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Chapter 1 - Guidance, Regulations and Roles of Key Personnel

This Biosafety Manual (BSM) provides a review of pertinent federal guidance and regulations, state regulations, information about safe work practices, safety equipment and personal protective equipment, and specific guidance for research with recombinant DNA (rDNA). Clemson’s Biological Safety Program is based on the premise that every member of the research community shares the responsibility for safety.

US Center for Disease Control's (CDC) *Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition* is the key guidance document for Biosafety. Although BMBL5 only recommends practices (“We wish to emphasize that the 5th edition of the BMBL remains an advisory document recommending best practices for the safe conduct of work in biomedical and clinical laboratories from a biosafety perspective, and is not intended as a regulatory document though we recognize that it will be used that way by some.” BMBL5 Forward), unless otherwise specifically stated herein, **compliance with all BMBL5 recommendations is mandatory for all laboratory biological activities (research, teaching, public service).**

www.cdc.gov/biosafety/publications/bmbl5/

*NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* is also a key guidance document for this manual. Whereas BMBL5 recommends practices, NIH Guidelines specifies practices; “The purpose of the NIH Guidelines is to specify practices...” (NIH Guidelines Section I-A. Purpose). Although these NIH Guidelines closely follow the recommendations of BMBL5, in some cases the guidelines are more restrictive. **In certain cases, this manual will adopt the more restrictive practices of the Guidelines, regardless of whether rDNA (recombinant DNA) is involved.** The advent of multi-PI labs precludes separate consideration of BMBL5 and the Guidelines. In such cases, the specific guideline will be referenced and explained. [https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf](https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf)


The Institutional Biosafety Committee (IBC) reviews, approves and oversees rDNA research to ensure compliance with the NIH Guidelines, determines necessity of health surveillance of personnel, sets biosafety containment levels as required by the Guidelines, ensures proper training for IBC members, staff, and PIs, and reports any significant problems, violations or significant research-related accidents or illnesses to NIH within 30 days. See Appendix F.

The Infection Control Committee manages the infectious waste stream from generation until offered for transport. See Chapter 9.

The Biological Safety Officer (BSO) must: conduct lab inspections; develop emergency and reporting procedures; investigate lab accidents; report recombinant DNA incidents, violations of the Guidelines to the IBC; provide general biosafety training.

The Principal Investigator (PI) must: be proficient in good microbiological techniques; supervise staff to ensure safety practices are followed; instruct laboratory staff on the risk of agents used in the lab, safe work practices, emergency procedures for spills and exposures, and the reasons for vaccinations and serum collection, when applicable. The PI must ensure that: laboratory workers undergo required training, proper biosafety, biowaste, and shipping/transport procedures are followed by staff; lab-specific SOPs are developed and followed for spills, exposure, loss of containment as needed, and reporting research-related accidents and illnesses; biological containment is maintained; unsafe work practices are corrected.
Chapter 2- Biological Risk Assessment

Risk assessment is a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person’s exposure to an agent, the likelihood that such exposure will cause a laboratory acquired infection (LAI), and the probable consequences of such an infection. The information identified by risk assessment will provide a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs. There are four primary resources to assist in the risk assessment process.

The primary factors to consider in risk assessment and selection of precautions fall into two broad categories: agent hazards and laboratory procedure hazards. The agent summary statements contained in BMBL5 identify the primary agent and procedure hazards for specific pathogens and recommend precautions for their control. A review of the summary statement for a specific pathogen is the starting point for assessment of the risks of working with that agent and those for a similar agent. Please see section VII of the BMBL:

www.cdc.gov/biosafety/publications/bmbl5/

The principal hazardous characteristics of an agent are: its capability to infect and cause disease in a susceptible human or animal host, its virulence as measured by the severity of disease, and the availability of preventive measures and effective treatments for the disease. The NIH Guidelines assigns human etiological agents into four risk groups on the basis of hazard. Agents are classified into four Risk Groups (RGs) according to their relative pathogenicity for healthy adult humans by the following criteria: Risk Group 1 (RG1) agents are not associated with disease in healthy adult humans. Risk Group 2 (RG2) agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available. Risk Group 3 (RG3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available. Risk Group 4 (RG4) agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.


The American Biological Safety Association also maintains a risk database: https://my.absa.org/Riskgroups

The Public Health Agency of Canada maintains Pathogen Safety Data Sheets on infectious agents:

BL 2 practices and containment must be followed when handling human materials that may contain bloodborne pathogens, e.g. HBV, HCV and HIV. The OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030) applies to all occupational exposure to blood or other potentially infectious materials. Under the OSHA BBP Standard employers are required to develop a written Exposure Control Plan, offer employees the hepatitis B vaccination, and provide annual training. For more information on the OSHA BBP standard see the Clemson University Exposure Control Plan:
http://media.clemson.edu/research/safety/Exposure%20Control%20Plan%20for%20Clemson%20University%20Research.pdf

OSHA considers both primary and established human cell lines to potentially contain bloodborne pathogens unless tests have shown them to be free of BBP. Cultured cells that are known to contain or be contaminated with a biohazardous agent (e.g. bacteria or viral) are classified in the same biosafety level as the agent. All cell and organ cultures of human origin, including well established cell lines, human bodily fluids, and non-human primate cells and tissues should be handled in accordance with the OSHA Bloodborne Pathogens Standard and under Biosafety Level 2 (BL2) containment unless shown to be free of infectious agents.

Researchers working with other mammalian cell lines and tissues should do a risk assessment specific for their sample of study. For animal cell culture, risk is defined as the likelihood that disease will be transmitted to humans. To evaluate this risk, consider the following: type of sample, source of sample, how well characterized the sample is, if there was any intentional exposure of the sample to a pathogen, and possible
zoonotic disease transmission. For help determining this risk, contact the Biosafety Officer; kkwist@clemson.edu.

The end product of risk assessment is the assignment of an appropriate biosafety level (BL or BSL) for an experiment. BL's will be discussed in detail in Chapter 4. The initial risk assessment from the preceding resources should be followed by a thorough consideration of how the agent is to be manipulated. Factors to be considered in determining the BL include agent factors such as: virulence, pathogenicity, infectious dose, environmental stability, route of transmission, communicability, operations, quantity, availability of vaccine or treatment, and gene product effects such as toxicity, physiological activity, and allergenicity. Any strain that is known to be more hazardous than the parent (wild-type) strain should be considered for handling at a higher BL. Certain attenuated strains or strains that have been demonstrated to have irreversibly lost known virulence factors may qualify for a reduction of the containment level compared to the RG assigned to the parent strain.

A final assessment of risk based on these considerations is then used to set the appropriate BL for the experiment. The containment level required might be equivalent to the RG classification of the agent or it might be raised or lowered as a result of the above considerations. The Institutional Biosafety Committee must approve the risk assessment and the BL for recombinant DNA.

Chapter 3- Principles of Biosafety

A fundamental objective of any biosafety program is the containment of potentially harmful biological agents. The term “containment” is used in describing safe methods, facilities and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents. The most important element of containment is strict adherence to standard microbiological practices and techniques. Knowledge of standard microbiological practices and techniques is gained through a combination of training and experience.

Appropriate facility design and engineering features, safety equipment, and management practices must supplement laboratory safety practices and techniques. Safety equipment includes biological safety cabinets (BSCs), enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The BSC is the primary barrier used to provide containment of infectious droplets or aerosols generated by many microbiological procedures. BSCs are discussed in Appendix A. An example of another primary barrier is the safety centrifuge cup, an enclosed container designed to prevent aerosols from being released during centrifugation. To minimize aerosol hazards, containment controls such as BSCs or centrifuge cups must be used when handling infectious agents.

Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Personal protective equipment (PPE) is often used in combination with BSCs and other devices that contain the agents, animals, or materials being handled. In some situations, in which it is impractical to work in BSCs, personal protective equipment may form the primary barrier between personnel and the infectious materials. Examples include certain animal studies, animal necropsy, agent production activities, and activities relating to maintenance, service, or support of the laboratory facility.

The design and construction of the facility provides a secondary barrier to protect persons outside the laboratory. PI's are responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated. The recommended secondary barrier(s) will depend upon the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in BSL-1 and BSL-2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave) and hand washing facilities.
Chapter 4- Laboratory Biosafety Level Criteria

Four biosafety levels (BLs or BSLs) are described in BMBL5; each consists of a combination of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and the laboratory function or activity. The BLs described in BMBL5 should be differentiated from Risk Groups, as described in the NIH Guidelines and the World Health Organization Laboratory Biosafety Manual. Risk groups are the result of a classification of microbiological agents based on their association with, and resulting severity of, disease in humans. The risk group of an agent should be one factor considered in association with mode of transmission, procedural protocols, experience of staff, and other factors in determining the BL in which the work will be conducted.

The recommended biosafety level(s) for the organisms in BMBL5 Section VIII (Agent Summary Statements) represent those conditions under which the agent ordinarly can be safely handled. Not all of the organisms capable of causing disease are included in Section VIII; the other resources listed in Chapter 2 of this manual must be utilized to perform risk assessments for agents not listed in Section VIII. Generally, work with known agents should be conducted at the biosafety level recommended in Section VIII. When information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent practices may be specified. Often an increased volume or a high concentration of agent may require additional containment practices.

**Biosafety Level 1** practices, safety equipment, and facility design and construction are appropriate for undergraduate training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. *Bacillus subtilis, Naegleria gruberi, infectious canine hepatitis virus, and exempt organisms under the NIH Guidelines are representative of microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Vaccine strains that have undergone multiple *in vivo* passages should not be considered avirulent simply because they are vaccine strains. BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing.

**Biosafety Level 2** practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. It is recommended that work with these organisms be done in a Biosafety Cabinet (BSC). In certain cases, work may be done on the open bench with approval from the Biosafety Officer and the potential for producing splashes or aerosols is low. Hepatitis B virus, HIV, the *Salmonella,* and *Toxoplasma* are representative of microorganisms assigned to this containment level. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Laboratory personnel working with human- derived materials should refer to the OSHA Bloodborne Pathogen Standard for specific required precautions). Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or centrifuges with safety cups. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves. Secondary barriers, such as hand washing sinks and waste decontamination facilities, must be available to reduce potential environmental contamination.

**Biosafety Level 3** practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection.
Mycobacterium tuberculosis, St. Louis encephalitis virus, and Coxxiella burnetii are representative of the microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At BSL-3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

**Biosafety Level 4** is beyond the scope of this manual. Standard and special practices, safety equipment, and facility requirements applicable to each BL are detailed in: [http://www.cdc.gov/biosafety/publications/bmbl5/bmbl5_sect_iv.pdf](http://www.cdc.gov/biosafety/publications/bmbl5/bmbl5_sect_iv.pdf)

**Multi-User Research Labs**

The advent of large open labs (Biosystems Research Complex (BRC)) and lab suites (Life Science Facility (LSF)) creates problems for separation of BL1 and BL2 areas and activities. The following **NIH Guidelines**, which shall apply to all biological activities at Clemson, make a multi-PI lab problematic.

- **For BL1**, Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress. [Appendix G-II-A-1-a]
- **For BL2**, Access to the laboratory is limited or restricted by the Principal Investigator when experiments are in progress. [Appendix G-II-B-1-a]
- **For BL2**, Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory. [Appendix G-II-B-1-h]
- **Any research group working with agents that are known or potential biohazards shall have an emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment.** The Principal Investigator shall ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan.” [Appendix G-I. Standard Practices and Training]
- The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms. [Appendix G-II-B-2-c]
- For BL2, Laboratory coats, gowns, smocks, or uniforms and safety glasses are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory. [Appendix G-II-B-2f]

Personnel in the student desk area of the BRC may be exempt from the requirement to wear lab coats and safety glasses depending on the work in their area. Contact Research Safety with questions for particular situations.

The BSO shall work with the occupants of each multi-PI lab to determine the most effective way of complying with the cited Guidelines for each lab. The default solution is to designate the entire lab BL2, with clearly demarcated BL1 section(s) as required. Note that BL2 waste must be kept in the BL2 area until decontaminated. Also, all Risk Group 2 agents must remain secure (under the control of the researcher using the agent only). The highlighted training requirement above will be met by maintaining a notebook containing a Pathogen Safety Data Sheet (PSDS) for each BL2 agent in use. See [http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php](http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php)

**Chapter 5- Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities**

*BMBL5* provides guidance for the use of experimentally infected animals housed in indoor research facilities (e.g., vivaria), and is also useful in the maintenance of laboratory animals that may naturally harbor zoonotic
infectious agents. Laboratory animal facilities are a special type of laboratory. As a general principle, the biosafety level (facilities, practices, and operational requirements) recommended for working with infectious agents *in vivo* and *in vitro* are comparable.

The animal room can present unique problems. In the animal room, the activities of the animals themselves can present unique hazards not found in standard microbiological laboratories. Animals may generate aerosols, they may bite and scratch, and they may be infected with a zoonotic agent. The co-application of Biosafety Levels and the Animal Biosafety Levels are determined by a protocol-driven risk assessment.

Facilities for laboratory animals used in studies of infectious or non-infectious disease should be physically separate from other activities such as animal production and quarantine, clinical laboratories, and especially from facilities providing patient care. Traffic flow that will minimize the risk of cross contamination should be incorporated into the facility design.

The recommendations detailed in *BMBL* Section V describe four combinations of practices, safety equipment, and facilities for experiments with animals involved in infectious disease research and other studies that may require containment. These four combinations, designated Animal Biosafety Levels (ABSL) 1-4, provide increasing levels of protection to personnel and to the environment, and are recommended as minimal standards for activities involving infected laboratory animals. The four ABSLs describe animal facilities and practices applicable to work with animals infected with agents assigned to Biosafety Levels 1-4, respectively. Investigators that are inexperienced in conducting these types of experiments should seek help in designing their experiments from individuals who are experienced in this special work.

In addition to the animal biosafety levels described in Section V, the USDA has developed facility parameters and work practices for handling agents of agricultural significance. Appendix D of *BMBL* includes a discussion on Animal Biosafety Level 3 Agriculture (BSL-3-Ag). USDA requirements are unique to agriculture because of the necessity to protect the environment from pathogens of economic or environmental impact. Appendix D also describes some of the enhancements beyond BSL/ABSL-3 that may be required by USDA-APHIS when working in the laboratory or vivarium with certain veterinary agents of concern.

Animal facility standards and practices are detailed in section V of the BMBL: https://www.cdc.gov/labs/BMBL.html

Care of research animals is the responsibility of the Institutional Animal Care and Use Committee. http://www.clemson.edu/research/compliance/iaucu/

**Chapter 6- Select Agents/ Toxins and Principles of Laboratory Biosecurity**

Select Agents are federally regulated agents that have potential use in biological warfare. Health and Human Services (HHS) regulates select agents targeting humans, the United States Department of Agriculture (USDA) regulates select agents targeting animals, and the USDA Plant Protection and Quarantine (PPQ) regulates select agents targeting plants. Before possessing, using, sending, or receiving select agents, the institution and Principal Investigator must register with CDC, APHIS, and/or USDA to receive official authorization for each individual requesting access to select agents. Requirements include background checks on those authorized to access select agents, security plans and inventories. Immediately notify the BSO if you discover select agents in your laboratory that have not been registered. View the Select Agent/Toxin List here: https://www.selectagents.gov/SelectAgentsandToxinsList.html

Anyone desiring to work with these materials must have security clearance to do so and have special security procedures in place for their labs. Investigators wishing to work with any of these agents MUST contact Tami Hemingway (the duly authorized responsible official) prior to doing so, and all security clearances and procedures must be in place prior to requesting the Select Agents (note that requests for Select Agents MUST be placed by the Responsible Official). Contact Tami Hemingway Phone: 864-656-4084 Fax: 864-656-4475 E-mail: theming@clemson.edu for assistance with Select Agent requirements.
The term “biosecurity” has multiple definitions. In the animal industry, the term biosecurity relates to the protection of an animal colony from microbial contamination. In some countries, the term biosecurity is used in place of the term biosafety. For the purposes of this section the term “biosecurity” will refer to the protection of microbial agents from loss, theft, diversion or intentional misuse. This is consistent with current WHO and American Biological Safety Association (ABSA) usage of this term.

Biosafety and biosecurity programs share common components. Both are based upon risk assessment and management methodology; personnel expertise and responsibility; control and accountability for research materials including microorganisms and culture stocks; access control elements; material transfer documentation; training; emergency planning; and program management. Biosafety and biosecurity program risk assessments are performed to determine the appropriate levels of controls within each program. Biosafety looks at appropriate laboratory procedures and practices necessary to prevent exposures and occupationally-acquired infections, while biosecurity addresses procedures and practices to ensure that biological materials and relevant sensitive information remain secure.

A detailed discussion of a lab biosecurity program may be viewed in section IV of the BMBL at:
https://www.cdc.gov/labs/BMBL.html

LABS MUST BE KEPT LOCKED WHEN NOT IN USE.

Chapter 7- Medical Surveillance

A medical surveillance program is provided through the Sullivan Center for personnel who are occupationally at risk of exposure to bloodborne pathogens (BBP) and/or direct contact with research animals. The program includes free hepatitis B vaccine offer and post-exposure evaluation with follow up. See
https://www.clemson.edu/cbshs/centers-institutes/sullivan/patients/services/medical-surveillance.html

Vaccination for various infectious agents used in the laboratory, e.g. vaccinia, attenuated rabies, and measles, is offered to all at-risk employees as recommended by the IBC. Clemson provides medical treatment and post-exposure evaluation after an exposure to infectious agents or rDNA for students and employees working in BL1 or BL2 laboratories.

Chapter 8 Emergencies and Reporting

Spills
In the event of a large spill or if lab personnel is not comfortable cleaning up the spill, evacuate the area and call 911 for HazMat response. However, many spills can be effectively handled by researchers and items to clean up a spill should be available in any laboratory area where biohazardous materials are used or stored. The following are recommended procedures for evaluating and handling a spill:

- An appropriate disinfectant that works against the BSL-2 agents of concern must be used.
- Allow sufficient contact time for the disinfectant to work. Follow manufacturer’s directions.
- Procedures vary with location depending upon if inside or outside of a containment device.
- Cleaning spills requires personal protective equipment (PPE) including a lab coat or gown, safety glasses, and gloves. A face shield, shoe covers or a respirator may be required.

NOTE: All spills must be reported to the Laboratory Supervisor and/or Principal Investigator. Contact the Biosafety Safety Officer (kkwist@clemson.edu or 864-656-7686) for any questions about spill cleanup procedures.
Spills outside of a containment device
If the spill is not inside of a Biological Safety Cabinet (BSC), Centrifuge, Refrigerator, Incubator, Freezer, Lab instrument etc.

1. Close off spill area to traffic and notify coworkers.
2. If the spill may involve an aerosol, (e.g. event involving dropping material onto floor, high mechanical force, a forceful expulsion of liquid) leave the room for 30 minutes to allow aerosols to settle.
3. Remove contaminated lab coat or clothing and wash exposed skin.
4. Make sure proper PPE is worn (gloves, safety glasses, lab coat).
5. Prepare enough volume of a 1:9 dilution of chlorine bleach or other approved disinfectant to saturate the contaminated area. If dilution is not possible, undiluted household bleach can be used but take special care when using to prevent splashes to skin or clothing.
6. Contain the spill with paper towels or other absorbent material e.g. "bench kote", blue "diaper pads"
7. Flood the spill area with disinfectant. Leave on for 10 minutes.
8. Push the absorbent material at the edge of the spill into the spill’s center. Add more paper towels as needed. If glass is present, do not use bare hands! Use tongs (large pieces) forceps (small pieces) followed by a dustpan to remove pieces. Place the paper towels and gloves in a Zip-lock bag and discard bag as hazardous waste. If contact with bleach occurs with skin, mucous membranes or eyes, flush area with copious amounts of water.
9. Wash hands thoroughly. Autoclave any overtly contaminated lab coat to prior to placing into laboratory laundry bag.
10. Report incident to supervisor and Principal Investigator.

Spills inside of a Biological Safety Cabinet (BSC)
1. Leave BSC on.
2. Make sure proper PPE is worn (gloves, safety glasses, lab coat) and gather paper towels for cleaning.
3. Prepare a fresh dilution of 1:9 bleach or approved disinfectant. Prepare enough solution to cover the entire contaminated area. Always follow manufacturer's directions. Do not use 70% ethanol for human materials as it evaporates too quickly to allow adequate surface contact time.
4. Flood the area with disinfectant solution and allow to remain in contact for required time: 10% Bleach for 10 minutes; or, Cavicide (full strength) for 3 minutes; or, Beaucoup (diluted according to manufacturer's directions) for 10 minutes.
5. Spray and or wipe down the cabinet interior and any items inside the BSC with a towel dampened with disinfectant.
6. Place the paper towels and gloves in a Zip-lock bag and discard bag as hazardous waste.
7. Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination; consult BSO immediately at (kkwist@clemson.edu), for guidance. If a spill such as this has occurred, do not turn the BSC off.
8. Report incident to supervisor and Principal Investigator.

Spills in a centrifuge
Biohazardous spills in centrifuges can be quite difficult to disinfect. Some but not all centrifuges have closed rotors, buckets or other carriers with leak proof lids, designed to contain spills and allow efficient, safe emptying and decontamination. However, not all centrifuges are equipped with these containment devices. A spill resulting from primary container breakage requires immediate suspension of use. PI notification and assistance from the Biological Safety Officer.

If unusual sounds from a centrifuge suggest that breakage and a spill has occurred, or, if breakage and a spill is discovered after the machine has stopped, wait at least 30 minutes after centrifuge has stopped before opening. This will allow hazardous aerosols to settle in the centrifuge.

1. Don lab coat, gloves, and face shield prior to opening centrifuge and then open carefully to assess the situation. Use of a respirator is recommended and double gloving is advisable if glass tubes were used and broken.
2. Attempt to determine if the spill is contained in a closed cup, bucket or tray carrier, or within a closed
rotor.

3. If the spill is contained as described in (2), spray the exterior with disinfect and allow adequate contact time. Take the carrier to the nearest BSC approved for use with this agent. NOTE: If a BSC is not available or if the rotor cannot be removed, the centrifuge should remain closed. Post a sign indicating "contaminated-do not use”. Notify lab director and or PI and contact BSO at (kkwist@clemson.edu or 864-656-7686), for assistance.

4. Obtain and place into the BSC containers suitable for holding tubes, broken glass or other containers while cleaning centrifuge components.

5. Carefully retrieve unbroken tubes, wipe outside with disinfectant, and place them into the other empty container in the BSC, out of the way. The broken glass tube(s) must be removed with a forceps or other instrument and immersed in a beaker of disinfectant solution for a time appropriate to achieve disinfection. The pieces can then be disposed of in a sharps container.

6. After proper decontamination, carriers, rotors etc. can be washed with a mild detergent according to the manufacturer's instructions.

7. Thoroughly wipe the inside of the centrifuge chamber with disinfectant saturated towels. Allow for adequate contact time before wiping up excess liquid. Place the paper towels and gloves in a ziplock bag and discard as hazardous waste.

Biological/Radiological emergencies/spills

Radiation Safety Officer (kpovod@clemson.edu or 864-656-3516) must be notified and will assist in the cleanup of a biological/radiological spill. Determine if anyone has been contaminated; remove contaminated clothing and wash contaminated skin with soap and water. Proceed with clean up as instructed by the Radiation Safety Officer. The infectious agent will be deactivated first, taking care in choosing a disinfecting agent to avoid chemical incompatibility. Chlorine compounds such as bleach must NOT be used to disinfect anything containing I-125 because the chlorine will cause the volatilization of radioactive iodine.

Exposure to Biohazardous Agents or Material

An exposure is defined as: BSL-2 agent contact with broken skin, eyes, nose, mouth, other mucous membranes, a percutaneous injury with a contaminated sharp, or contact with an infectious agent over a large area of apparently intact skin. Individuals are encouraged to report any conditions that may increase risk or consequences of a laboratory acquired infection (e.g. pregnancy, a medical condition which compromises immunity) to their PI.

In the event of exposure:

1. If the exposure creates a medical emergency, go to the closest emergency medical center or call 911.
2. Wash the area with soap and water or flush eyes, nose or mouth with large amounts of water for 15 minutes.
3. Students not employed by the university should report to Redfern during business hours for personal exposures. During off hours, an outpatient facility may be used if necessary.
4. All exposures must be reported to the immediate supervisor and Principal Investigator when able. The Principle Investigator is responsible for calling CorVel (1-866-282-2674) as soon as possible. They also should contact Risk Management (864-656-3365) and Research Safety (864-656-0351) and report the exposure. CorVel will instruct the PI as to where the exposed employee should report.
5. Principal Investigators are responsible for reporting exposure incidents to Biosafety Officer.
6. The Biosafety Officer will perform a follow-up investigation of the incident and report findings to the Institutional Biosafety Committee (IBC).

Security incidents

Security incidents such as suspicious visitors, missing chemicals, or missing biological agents must be promptly reported to the Principal Investigator. University Security or Police should be notified. Principal Investigators are responsible for reporting incidents to the Biosafety Officer.

Chapter 9 Disinfection, Sterilization, Incineration and Waste Management
CLEMSON UNIVERSITY INFECTION CONTROL PROTOCOL

This protocol was developed by the Infection Control Committee (ICC Chair-Kerri Kwist, Biosafety Officer) and reviewed by the Institutional Biosafety Committee (IBC Chair- Dr. James Morris). It shall be reviewed annually by the ICC; all changes should be reviewed by the IBC.

SCOPE

The Scope of the Infection Control Protocol is defined by both regulatory and safety requirements. The requirement for an Infection Control Committee comes from SC Regulation 61-105 (R61-105), South Carolina Infectious Waste Management (June 2010): http://www.scdhec.gov/environment/lwm/regs/R61-105.pdf According to the regulation:

Each generator must have a designated infection control committee with the authority and responsibility for infectious waste management. This committee must develop or adopt a written protocol to manage the infectious waste stream from generation until offered for transport.

This protocol has been expanded beyond the narrow definition of infectious, which is limited to human pathogens, and addresses the management of biohazardous waste, defined below. This management protocol applies to activities at all main campus facilities. Off campus biohazardous waste is dealt with separate permits, if applicable.

Biohazardous waste is waste that requires inactivation of the biological material in an approved manner prior to final disposal. It includes the following:

- **Sharps Waste.** All hypodermic needles, syringes with needles attached, IV tubing with needles attached, scalpel blades, broken glass contaminated with a biohazard and lancets that have been removed from the original package.
- **Cultures and Stocks of Etiologic Agents and Associated Biologicals.** This includes, but is not limited to, specimen cultures, cultures and stocks of etiologic agents and agents requiring biosafety level (BSL) 1, 2 and 3 containment, wastes from production biologicals and serums, and discarded live and attenuated vaccines.
- **Human Pathological Waste.** This includes human tissues and anatomical parts that emanate from surgery, obstetrical procedures, autopsy, teaching and research laboratories. This does not include extracted teeth, hair, toenails, fingernails, human corpses, remains and anatomical parts that are intended for interment or cremation.
- **Human Body Fluids.** This includes, but is not limited to, blood and blood products, serum and plasma, cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, and amniotic fluid.
- **Infected Human Body Substances.** This includes wastes that have come into contact with human body fluids or tissues from humans infected with, or isolated to protect others from, highly communicable infectious diseases.
- **Non-Human Primate Waste.** This includes, but is not limited to, non-human primate blood, carcasses, tissues, body fluids, and bedding.
- **Animal Waste.** This includes, but is not limited to, animal carcasses, body parts, bedding of animals, and cell lines from mammals. These samples may or may not be infected with pathogenic microorganisms
- **Recombinant/Synthetic DNA.** Recombinant or rDNA is formed by combining genetic material from multiple sources. rDNA has unique environmental or pathological hazards regardless to whether or not genetic material from infectious sources. Because of this, all rDNA waste must be decontaminated prior to disposal in the sewer or trash.
- **Laboratory Waste Which Has Come in Contact with a Biohazard (as listed above).** This includes, but is not limited to, disposable laboratory personal protective equipment (gloves, gowns, shoe covers, masks), disposable laboratory plastic ware (culture dishes, plates and flasks, pipettes, and pipette tips), blood specimen tubes, devices used to transfer, inoculate and mix cultures; and paper and cloth which have come
into contact with cultures and stocks of etiologic agents.

Additional wastes may be added at the discretion of the Infectious Control Committee, in consultation with the Institutional Biosafety Committee (IBC).

Note that although not defined as biohazardous waste, rDNA experiments involving plants have NIH imposed requirements for waste. For both BL1-P and BL2-P, experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility. See Appendix H for more information.

DEFINITIONS

Disinfection. Disinfection is generally a less lethal process than sterilization. It eliminates nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. Disinfection does not ensure an "overkill" and therefore lacks the margin of safety achieved by sterilization procedures. The effectiveness of a disinfection procedure is controlled significantly by a number of factors, each one of which may have a pronounced effect on the end result. Among these are: the nature and number of contaminating microorganisms (especially the presence of bacterial spores); the amount of organic matter present (e.g., soil, feces, and blood); the type and condition of instruments, devices, and materials to be disinfected; the temperature.

Sterilization. Any item, device, or solution is considered to be sterile when it is completely free of all living microorganisms, endospores and replication competent viruses. The definition is categorical and absolute (i.e., an item is either sterile or it is not). A sterilization procedure is one that kills all microorganisms, including high numbers of bacterial endospores. Sterilization can be accomplished by heat, ethylene oxide gas, hydrogen peroxide gas, plasma, ozone, and radiation (in industry). From an operational standpoint, a sterilization procedure cannot be categorically defined. Rather, the procedure is defined as a process, after which the probability of a microorganism surviving on an item subjected to treatment is less than one in one million (10^-6). This is referred to as the "sterility assurance level."

Incineration is complete combustion of the waste and packaging to carbonized or mineralized ash.

Biohazard Bag is a red or orange disposable, leak-proof bag having enough strength to prevent ripping, tearing, breaking, or bursting under normal use. These red or orange bags are marked with the biohazard symbol and the word "Biohazard." Biohazard bags are provided by Research Safety.

Biohazard Bin is semi-rigid, leak resistant secondary container that is impervious to moisture, marked with the biohazard symbol plus the word "Biohazard" and lined with a biohazard bag. Biohazard bins are provided by Research Safety. The bin may be plastic or cardboard.

Biohazard Bottle is a poly leak-proof, rigid poly container labeled with the biohazard symbol and the word "Biohazard." Biohazard bottles are provided by Research Safety.

Sharps Container is a red leak-proof, rigid, puncture-resistant, durable plastic container labeled with the biohazard symbol and equipped with a tight-fitting lid for use during handling and transport. Sharps containers are provided by Research Safety.

Unmarked autoclave bag is a clear (not red or orange) bag that does not bear the biohazard symbol.

All other terms used herein shall have the meaning defined by R61-105.

RESPONSIBILITIES

Individual principal investigators and/or departmental managers/supervisors are responsible for identifying the biohazardous waste generated by their activity, segregating it into the appropriate waste stream, managing it in accordance with this protocol and providing appropriate training to research personnel under their supervision. The second level of supervision is the Biological Safety Officer (BSO) who is responsible for ensuring, through training and inspections that all laboratory personnel are knowledgeable of and comply with procedures outlined in this protocol. Research Safety is responsible for picking up those biohazardous
wastes requiring offsite treatment from laboratories in a timely manner, final packaging, manifesting and shipping them for incineration and recordkeeping in compliance with SC R61-105.

**TRAINING**

Personnel who work in a lab with biohazards will either generate waste or have the potential to generate waste. In order to be able to determine if the waste is infectious or not, all personnel will need to be trained. This training will be managed by the BSO as a one-time training, either online or in person.

**DISINFECTION**

SC Regulation 61-105 requires that any material or surface that comes in contact with an infectious substance must be disinfected prior to reuse. Disinfection is permitted by appropriate use of an EPA registered disinfectant used according to the label instructions at the tuberculocidal (TB) strength. Clemson's IBC requires that disinfectants used for Bloodborne Pathogens be registered for HIV-1 and HBV as well as TB. The list used for disinfection at Clemson is EPA Disinfectant List E (HIV-1, HBV, TB); see https://www.epa.gov/pesticide-registration/list-e-epas-registered-antimicrobial-products-effective-against-mycobacterium

The most familiar disinfectants on this list are Clorox Bleach (active ingredient sodium hypochlorite) at a concentration of 10% (1 part household bleach mixed with 9 parts water, hitherto called 10% bleach), Cavicide, and 70% isopropyl alcohol. When working with other organisms, the Pathogen Safety Data Sheet (PSDS) should be consulted for effective disinfectants.

Spills shall be disinfected immediately with 10% bleach or other effective disinfectant. As diluted bleach degrades rapidly, this solution must have been prepared within 8 hours of use. See Chapter 8 for detailed spill procedures.

Reusable containers that have been used to contain biohazardous waste must be disinfected immediately after being emptied.

**TREATMENT**

SC Regulation 61-105 requires that infectious waste be treated by chemical disinfection, incineration or steam sterilization before disposal. Clemson University requires that all biohazardous waste be treated with 10% bleach (liquids only), sterilized by autoclave or packaged for offsite contract incineration.

**BIOHAZARDOUS WASTE SEGREGATION/CONTAINMENT/TREATMENT/DISPOSAL**

Sharps are deposited in a sharps container. All sharps waste is treated offsite by incineration; disposal is by the University's Infectious Waste Contractor. Pretreatment is discouraged.

Re-usable sharps are safely segregated and contained in leak-proof, rigid, puncture-resistant containers while awaiting cleaning, decontamination, and sterilization before re-use.

Liquid biohazardous waste is chemically treated with Bleach and disposed via sanitary sewer or autoclaved and disposed via sanitary sewer or contained in a biohazard bottle which is placed in a biohazard bin. Biohazard bins are treated offsite by incineration; disposal is by the University's Infectious Waste Contractor.

Solid biohazardous waste is either autoclaved in an unmarked autoclave bag and disposed of as ordinary trash or placed in a biohazard bin. Biohazard bins are treated offsite by incineration; disposal is by the University’s Infectious Waste Contractor. Red or orange bags, or bags marked as biohazard cannot be disposed as ordinary trash, regardless of whether they were autoclaved.

Infectious animal body parts or carcasses are contained in biohazard bags, kept in designated freezers or refrigerators until pickup by Research Safety. They’re placed in a biohazard bin before removal from the lab. Biohazard bins are treated offsite by incineration; disposal is by the University’s Infectious Waste Contractor.
Chemical Treatment Standards

Household bleach is 5 - 10% sodium hypochlorite. The appropriate concentration of sodium hypochlorite for disinfecting liquid waste, e.g. supernatants from cell culture, is 5000 ppm, approximately 0.5%. A 1:9 (v/v) dilution of bleach (EPA registration number 5813-50) to liquid biological waste is required. A minimum contact time of sodium hypochlorite with liquid waste is 10-20 minutes. After contact time, disinfected liquid waste is poured into the sanitary sewer. Bleach should be stored between 50 and 70°F. According to Clorox, undiluted household bleach has a shelf life of six months to one year from the date of manufacture, after which bleach degrades at a rate of 20% each year until totally degraded to salt and water (exposure to sunlight or additional heat increases the degradation rate substantially), and a 1:9 bleach solution has a shelf life of 24 hours. However, this protocol requires that the dilution of bleach be mixed within the previous 8 hours.

All wastes that meet the definition of infectious waste and are treated chemically or by steam sterilization MUST be logged before disposal. The date and amount of waste treated and disposed must be included. A monthly log must be kept and sent to the BSO on a regular basis.

Autoclave Standards

All steam sterilizers must,

(a) use the biological indicator Bacillus stearothermophilus placed at the center of a load processed under standard operating conditions to confirm the attainment of adequate sterilization conditions. Indicator organisms must be used monthly at a generator facility in each steam sterilizer;

(b) record the temperature and time during each complete cycle to ensure the attainment of a temperature of 121 degrees Centigrade (250 degrees Fahrenheit) for 45 minutes or longer (refer to Appendix I of the Biosafety Manual for large quantities) at fifteen (15) pounds pressure, depending on quantity and density of the load, in order to achieve sterilization of the entire load; (Thermometers shall be checked for calibration at least annually.)

(c) have a gauge that indicates the pressure of each cycle.

(d) use heat sensitive tape that is lead-free or other device for each container that is processed to indicate that the steam sterilization temperature has been reached. The waste will not be considered appropriately treated if the indicator fails.

(e) maintain records of the procedures specified in (b) above for a period of not less than three (3) years.

(f) assure that treatment residues are disposed of in accordance with applicable State and Federal Requirements.

(g) certify the autoclave by an external vendor one a year.

For more on use of autoclaves, see Appendix H.

Appendix A Biological Safety Cabinets

Biological Safety Cabinets and other primary containment devices are an essential component of conducting biological research. As a primary safety barrier, the effectiveness of the BSC is limited by the techniques employed by the researcher (e.g. good microbiological techniques), an understanding of how the cabinet functions, and the location of the biological safety cabinet within the facility. As a general rule, keep biosafety cabinets away from doors, high traffic areas and supply diffusers. The Biosafety Officer can provide consultation and guidance on the selection, operation and use that meet your specific research needs.

A Class II, A2 Biosafety Cabinet is recommended and the most common type of BSC in use, as it is appropriate for most biohazardous work applications. Class II BSC provide personnel protection from biohazardous
materials using HEPA filtered air prior to release into the room, in addition to providing product protection (to maintain sterility). Information on specific classes and types of Biosafety Cabinets can be found in appendix A of the BMBL: https://www.cdc.gov/labs/BMBL.html

For most applications, it is not necessary to connect a Class II A2 BSC to the building ventilation system. Class II A2 cabinets are designed to be “convertible” units and can be connected to the ventilation system using a thimble connection designed with an air gap. This is recommended only in certain situations that merit connection to the HVAC system.

Biological Safety Cabinets must be certified annually and whenever moved; they must be decontaminated (chlorine dioxide gas or hydrogen peroxide vapor) before being moved. BSCs are certified (and decontaminated) by an outside contractor at the expense of the Principal Investigator. The Biosafety Officer will provide a list of designated, NSF-certified vendors from which to choose. Laboratory staff is responsible for coordinating BSC certification service directly with the vendor.

Instructions on the proper use of the Biological Safety Cabinet (BSC)

- Biosafety cabinets are designed to run 24 hours a day, and for frequent work with BSL-2 agents in the BSC, it is recommended that blowers remain on at all times.
- If it is necessary to turn off the blower, allow sufficient time to purge airborne contaminants from the work area (Centers for Disease Control and the Public Health Agency of Canada recommend a minimum of 5 minutes before and 5 minutes after work, taking into account sufficient time for settling of aerosols).
- Minimize other activities in the room (e.g., rapid movement, open/closing room doors, etc.) to avoid disrupting the cabinet air barrier.
- Laboratory coats are worn buttoned over street clothing; gloves are worn to provide hand protection.
- Before beginning work, the investigator must adjust the stool height so that his/her face is above the front opening.
- Plastic-backed absorbent toweling can be placed on the work surface (but not on the front or rear grille openings). This toweling facilitates routine cleanup and reduces splatter and aerosol formation during an overt spill.
- Closure of the drain valve under the work surface must be done prior to beginning work so that all contaminated materials are contained within the cabinet should a large spill occur.
- Place necessary materials in the BSC before beginning work. This serves to minimize the number of arm-movement disruptions across the air barrier of the cabinet. All materials must be placed as far back in the cabinet as practical, toward the rear edge of the work surface and away from the front grille of the cabinet.
- The front grille must not be blocked with research notes, discarded plastic wrappers, pipetting devices, etc.
- Aspirator suction flasks must contain an appropriate disinfectant, and a High Efficiency Particulate Air (HEPA) in-line filter. This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution such as bleach, into the flask to kill the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of as noninfectious waste.
- Horizontal pipette discard trays containing an autoclave bag or an appropriate chemical disinfectant should be used within the cabinet. Upright pipette collection containers placed on the floor outside the cabinet or autoclave-safe biohazard collection bags taped to the outside of the cabinet should not be used. The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection.
- All operations should be performed on the work surface at least four (4) inches from the inside edge of the front grille.
- Active work should flow from the clean to contaminated area across the work surface. Bulky items such as biohazard bags, discard pipette trays and suction collection flasks must be placed to one side
Use of glass Pasteur pipettes is discouraged. Glass pipettes should be replaced with safer alternatives (i.e., plastic) as recommended by the U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health in Biosafety in Microbiological and Biomedical Laboratories, 5th Edition Fifth Edition and by The World Health Organization Biosafety Manual. Contact the Biosafety Officer for more information on safer alternatives.

Open flames (i.e., Bunsen burners) are rarely necessary in the near microbe-free environment of a biological safety cabinet and are an artifact left over from usage of A1 cabinets (e.g., provided only personnel, not product protection) several decades ago. An open flame creates turbulence that disrupts the pattern of HEPA-filtered air supplied to the work surface. When deemed absolutely necessary, touch-plate micro-burners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Micro-incinerators (electric) are the best alternative for use in the BSC.

Use of ultraviolet light (UV) in the BSC is strongly discouraged. UV bulbs in the BSC must be cleaned and monitored regularly, as dust and debris inhibit effectiveness as well as gradual degradation of the UV bulb over time and should never be used as a primary or sole means of disinfecting the unit. Therefore, chemical surface disinfection must be the primary means of decontaminating the BSC.

Clean Up: Upon completion of work, the final surface decontamination of the cabinet must include a wipe-down of the interior surfaces. Investigators must remove their gloves and gowns in a manner to prevent contamination of unprotected skin and aerosol generation and wash their hands as the final step in safe microbiological practice. Investigators must determine the appropriate method of decontaminating materials that will be removed from the BSC at the conclusion of the work.

Appendix B Shipping of Infectious Substances

The Department of Transportation regulates shipment of infectious substances by commercial carrier through 49CFR173.134 as follows.

Title 49: Transportation PART 173—SHIPPERS—GENERAL REQUIREMENTS FOR SHIPMENTS AND PACKAGINGS § 173.134 Class 6, Division 6.2—Definitions and exceptions.

(a) Definitions and classification criteria. For the purposes of this subchapter, the following definitions and classification criteria apply to Division 6.2 materials.

(1) Division 6.2 (Infectious substance) means a material known or reasonably expected to contain a pathogen. A pathogen is a microorganism (including bacteria, viruses, rickettsiae, parasites, fungi) or other agent, such as a proteinaceous infectious particle (prion) that can cause disease in humans or animals. An infectious substance must be assigned the identification number UN 2814, UN 2900, UN 3373, or UN 3291 as appropriate, and must be assigned to one of the following categories:

(i) Category A: An infectious substance in a form capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs. An exposure occurs when an infectious substance is released outside of its protective packaging, resulting in physical contact with humans or animals. A Category A infectious substance must be assigned to identification number UN 2814 or UN 2900, as appropriate. Assignment to UN 2814 or UN 2900 must be based on the known medical history or symptoms of the source patient or animal, endemic local conditions, or professional judgment concerning the individual circumstances of the source human or animal.

(ii) Category B: An infectious substance that is not in a form generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs. This includes Category B infectious substances.
transported for diagnostic or investigational purposes. A Category B infectious substance must be described as “Biological substance, Category B” and assigned identification number UN 3373. This does not include regulated medical waste, which must be assigned identification number UN 3291.

(2) **Biological product** means a virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, or arsphenamine or derivative of arsphenamine (or any other trivalent arsenic compound) applicable to the prevention, treatment, or cure of a disease or condition of human beings or animals. A **biological product** includes a material subject to regulation under 42 U.S.C. 262 or 21 U.S.C. 151-159. Unless otherwise excepted, a biological product known or reasonably expected to contain a pathogen that meets the definition of a Category A or B infectious substance must be assigned the identification number UN 2814, UN 2900, or UN 3373, as appropriate.

(3) **Culture** means an infectious substance containing a pathogen that is intentionally propagated. Culture does not include a human or animal patient specimen as defined in paragraph (a) (4) of this section.

(4) **Patient specimen** means human or animal material collected directly from humans or animals and transported for research, diagnosis, investigational activities, or disease treatment or prevention. Patient specimen includes excreta, secretions, blood and its components, tissue and tissue swabs, body parts and specimens in transport media (e.g., transwabs, culture media, and blood culture bottles).

(5) **Regulated medical waste or clinical waste or (bio) medical waste** means a waste or reusable material derived from the medical treatment of an animal or human, which includes diagnosis and immunization, or from biomedical research, which includes the production and testing of biological products. Regulated medical waste or clinical waste or (bio) medical waste containing a Category A infectious substance must be classed as an infectious substance, and assigned to UN 2814 or UN 2900, as appropriate.

(6) **Sharps** means any object contaminated with a pathogen or that may become contaminated with a pathogen through handling or during transportation and also capable of cutting or penetrating skin or a packaging material. Sharps includes needles, syringes, scalpels, broken glass, culture slides, culture dishes, broken capillary tubes, broken rigid plastic, and exposed ends of dental wires.

(7) **Toxin** means a Division 6.1 material from a plant, animal, or bacterial source. A toxin containing an infectious substance or a toxin contained in an infectious substance must be classed as Division 6.2, described as an infectious substance, and assigned to UN 2814 or UN 2900, as appropriate.

(8) **Used health care product** means a medical, diagnostic, or research device or piece of equipment, or a personal care product used by consumers, medical professionals, or pharmaceutical providers that does not meet the definition of a patient specimen, biological product, or regulated medical waste, is contaminated with potentially infectious body fluids or materials, and is not decontaminated or disinfected to remove or mitigate the infectious hazard prior to transportation.

**Note the following EXCEPTIONS.**

(b) **Exceptions.** The following are not subject to the requirements of this subchapter as Division 6.2 materials:

(1) A material that does not contain an infectious substance or that is unlikely to cause disease in humans or animals.

(2) Non-infectious biological materials from humans, animals, or plants. Examples include non-
infectious cells, tissue cultures, blood or plasma from individuals not suspected of having an infectious disease, DNA, RNA or other non-infectious genetic elements.

(3) A material containing microorganisms that are non-pathogenic to humans or animals.

(4) A material containing pathogens that have been neutralized or inactivated such that they no longer pose a health risk.

(5) A material with a low probability of containing an infectious substance, or where the concentration of the infectious substance is at a level naturally occurring in the environment so it cannot cause disease when exposure to it occurs. Examples of these materials include: Foodstuffs; environmental samples, such as water or a sample of dust or mold; and substances that have been treated so that the pathogens have been neutralized or deactivated, such as a material treated by steam sterilization, chemical disinfection, or other appropriate method, so it no longer meets the definition of an infectious substance.

If you're unsure whether your shipment is regulated (infectious) or exempted, contact the Biosafety Officer, kkwist@clemson.edu.

Federal regulations require that the employer train and certify employees before they ship infectious substances. For training as required by federal regulations or more information, please contact the Hazardous Materials Manager, June-Brock Carroll, at juneb@clemson.edu or 864-633-6357 or the Office of Research Safety at 864-656-0341.

Please note that if infectious material is shipped in a chemical (i.e. formalin, dry ice, etc) then the shipment may fall under hazardous materials shipment requirements.

**Appendix C Transport of Biohazardous Material**

The DOT Hazardous Material Regulations (49 CFR Parts 171-180) regulates the movement of Division 6.2 Infectious Substances when a commercial carrier is used. This is covered in Appendix B: Shipment of Infectious Substances. Transport refers to movement of Clemson material by Clemson personnel (faculty, staff or students) in a vehicle or hand carry between buildings.

Substances that meet the DOT definition of infectious (see Appendix B) and that are being transported in a vehicle, must be packaged per DOT regulations by a person certified to ship infectious substances. Pay particular attention to the exemptions in Appendix B; most substances requiring transport at Clemson are exempt from the DOT definition of infectious.

Substances not meeting the DOT definition of infectious, but requiring BL-2 (or higher) containment or covered by Clemson’s definition of biohazardous (see Chapter 9 of this Manual) must be packaged as follows for transport either by vehicle or hand carry: Place material in a primary (specimen) container that is leak-proof and secured with a tight-fitting cap; place absorbent material (diapers, absorbent towels, pads) around the primary containers for transport of liquids; place the primary container(s) in a secondary transport container that is also sealed and labeled with a biohazard symbol.

Clemson encourages the use of university-owned vehicles when transporting materials off campus. Accidents during movement of any of these materials can result in harm to persons and property. Release and spills of these materials may involve police and HAZMAT responders including clean-up and cost of recovery.

Please note that if biohazardous material is transported in a chemical (i.e. formalin, dry ice, etc) then the transport may fall under hazardous materials guidelines. Under NO circumstances may public transportation be used for transport of any Biohazardous or Hazardous Materials.
Appendix D  Use of Human, Non-human Primate and other Mammalian Cells and Tissues

**Background:** In 1991, the Occupational Safety and Health Administration (OSHA) issued the Bloodborne Pathogens (BBP) Standard to protect employees who have occupational exposure to human blood or other potentially infectious materials. While human blood, most body fluids, unfixed human tissues and organs were clearly included within the scope and application of the standard, the inclusion of human cell lines was ambiguous.

In 1994, OSHA issued an interpretation of the applicability of the BBP Standard towards human cell lines. According to the interpretation, human cell lines are considered to be potentially infectious and within the scope of the BBP Standard unless the specific cell line has been characterized to be free of hepatitis viruses, HIV, Epstein-Barr virus, papilloma viruses and other recognized bloodborne pathogens. In alignment with this interpretation, the American Type Culture Collections (ATCC) recommends that all human cell lines be accorded the same level of biosafety consideration as a line known to carry HIV. ATCC also states “However, in general, we do not test for animal viruses, therefore not all of our cell lines have been tested for animal viruses and should be treated accordingly”.

ATCC assigns biosafety level (BSL) designations for the purposes of safe shipment, according to whether or not at the time of accessioning it is known that the cell lines is harboring any known virus or any portion of a virus which causes human disease. “While ATCC does provide the biosafety level for each ATCC culture, we do not provide information on biosafety practices. Each researcher should determine the appropriate BSL using a risk assessment based on the characteristics of the cell line and how the cell line will be used.” This includes, but is not limited to, laboratory manipulations employed during its handling.

Human, primate or mammalian cell lines, even in the absence of overt contamination, may contain adventitious viruses and/or other opportunistic pathogens or zoonotic agents. Since it is extremely difficult to screen for every pathogen, all human and primate cell lines must be handled with standard precautions i.e. treated as though they are contaminated with infectious agents and utilize BSL2 practices. Mammalian cells lines should be handled depending on their individual risk assessment.

In addition to viral and bacterial threats, the European Union – European Medicines Agency document “Viral Safety Evaluation of Biotechnical Products Derived from Cells lines of Human or Animal Origins” also indicates the potential for the introduction of bovine spongiform encephalopathy prions (BSE) contamination into established cell lines via use of contaminated animal-derived products (including animal serum products) and/or through the use of improper work methods. “However, in contrast to most of the infectious agents, prions are particularly difficult to inactivate. In fact, no method can guarantee total inactivation of these agents. So, one should bear these considerations in mind when using growth media of bovine origin”.

In consideration of the aforementioned regulatory interpretations, consensus guidelines, best practices, and other factors, the CU Institutional Biosafety Committee has adopted the following guidance:

- All cell and organ cultures of human origin, including well established cell lines, human bodily fluids and non-human primate cells and tissues should be handled in accordance with the OSHA Bloodborne Pathogens Standard and under Biosafety Level 2 (BSL2) containment unless tested and found to be pathogen free. Mammalian tissues and cells lines should be handled according to the individual risk assessment.

Members of the research team working with human or non-human primate cells and tissues shall take Clemson University's Bloodborne Pathogens (BBP) training for Research Labs annually. The initial training should be a “LIVE” training. Contact the biosafety officer (kkwist@clemson.edu) for the next scheduled session. Refresher trainings can be taken online on the Research Safety website.
Lab members working with human or non-human primate materials should also enroll in the CU Medical Surveillance Program (https://www.clemson.edu/cbshs/centers-institutes/sullivan/patients/services/medical-surveillance.html). This service is open to faculty, staff and students who work in these labs.

Appendix E Guidelines for Work with Toxins of Biological Origin

Biological toxins comprise a broad range of poisons, predominantly of natural origin but increasingly accessible by modern synthetic methods, which may cause death or severe incapacitation at relatively low exposure levels. Laboratory work with most toxins, in amounts routinely employed in the biomedical sciences, can be performed safely with minimal risk to the worker and negligible risk to the surrounding community. Toxins do not replicate, are not infectious, and are difficult to transmit mechanically or manually from person to person. Many commonly employed toxins have very low volatility and, especially in the case of protein toxins, are relatively unstable in the environment; these characteristics further limit the spread of toxins.

Toxins can be handled using established general guidelines for toxic or highly-toxic chemicals with the incorporation of additional safety and security measures based upon a risk assessment for each specific laboratory operation. The main laboratory risks are accidental exposure by direct contamination of mouth, eyes or other mucous membranes; by inadvertent aerosol generation; and by needle-sticks or other accidents that may compromise the normal barrier of the skin.

A risk assessment should be conducted to develop safe operating procedures before undertaking laboratory operations with toxins; suggested “pre-operational checklists” for working with toxins are available. For complex operations, it is recommended that new workers undergo supervised practice runs in which the exact laboratory procedures to be undertaken are rehearsed without active toxin. If toxins and infectious agents are used together, then both must be considered when containment equipment is selected and safety procedures are developed. Likewise, animal safety practices must be considered for toxin work involving animals.

Detailed information and guidance is contained in appendix I of the BMBL https://www.cdc.gov/labs/BMBL.html

Appendix F NIH Oversight of Research Involving Recombinant DNA

The NIH Guidelines for Research Involving Recombinant DNA Molecules (https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf) contain procedures for the containment of rDNA research. The Guidelines apply to all institutions that receive NIH funding for rDNA. 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 rDNA research at the institution, or a requirement for prior NIH approval of rDNA projects at the institution.

The original guidelines were issued in 1976 due to public concern for safety, environmental impact, and ethical implications of rDNA research. The purpose of the guidelines is to specify safe handling practices and containment levels for rDNA molecules, organisms and viruses containing rDNA molecules, and transgenic animals.

Responsibilities under the Guidelines:

- The Institution must: establish an Institutional Biosafety Committee (see http://www.clemson.edu/research/compliance/ibc/), ensure compliance with the NIH Guidelines
by investigators and report any significant problems, violations or significant research-related accidents or illnesses to NIH within 30 days.

- The Institutional Biosafety Committee must: review, approve and oversee rDNA research to ensure compliance with the Guidelines, determine necessity of health surveillance of personnel, ensure training for IBC members, staff, PIs, and laboratory staff,
- Set biosafety containment levels as required by the Guidelines for some experiments under III-D-4-b

**Incident Reporting to NIH**

The following incidents must be reported to NIH OBA within 30 days: any significant problems or violations of the NIH Guidelines, e.g. failure to adhere to the containment and biosafety practices in the Guidelines, 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.

The following incidents require immediate reporting to NIH OBA: spills or accidents involving rDNA requiring BL2 containment resulting in an overt exposure, e.g. needlestick; splash in eyes, nose, mouth; or accidental aerosolization/inhalation.

**Appendix G Recombinant DNA Experiments Involving Plants**

Appendix P *NIH Guidelines* specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant DNA-containing plants, plant-associated microorganisms, and small animals. All provisions of the *NIH Guidelines* apply to plant research activities with the following modifications:

Appendix P supersedes Appendix G *NIH Guidelines (Physical Containment)* when the research plants are of a size, number, or have growth requirements that preclude the use of containment conditions described in Appendix G. The plants covered in Appendix P include but are not limited to mosses, liverworts, macroscopic algae, and vascular plants including terrestrial crops, forest, and ornamental species.

Plant-associated microorganisms include viroids, virusoids, viruses, bacteria, fungi, protozoans, certain small algae and microorganisms that have a benign or beneficial association with plants, such as certain *Rhizobium* species and microorganisms known to cause plant diseases. The appendix applies to microorganisms that are being modified with the objective of fostering an association with plants.

Plant-associated small animals include those arthropods that: (i) are in obligate association with plants, (ii) are plant pests, (iii) are plant pollinators, or (iv) transmit plant disease agents, as well as other small animals such as nematodes for which tests of biological properties necessitate the use of plants. Microorganisms associated with such small animals (e.g., pathogens or symbionts) are included.

*For both BL1-P and BL2-P, experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.*

**Appendix H Autoclaves**

Autoclaves are safe and highly effective when used properly. They sterilize equipment and supplies, and they kill biologicals and denature proteins. Autoclaves do not remove chemical contamination and should not be used in conjunction with chemical disinfectants.

**Autoclave Cycles**

There are three basic autoclave cycles:
1) Gravity Cycle (aka Fast Exhaust): This cycle is used for dry items such as glassware or metal instruments. This cycle cannot be used for liquids because the rapid pressure release will cause any liquids to boil.

2) Liquid Cycle (aka Slow Exhaust): This cycle is used to sterilize liquids. The slow pressure release at the end of the cycle keeps liquids (which have been heated to 121C) from boiling. A table of autoclave times based on liquid volume is given below.

3) Pre-Vacuum Cycle: This cycle is used for porous materials, animal bedding, etc. Some of the air in the chamber is evacuated to create a negative pressure space. This allows the steam to penetrate deeper into the materials placed in the autoclave to help ensure that even the deepest pockets within these materials become sterile.

When an autoclave is used to kill biologicals prior to disposal, follow the procedures set forth in the appropriate chapter of the Biosafety Manual.

Safety Rules for Autoclaves

- Hot glassware being removed from the autoclave must be handled with dry, heat-resistant gloves.
- Use the liquid (slow-exhaust) cycle for liquids and let them stand for 10 minutes before removal from the autoclave.
- For media bottles (glassware with a sealable cap), the cap must remain loose while the bottle is in the autoclave. This will prevent it from building pressure and exploding.
- Secondary containment must be used for items being placed in the autoclave. Items should never be placed directly on the floor of the autoclave.
- Not all plastics can be autoclaved. Polypropylene and polycarbonate may survive the autoclave; polyethylene, even high-density polyethylene will not. Be sure your item is autoclave safe before placing in the autoclave. If there is any doubt, use secondary containment.
- To ensure adequate steam penetration, add 1 cup of extra water for dry loads and leave bags partially open.
- Red biohazard bags must never be used in an autoclave, even if they are autoclave-safe. Clear autoclave bags must be used.
- Never open an autoclave until the chamber pressure is zero.
- Stand back from the door upon opening to allow excess steam to be released safely.
- An indicator, such as autoclave tape, must be used with each load to ensure the chamber has reached the appropriate temperature.

Other Autoclave Operating Suggestions

- Placing 1-2 inches of water in the bottom of the secondary container with bottles will help prevent bottle bottoms from breaking.
- Autoclaving new glassware for 90 minutes will partially temper it, making it stronger.
- If there is doubt about whether or not a piece of glassware is autoclave safe, it should be wrapped in foil. This will capture the pieces of glass if the glassware were to crack or shatter.

Autoclave times based on liquid volume
These autoclave times are only given as guidance. More time may be required for certain organisms or other biologicals. Allow 10-20 minutes extra for crowded items.

<table>
<thead>
<tr>
<th>&lt;500 mL</th>
<th>500mL – 1L</th>
<th>1L – 2L</th>
<th>2L-4L</th>
<th>4L</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 minutes</td>
<td>40 minutes</td>
<td>45-55 minutes</td>
<td>55-60 minutes</td>
<td>60 minutes</td>
</tr>
</tbody>
</table>

For any additional questions regarding autoclave use and safety, contact the BSO at kkwist@clemson.edu.
Appendix I: Clemson University Integrated Pest Control Policy for Biological Research labs

I. Policy

a. Clemson University's Integrated Pest Control Policy is based on the Biosafety in Microbiological and Biomedical Laboratories (BMBL) Appendix G and the NIH's Biomedical and Animal Research Facilities Design Policies and Guidelines.
   i. Pest control is important because insect and rodent pests:
      ii. Carry disease and organisms on or in their bodies
      iii. Cause physical damage to building facilities (by chewing/gnawing).
      iv. Contaminate and compromise the research environment.
      v. Are unacceptable in the workplace.

II. Information

a. The BMBL via the CDC and the NIH recommends Integrated Pest Management, meaning Pest Control has several parts and these organizations cautions against the use of pesticides as the main way to control these issues. This comprehensive Pest Control starts with building construction, then uses building maintenances and housekeeping to prevent infestation. Pesticides are used only in certain cases.

III. Procedure

a. Construction design and maintenance
   i. Facility design – Set up and materials used discourage pest infestation
   ii. Maintenance – Repairs are made in a timely manner to exclude pest

b. Housekeeping
   i. Storage of food and other personal items are done in designated areas and in a way to not attract pests
   ii. Trash and recycling is kept in well maintained bins, emptied, and picked up on a regular schedule.

c. Monitoring
   i. All building users are required to report any signs of pests to the appropriate person (building manager or PI)
   ii. When sightings are reported, action should be taken.
   iii. Certain devices (glue boards, traps) are acceptable for monitoring.

d. Action
   i. Non-pesticide includes trapping, exclusion, caulking, and washing
   ii. Pesticide use must be done by licensed professional and be done to consider other research projects as well as the environment.

IV. Communication
a. Lab PI is first point of contact
b. Building manager will coordinate pest control efforts
c. Contracted pest control service is Gregory Pest Control and their services can be ordered through BuyWays.
d. Facilities can be contacted to fix cracks or holes to prevent entry.

Appendix J: Biosafety in Teaching Labs at Clemson University

This guidance document applies to all biological teaching labs on campus

Overview

This document will identify Clemson specific teaching lab policies created in accordance with the work to support the American Society of Microbiology (ASM) Guidelines for Biosafety in Teaching labs. A copy of the original teaching lab guidelines can be found here: https://www.asm.org/getattachment/3c1eb38c-84d7-472f-aa9b-5d695985df21/ASM-Biosafety-Guidelines.pdf This ASM document was finalized in 2013 and if there are any questions about its contents, please contact the Biosafety Officer, Kerri Kwist kkwist@clemson.edu

Teaching labs working with biohazardous material at Clemson University do NOT have to be registered with the Institutional Biosafety Committee. However, as new labs are developed and space allocation changes, it is recommended that the Office of Research Safety is consulted. Labs involving the use of animals DO need to be registered with the Institutional Animal Care and Use Committee (IACUC).

Medical Concerns

Instructors are not allowed to ask about student health status. A list of all cultures and the Pathogen Safety datasheet (PSDS) (https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html) must be provided to each student prior to or during the first class so they are informed of agents that could affect their health status. If needed, students are encouraged to take the list to their private medical provider. The Clemson University Occupational Health Nurse’s (OHN) name and phone number should be provided in the syllabus of each lab. If there are questions about the safety of any of the microorganisms, concerned students can register for medical surveillance or contact the OHN with questions. Contact information and other details on the medical surveillance program (MSP) can be found here: https://www.clemson.edu/cbshs/centers-institutes/sullivan/patients/services/medical-surveillance.html

Lab Practices

Good lab practices should always be encouraged. Proper Personal Protective Equipment (PPE) including long pants and closed toed shoes must be worn as appropriate. Students should be taught to disinfect their work space before work begins using the correct disinfectant and the correct contact time. They should also be required to clean and disinfect surfaces and equipment before leaving the lab. If a space should be used for more than one purpose, materials and samples should be able to be safely stored away. All chemicals and cultures should be clearly labeled with contents and the date. All waste must be properly stored, segregated and disposed of correctly. Handwashing should be encouraged during and before leaving the lab.

Training

Most training for lab work will be covered through the lab curriculum. If a lab will use ANY human bodily fluid, including from the participating student, a version of Blood Borne Pathogen (BBP) training must be provided. This training can be set up with the Biosafety Officer from Research Safety or done by the TA.
Unless otherwise specified, this training will only cover the basics of blood borne pathogen use and cannot be used to meet the requirement for BBP in Research Labs. This training must include at a minimum: what are blood borne pathogens, examples, modes of transmission, and methods of control. The full training can be given if asked or slides for this training can be provided by request. Other trainings can be given on a case by case basis (Hazardous Waste, Biohazardous Waste, Hazardous Materials Shipping, Safety Overview).

Students must be given the ability to “opt out” of labs using body fluids, including their own. Synthetic or purchased fluids could be reviewed as a possible alternative.

**Biosafety Considerations**

For Teaching Labs, every effort should be made to keep work at Biosafety Level 1 (BSL 1) containment. In most cases, Risk Group 1 (RG1) organisms can be substituted for risk group 2 (RG2) organisms. Even so, care should be taken during the lab to minimize aerosols and the use of sharps. Encourage students to come prepared to class, so the lesson can be moved through in an organized manner. Provide all equipment and materials before the lab begins so the work can stay localized.

Labs that plan on culturing unknown samples from the environment are recommended to consult with the biosafety officer about safe ways to do so. Many potentially pathogenic microorganisms exist in this habitat and it is entirely possible that a microbe that would normally be considered Risk Group 2 (RG2) be isolated in this manner. Experiments like this still can be carried out in BSL-1 containments, but after culturing efforts should be made to prevent exposure. The Biosafety Officer can give more guidance about these types of work practices.

Cross contamination has been found to be an issue with teaching labs, with writing instruments and cell phones being some of the main sources. Ways to prevent contamination can include the following:
- Have a note taking space separate from the work area.
- Designate a note taker for lab groups.
- Provide in class protocols for use on the bench separate from the lab notebook that can either be discarded or disinfected at the end of the lab.
- Teach the students about the one glove method, so even if they must take notes, they are using proper techniques to keep their materials clean.
- Use “lab only” writing utensils.

**Equipment**

If labs require using BSL2 containment, certain safety controls must be in place including but not limited to extra training, biosafety cabinets, centrifuge cups, posting the biohazard sign and the logging of waste disposal. For guidance on these issues, please contact the Biosafety Officer.

**Incidents**

In the event of an exposure or breach of containment, follow the steps listed:
1. If it is an emergency, call 911.
2. Section off the affected area to maintain the safety of the rest of the lab.
3. Contact the PI in charge of the lab.
4. The student should go to Redfern for treatment. They can be escorted by another lab member or the TA if needed. Labs that meet after hours should consult with their department for incident response.
5. Fill out the following incident report forms:
   a. [https://www.clemson.edu/administration/risk/documents/Incident-Report-Form.pdf](https://www.clemson.edu/administration/risk/documents/Incident-Report-Form.pdf)
   b. [https://media.clemson.edu/research/safety/DSCs/Incident_Report_Form.pdf](https://media.clemson.edu/research/safety/DSCs/Incident_Report_Form.pdf)
6. The PI will follow the steps in the Exposure Control plan and the Post-Exposure Control plan.

In the event of a spill:
1. If it is a large spill or if the people involved do not feel comfortable cleaning up the spill, evacuate the area and call 911 for HazMat response.
2. If the spill is manageable, cover the spill with absorbent material and keep others away from the area.
3. Wear proper PPE.
4. Soak absorbent material with proper disinfectant. Allow to sit for proper contact time.
5. Clean up spill material and dispose of as hazardous waste.
6. Report spill to PI.

Additional Information

The goal of Research Safety is to work with our teaching labs to provide relevant and safe learning experiences at Clemson. If there is a topic that is outside the scope of this document or if there are any questions or concerns, please contact the Office of Research Safety.