced warnings are necessary. The template for additional placards must be requested from the EH&S Biosafety Officer.

Door signs and placards should not be posted for laboratories where containment is limited only to BSL-1 or BL-2P (plant) work.

Disinfection

Characteristics of microorganisms affect their resistance to disinfection. The Disinfection Selection FAQ (in revision) and the table below provide a starting point for identifying appropriate chemicals for disinfection depending on the circumstances and type of biohazard. To locate information on proprietary disinfectants, refer to the EPA-registered disinfectants website at http://www.epa.gov/oppad001/chemregindex.htm to review efficacy claims against microbes of interest.

OSHA requires use of an EPA-registered disinfectant under the Bloodborne Pathogens Standard. Note that 70% ethanol is not an EPA-registered disinfectant. It evaporates too quickly to be an effective disinfectant. 70% ethanol can be used as a cleaner, for example, to remove excess bleach or other EPA-registered disinfectants. Alternative disinfectants include Clorox, Amphyl, Lysol, and Sporicidin.

At NC State University liquid biohazard waste is autoclaved with a test indicator and disposed down the sanitary sewer. Chemicals may NOT be directly poured down the drain. For example, any greater concentration than a 1:5 dilution of bleach (5.25% sodium hypochlorite) to the final volume needs review of disposal options with the EHS hazardous waste manager. If chemicals 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.

When decontaminating small tubes such as epi tubes, empty them out into a plastic container in a sink, add a 1:10 dilution of household bleach (5.25% sodium hypochlorite) to water or another IBC approved disinfecting solution. After the appropriate contact time has been achieved (this is listed on the BUA), it may then be poured down the drain.
**List of disinfectants**

<table>
<thead>
<tr>
<th>USE PARAMETERS</th>
<th>Ethylene Oxide</th>
<th>Paraformaldehyde (gas)</th>
<th>Quaternary Ammonium Cmpds.</th>
<th>Phenolic Cmpds.</th>
<th>Chlorine Cmpds.</th>
<th>Iodophor Cmpds.</th>
<th>Alcohol (ethyl or isopropyl)</th>
<th>Formaldehyde</th>
<th>Glutaraldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. of active ingredient</td>
<td>400-800 mg/liter</td>
<td>0.3 g/ft³</td>
<td>0.1-2%</td>
<td>0.2-3%</td>
<td>0.01-5%</td>
<td>0.47%</td>
<td>70-85%</td>
<td>4-8%</td>
<td>2%</td>
</tr>
<tr>
<td>Temp. (°C)</td>
<td>35-60</td>
<td>&gt;23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative humidity (%)</td>
<td>30-60</td>
<td>&gt;60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact time (min.)</td>
<td>105-240</td>
<td>60-180</td>
<td>10-30</td>
<td>10-30</td>
<td>10-30</td>
<td>10-30</td>
<td>10-30</td>
<td>10-600</td>
<td></td>
</tr>
<tr>
<td>EFFECTIVE AGAINST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Vegetative Bacteria</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bacterial Spores</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Lipo Viruses</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hydrophilic viruses</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>Tubercle bacilli</td>
<td>+</td>
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<td>+</td>
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<td>HIV</td>
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<td>HBV</td>
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<tr>
<td>APPLICATIONS</td>
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<td></td>
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<tr>
<td>Contaminated liquid discard</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Contaminated glassware</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Contaminated instruments</td>
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<td>+</td>
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</tr>
<tr>
<td>Equipment total decontamination</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*These chemical disinfection methods are recognized by the National Institutes of Health, the CDC, or the American Biological Safety Association.

+ denotes very positive response

± denotes a less positive response

Blank denotes a negative response or not applicable.
Chapter 8: Biohazard Waste Management

The procedures for Biological Waste and Animal Tissue disposal at NC State 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.

All biohazard waste generated in NC State University or tenant research, diagnostic, and/or teaching laboratories must be properly treated prior to its disposal in designated red dumpsters. If treatment of waste is not an option complete an EH&S hazardous waste collection request. If a designated red dumpster is not available, notify EH&S at 919-515-7915.

Biohazard waste that requires treatment prior to disposal in designated red dumpsters includes unless otherwise determined in your approved Biological Use Authorization:

- Materials contaminated or potentially contaminated during the manipulation or clean-up of material generated during research, diagnostic, and/or teaching activities requiring biosafety level 1, 2, or 3 or animal or plant biosafety level 1, 2, or 3. 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 BSL2 and BSL3 agents.

Tissue, anatomical remains, and sharps containers (see below) require removal by EHS.

Refer to this quick reference chart regarding disposal practices of biohazard waste at NC State University.

Disposal practices for research involving whole animals

Appendix Q of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules specifies disposal practices for research involving whole animals where:

- the animal's genome has been altered by stable introduction of recombinant or synthetic nucleic acid molecules, or DNA derived therefrom, into the germ-line (transgenic animals); and/or
- experiments involving viable recombinant or synthetic nucleic acid molecule-modified microorganisms are tested on whole animals.

Appendix Q-I-B-1. When an animal covered by Appendix Q containing recombinant or synthetic nucleic acid molecules or a recombinant or synthetic nucleic acid molecule-derived organism is euthanized or dies, the carcass shall be disposed of to avoid its use as food for human beings or animals unless food use is specifically authorized by an appropriate Federal agency.

Appendix Q-I-B-2. A permanent record shall be maintained of the experimental use and disposal of each animal or group of animals.
Solid biohazard waste collection and handling procedures:

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

2. Collect BSL-1 and BSL-2 waste in red, hard-walled biohazard waste collection containers not to exceed 15 gallons and lined with a clear autoclavable bag. The lid must remain on the container when not in use (e.g., overnight, etc.). The lid and container each must bear the biohazard symbol with the “Biohazard”.

3. Autoclave bags must be clear and also have the biohazard symbol and the word “Biohazard” on the outside of the bag. Orange or red colored bags are not to be used. Each bag must be labeled with the first and last name of the Principal Investigator on the BUA and/or Safety Plan.

4. Bags must be removed from collection containers prior to being 2/3 full to allow headspace to seal the bag for transport to the autoclave. Never overfill biohazard waste collection containers. 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 (click the link for more information on hazardous waste requirements).

5. A log detailing autoclave performance verification must be completed on every load and maintained at the autoclave. See below for Autoclave Performance Verification details.

6. Place all items in heat-resistant secondary containers to secure and contain spills. Bags should be opened before autoclaving to insure sterilization.

**Autoclave Danger!**

No one should use an autoclave unless they have received recent instruction in procedure for the specific autoclave unit they are operating or are working under the direct supervision of a user experienced with the specific autoclave unit they are operating. Refer to the Manager’s Training Matrix within the Manager’s Safety Orientation Checklist for guidance in autoclave training.

**Autoclave Emissions**

Appropriate ventilation systems should be operating in areas where autoclaves are located. Avoid placing hazardous chemicals in the autoclave. For more information, read Hadar, Julia et al. 'Autoclave Emissions-Hazardous Or Not'. Journal of the American Biological Safety Association 2.3 (1997): 44-51 or contact the Biosafety Officer at EHS.
Unloading the 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.

1. Minimum PPE for unloading an autoclave include long-sleeved heat-insulating gloves (these gloves are compromised if wet or have holes), lab coat, eye protection, and proper shoes. A rubber apron and face shield may also be worn.

2. Ensure cycle has completed and both temperature and pressure have returned to a safe range.

3. Wearing 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.

4. 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.

5. Do not agitate containers of super-heated liquids or remove caps before unloading.

6. Wear PPE to 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.

7. Shut the autoclave door.

8. Allow autoclaved materials to cool to room temperature before transporting. Never transport superheated materials.

9. Any breakage of bags or leakage of contaminated materials should be reported to the laboratory director or supervisor at once for instructions on procedures for safe cleanup.

10. Reseal the bags with tape and remove from the building. Place in the designated red dumpster marked “Autoclaved” located near the rear of the building.

Autoclave performance verification

Compliance with state law dictates all biohazard waste at NCSU be treated for a minimum of 45 minutes at 121°C (250°F) at 15 psi. Each load of biohazardous waste processed in an autoclave must meet these operating conditions and be tested:

1. The operator will incorporate with each load a Chemical Integrator Test Pack (CITP), evaluate the performance of the autoclave based on color changed of the CITP; and document the results in a User Log. A sample Autoclave Use Log is available at the EHS website. All bags autoclaved with a failed CITP will be autoclaved again. 3M SteriGage Test Packs #41360 is currently the system accepted for this test.
2. Users should make sure that the autoclave is working properly before re-autoclaving. If the autoclave needs repair a tag "Out of Service" must be placed on the autoclave.

3. Monthly, a biological challenge will be performed with a standard load. The biological challenge needs to be incubated for 48 hours. Test results will be documented – date tested, initial of person doing test; test results.

Liquid biohazard waste for drain disposal

Liquid biohazard waste from a BSL-3 laboratory is autoclaved following lab-specific SOPs prior to disposal. Autoclaves in BSL-3 labs are validated weekly with biological indicators and a log is kept on-site per the North Carolina medical waste rules.

The preferred method for disinfecting rDNA, BSL-1 and BSL-2 liquid waste for drain disposal is autoclaving on the liquid cycle. If the liquid waste was used for propagating microbes, viral vectors, or toxins, chemical disinfection followed by drain disposal must be listed on your Biological Use Authorization for IBC approval.

Liquid biohazard waste treated in large quantities or in a bioreactor must follow the Procedure for Drain Discharge of Bioreactor Waste (click the link for the procedure).

Sharps waste collection and handling procedures:

Biohazard sharps waste at NC State is material used with rDNA, BSL-1, BSL-2, or BSL-3 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.

1. NC State labs collect biohazard sharps waste in labeled plastic sharps containers. The Wake County Landfill will not accept plastic sharps containers from NC State. To avoid injury, do NOT clip, bend, shear, or separate needles from syringes and do NOT recap needles.

2. When the container is ¾ full, cap it, autoclave as applicable, and complete an EH&S hazardous waste collection request. Do not overfill the biosharp container.

Mixed waste:

Mixed waste often requires special procedures. Please contact the EH&S Office for proper disposal procedures.

1. Mixed biological/chemical waste can be disinfected by using carefully selected chemical treatments only if compatible with the other chemicals in the experiment. Handle resulting waste as hazardous chemical liquid waste. Contact the EH&S office for advice on avoiding adverse chemical reactions.

2. Treat animal or human tissue in 10% formalin waste as liquid chemical waste and label the hazardous waste tag “10% formalin + non-infectious animal
tissue” or “10% formalin + non-infectious human tissue.”

3. Disinfect biologically contaminated radiological solid waste by soaking in a suitable disinfectant. Discard disinfectant waste in designated and posted sink if radiological contamination is within sink disposal limits.

4. Disinfect iodinated liquid waste with a phenolic disinfectant; e.g., Lysol™. Disinfect all other liquid waste with bleach (10% final concentration with 5.25% sodium hypochlorite.) If the waste is within radiological sink disposal limits, dispose of in designated and posted sink. If levels are above sink disposal limits, then package for hazardous waste collection and submit an online request for radiation/chemical waste pick-up.
Chapter 9: Emergencies and Incident Reporting

Each laboratory with an IBC-approved Biological Use Authorization is required to have an Emergency Plan that includes documentation how to cease, terminate and secure the laboratory in case of a lab or campus emergency; a spill response plan specific to the agents used in the lab; and reporting requirements to Environmental Health and Public Safety in case of incident. The Emergency plan should be posted in the laboratory and reviewed by all lab occupants on an annual basis. The Principal Investigator shall keep written records to document that all occupants have been trained in the Plan.

A biosafety incident or “release” is either an exposure to personnel or an event that results in the discharge of a bioagent outside the primary containment barrier. The event can be the result of a splash or spill of infectious material or a failure of the containment system.

All spills, releases, or accidents involving materials registered on a Biological Use Authorization, regardless of how minor the event or how remote the location, must be reported according to the NC State Accident Report Form Flowchart. For questions, contact the Biological Safety Officer at 919-515-6858.

An incident should be reported even if there is no obvious exposure resulting from the release. Laboratory acquired infections are often not associated with an apparent exposure. EHS recommends posting this diagram as a visual reminder of the appropriate steps if a concern arises regarding exposure to biological material at NC State University. It is possible that lab workers exposed to volumes and concentrations of microorganisms not typically found in nature can present with atypical signs and symptoms.

As a minimum, each Emergency Plan should include the following if applicable to the respective laboratory:

**Injury, Medical Emergency, Animal Bite**

**OBTAIN MEDICAL ATTENTION**
- For serious medical emergencies day or night, dial 911.
- Minor injuries -- employees: notify your supervisor.
- Minor injuries -- students: notify supervisor and report to Student Health Services.

**HAZARDOUS MATERIAL ON SKIN OR SPLASHED IN EYE**
- Remove contaminated clothing, shoes, jewelry, etc.
- 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.
- Immediately after rinsing, see above OBTAIN MEDICAL ATTENTION.
- After emergency telephone notifications are made, report the incident using the First Report of Injury form and notify the Biosafety Officer at 919-515-6858.
NEEDLESTICK OR CUT WITH CONTAMINATED SHARP ITEM

- Immediately wash the area with soap and water for at least 15 minutes.
- Immediately after rinsing, see above OBTAIN MEDICAL ATTENTION.
- If sharps or needles are used in the lab, post the needle stick protocol as an addendum to the Emergency Plan (see http://www.ncsu.edu/ehs/accidents/needleStickInjuries.pdf)

INJURY INVOLVING RESEARCH ANIMAL

- BITE/SCRATCH/CUT: wash the area with soap and water for at least 15 minutes.
- Immediately after rinsing, see above OBTAIN MEDICAL ATTENTION.
- As soon as practicable, notify the animal facility manager who will help complete the First Report of Injury form. Notify the Biosafety Officer at 919-515-6858.

ASSISTING IN MEDICAL EMERGENCY OR PERSONAL INJURY

- Dial 911 from a campus phone or cell phone.
- Do not move injured person unless there is a danger of further harm from remaining in the location. If the area is unsafe, then evacuate, close doors to area, and prevent access. Provide information to emergency responders.
- Remain with the injured person until medical assistance arrives. Initiate life-saving measures if necessary and if you have received appropriate training.

Spill procedures for biohazardous material

If the spill cannot be handled safely by laboratory employees with available absorbents and disinfectants, notify your supervisor and/or dial 911 to notify University Police for assistance.

SPILL INSIDE BIOSAFETY CABINET:

1. Contain spill with absorbent paper.
2. Dampen paper with disinfectant. Allow to stand for 20 minutes.
3. If sharps/glass are present, use mechanical means to collect the waste (e.g. forceps, cardboard flaps).
4. Remove gloves after area is decontaminated.
5. Wash hands.

LARGE SPILL INSIDE BIOSAFETY CABINET:

1. If splash has occurred outside the cabinet resulting in personnel exposure to infectious material, the Principal Investigator and EH&S (919-515-7915) should be notified immediately and the need for prophylactic treatment or other medical attention determined.
2. Contaminated clothing should be removed and containerized for autoclaving.
3. Thoroughly wash hands and face, if exposure has occurred.
4. Remove gloves after area is decontaminated
5. Chemical decontamination procedures should be initiated at once while the cabinet continues to operate to prevent escape of contaminants from the cabinet.
6. Spray or wipe walls, work surfaces, and equipment with appropriate disinfectant.
7. Flood top tray, drain pans, and catch basin below work surfaces with disinfectant and allow to stand 20 minutes.
8. Dump excess disinfectant from tray and drain pans into cabinet base.
9. Lift out tray and removable exhaust grille work. Wipe off top and bottom (underside) surfaces with disinfectant sponge or cloth. Replace in position.
10. Gloves, cloth or sponge should be discarded in an autoclave pan and autoclaved.
11. Drain disinfectant from cabinet base into an appropriate container and autoclave.
12. Remove gloves and wash hands.
13. This procedure does not decontaminate the interior parts of the cabinet such as the filters, blowers, and air ducts. If the entire cabinet is to be decontaminated with toxic gas refer, contact the Biosafety Officer at 919-515-6858.

**SPILL OUTSIDE BSC:**
1. Decontaminate and/or remove all personnel, clothing and exit laboratory.
2. Wash hands and any exposed skin thoroughly.
3. Alert others in the area. Notify PI and 911 if assistance is required.
4. If necessary, allow aerosols to settle for 30 minutes.
5. Re-enter wearing PPE (gloves, lab coat, and eye/face protection).
6. Cover spill with paper towels and carefully pour disinfectant, e.g., 10% bleach, around and over the spill from outside edges.
7. Allow contact time for disinfectant (e.g. 10% bleach for 20 mins).
8. Clean-up with paper towels. Pick up sharp items, e.g., broken glass or needles, with forceps or dust pan and brush and place in a sharps container.
9. Decontaminate or dispose of clean-up materials in biohazard bag.
10. Remove contaminated PPE and wash hands.

**Reporting Instructions**

All spills, releases, or accidents involving materials registered on a Biological Use Authorization, regardless of how minor the event or how remote the location, must be reported according to the NC State [Accident Report Form Flowchart](http://www.ncsu.edu/ehs/accidents/accinv1.htm#report). For questions, contact the Biological Safety Officer at 919-515-6858.

Report all other injuries, accidents, animal bites, and exposures according to standard practices as outlined in the [Accident Report Form Flowchart](http://www.ncsu.edu/ehs/accidents/accinv1.htm#report) beginning with the First Report of Injury form. All relevant forms may be found at [http://www.ncsu.edu/ehs/accidents/accinv1.htm#report](http://www.ncsu.edu/ehs/accidents/accinv1.htm#report).
Chapter 10: Shipping Biological Materials

Transporting Biological Materials

The US Department of Transportation is vigilantly reviewing companies, carriers, and academic settings for compliance in packaging and shipping hazardous materials. It is the responsibility of individual shippers to properly identify their material and package it accordingly. The latest information on shipping biological materials is available on the EHS website for Hazardous Materials Shipping Program.

If you are transporting the materials yourself in a vehicle, even across campus:
- Notify EHS at 919-515-2895 for training prior to initial transport
- Use a University Vehicle
- Collecting specimens? Don’t contaminate the vehicle.
- Check packaging: sealed secondary container? Biohazard label on the outside?
- Depending on volume, a spill clean-up kit may be required.

Training

Most biological materials require specific packaging, labeling, and documentation. Infectious materials (materials containing or expected to contain pathogens affecting humans) are regulated by the US Department of Transportation (DOT) and the International Air Transport Association (IATA). You must complete a hazardous materials shipping training course to be certified to ship infectious biological materials. This training is also required to be able to properly identify your materials according to DOT and IATA guidelines.

Click here for the EH&S biological material shipping training.

Individuals receiving packages should be familiar with this information on Suspicious Packages (click link)

Import and Transfer Permits

Some biological materials require a permit to be imported or transferred to another institution outside of NC State University. 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), Etiological Agent Import and Interstate Transfers. This permit applies to the etiological agents themselves, unsterilized biological material (ex: patient samples) containing an etiological agent, and animals that could be a host or vector of disease in humans.

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. For more information, review the [Guide to USDA Animal and Plant Health Inspection Service (APHIS) Permits](https://animalplantinspection.usda.gov/permits).


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)](https://www.fda.gov) 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.

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.

**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 review the Export Controls information found on the SPARCS web page at [http://www.ncsu.edu/sparcs/export/index.html](http://www.ncsu.edu/sparcs/export/index.html). Filing for export control license applications can take several weeks so identify any possible licenses you will need well in advance of your planned shipping date.

**Select Agent Transfers**

All movements of Select Agents need to be approved and documented even if it is within the University. Contact EH&S if you are considering bringing in a Select Agent, shipping one outside of the University, or moving one from one location on campus to another.
Chapter 11: General Biosafety References

American Biological Safety Association  Biosafety Links

Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), National Institutes of Health, March 2013

Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition, Centers for Disease Control and Prevention, National Institutes of Health, February 2007

Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, BMBL Appendix A, Centers for Disease Control and Prevention, National Institutes of Health

Federal Select Agent Program, Animal and Plant Health Inspection Service (APHIS) and the Centers for Disease Control and Prevention (CDC)

Select Agents and Toxins List

Bloodborne Pathogens Standard CFR 1910.1030, Occupational Safety and Health Administration, U.S. Department of Labor

NC Medical Waste Management Rules, North Carolina Division of Waste Management

North Carolina Biological Agents Registry, North Carolina Department of Health and Human Services

NC Department of Agriculture & Consumer Services, Plant Protection Section