



*Biological
Safety
Manual*

*Environmental Health and
Safety*

Executive Summary

The purpose of this manual is to provide guidance for personnel on how to perform work with biological materials safely. All research and teaching laboratories that use biological materials are expected to be in compliance with this manual in the areas where it applies. Where the guidance provided in this manual is not used, written documentation should be given showing that the alternative procedure or arrangement will provide a situation that is either as safe or safer than the guidance given in this manual.

The primary sources for the regulations and guidelines that apply to biological safety are the US Centers for Disease Control and Prevention's (CDC) Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition and the National Institute of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. There is also limited application of both the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) regulations. These authorities are cited where appropriate.

The University of Memphis is required by NIH guidelines to have an Institutional Biosafety Committee (IBC). The IBC is composed of faculty and staff from various departments at the university. The IBC oversees all research projects that involve recombinant DNA and all research projects where pathogenic microorganisms are used. The IBC has also reviewed and approved this manual; any changes to the manual are made available to the IBC for review prior to their implementation.

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Chapter 1 Guidance, Regulations and Roles of Key Personnel

1.1 Introduction

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). The University of Memphis's Biological Safety Program is based on the premise that every member of the research community shares the responsibility for safety.

1.2 Guidance

US Centers for Disease Control and Prevention's (CDC) Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition is the key guidance document for Biosafety. Although BMBL6 only recommends practices ("We wish to emphasize that the 6th 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." BMBL6 Forward), unless otherwise specifically stated herein, compliance with all BMBL6 recommendations is mandatory for all laboratory biological activities (research, teaching, public service). www.cdc.gov/biosafety/publications/BMBL6/

NIH Guidelines is also a key guidance document for this manual. Whereas BMBL6 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 BMBL6, 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 is involved. The advent of multi-PI labs precludes separate consideration of BMBL6 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

1.3 Other Regulations

US Department of Transportation's 49CFR Subchapter C Hazardous Materials Regulations governs shipment of infectious (biohazardous) materials. <https://www.ecfr.gov/cgi-bin/text-idx?SID=a64e9c6dd6a80579f36c9c451213d153&mc=true&tpl=/ecfrbrowse/Title49/49CSubchapC.tpl>

1.4 Roles of Key Personnel

1.4.1 Institutional Biosafety Committee (IBC)

The Institutional Biosafety Committee (IBC) should:

- Review, approve and oversee rDNA research to ensure compliance with the NIH Guidelines,
- Determine necessity of health surveillance of personnel,
- Set biosafety containment levels as required by the guidelines,
- Ensure proper training for IBC members, staff, PIs, and laboratory staff,
- Report any significant problems, violations or significant research-related accidents or illnesses to NIH

- within 30 days (see appendix F) and
- Provide expert advice to the BSO in helping ensure safe research with hazardous biological materials.

1.4.2 Biological Safety Officer

The Biological Safety Officer (BSO) should:

- 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,
- Serve on the IBC as a voting member and
- Ensure safe research with all hazardous biological materials.

1.4.3 Principal Investigator

The Principal Investigator (PI) should:

- Be proficient in good microbiological techniques,
- Supervise staff to ensure safety practices are followed,
- Develop lab-specific SOPs for spills, exposures and/or loss of containment,
- Immediately report research-related accidents and illnesses,
- Correct unsafe work practices,
- Ensure biological containment is maintained and
- 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.

Chapter 2 Biological Risk Assessment

2.1 Introduction

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. 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 BMBL6 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.

2.2 Hazardous Characteristics of Agents

2.2.1 Principal Hazardous Characteristics

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.

2.2.2 Risk Groups

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.

2.3 Risk Assessment for Human Cell Lines and Human-Derived Materials

Biosafety level 2 (defined in chapter 4) 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 University of Memphis Exposure Control Plan. 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, non-human primate and some mammalian cells and tissues should be handled in accordance with the OSHA Bloodborne Pathogens Standard and under Biosafety Level 2 (BSL-2) containment unless shown to be free of infectious agents.

2.4 Risk Assessment for Other Agents

The end product of risk assessment is the assignment of an appropriate biosafety level (BSL) for an experiment. BSLs 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. Agent factors to be considered in determining the BSL include:

- Virulence,
- Pathogenicity,
- Infectious dose,
- Environmental stability,
- Route of transmission,
- Communicability, operations, quantity,
- Availability of vaccine or treatment and
- Gene product effects such 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 BSL. 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.

Laboratory procedure factors to be considered in determining the BSL include:

- Quantity of agent produced/manipulated,
- Physical hazards of the procedure,
- Likelihood of producing aerosols and
- Environmental factors.

A final assessment of risk based on these considerations is then used to set the appropriate BSL 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 BSL for recombinant DNA.

Chapter 3- Principles of Biosafety

3.1 Introduction

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.

3.2 Engineering Controls

Engineering controls eliminate or reduce the exposure of individuals to hazards in the laboratory through the use of specialized equipment. In most cases, engineering controls will be the primary barrier between hazardous materials and the individual. Engineering controls include

- Biological safety cabinets (BSCs) (discussed in Appendix A),
- Other enclosures,
- Safety centrifuge cups and
- Retractable needles.

3.3 Personal Protective Equipment

Personal protective equipment (PPE) is equipment that the individual wears to protect them from exposure to hazards in the laboratory. PPE is generally intended to be a secondary barrier meaning that it is to be used in conjunction with engineering controls to protect the user. PPE may be used a primary barrier when engineering controls are not possible or feasible. PPE worn should be based on the hazard present and can include

- Gloves,
- Safety glasses or splash goggles,
- Lab coat,
- Gown,
- Shoe covers,
- Boots,
- Respirator (includes dust masks) and
- Face shield.

3.4 Facility Construction and Design

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

4.1 Introduction

Four biosafety levels (BSLs) are described in BMBL6; each consists of a combination of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations being performed, the documented or suspected routes of transmission of the infectious agents, and the laboratory function or activity.

The BSLs described in BMBL6 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 BSL in

which the work will be conducted.

The recommended biosafety level(s) for the organisms in BMBL6 Section VIII (Agent Summary Statements) represent those conditions under which the agent ordinarily 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.

4.2 Biosafety Level 1

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, *Nigeria 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.

4.3 Biosafety Level 2

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. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low.

Hepatitis B virus, HIV, *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 safety centrifuge 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.

4.4 Biosafety Level 3

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 *Coxiella 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. There are currently no BSL-3 activities occurring at the University of Memphis.

4.5 Biosafety Level 4

Biosafety level 4 is beyond the scope of this manual. There are currently no BSL-4 activities occurring at the University of Memphis.

4.6 Multi-User Research Labs

The advent of large open labs and lab suites creates problems for separation of BSL-1 and BSL-2 areas and activities. The following NIH Guidelines, which shall apply to all biological activities at The University of Memphis, make a multi-PI lab problematic.

For BSL-1, 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 BSL-2, access to the laboratory is limited or restricted by the Principal Investigator when experiments are in progress [Appendix G-II-B-1-a]. For BSL-2, 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 BSL-2, laboratory coats, gowns, smocks, or uniforms 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].

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 BSL-2, with clearly demarcated BSL-1 section(s) as required. Note that BSL-2 waste must be kept in the BSL-2 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 RG2 agent in use.

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

BMBL6 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 separated 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 BMBL6 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 specialized 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 BMBL6

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.

Care of research animals is the responsibility of the Institutional Animal Care and Use Committee.

<https://www.memphis.edu/research/researchers/compliance/animalcareanduse.php>

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 PPQ 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 EHS 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 Bobby Clark, the EHS Director, 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 Bobby Clark Phone: 901-678-4672 E-mail: rclark12@memphis.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.

Chapter 7- Medical Surveillance

According to the BMBL, “Laboratory personnel must be provided medical surveillance, as appropriate,

and offered immunization for agents handled or potential present in the laboratory." Medical surveillance and vaccinations should be considered when infectious agents are present in the lab at the recommendation of the IBC. Work with human cells may require offer of vaccination to lab workers. For more details, consult the department Exposure Control Plan.

Chapter 8 Emergencies and Reporting

8.1 Spills

8.1.1 General

In general, most spills can be effectively handled by researchers and a spill kit 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, and gloves. A face shield, shoe covers or a respirator may be required.
- All spills must be reported to the Laboratory Supervisor and/or Principal Investigator. Contact the Biosafety Safety Officer (rclark12@memphis.edu) for any questions about spill cleanup procedures.

8.1.2 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. follow this procedure:

- Close off spill area to traffic, and notify coworkers.
- 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.
- Remove contaminated lab coat or clothing and wash exposed skin and put on clean gloves and lab coat.
- 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. However, eye protection must be worn and care taken not to splash the bleach onto skin or clothing.
- Contain the spill with paper towels or other absorbent material e.g. "bench kote", blue "diaper pads"
- Flood the spill area with disinfectant. Leave on for 10 minutes.
- 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 in biohazard box (see Chapter 9). If contact with bleach occurs with

skin, mucous membranes or eyes, flush area with copious amounts of water.

- Wash hands thoroughly. Autoclave an overtly contaminated lab coat to prior to placing into laboratory laundry bag.
- Report incident to supervisor and Principal Investigator.

8.1.3 Spills inside of a Biological Safety Cabinet (BSC)

If the spill is inside a biological safety cabinet, follow the following procedure:

- Leave BSC on.
- Put on gloves and lab coat and gather paper towels for cleaning.
- 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 as it evaporates too quickly to allow adequate surface contact time.
- Flood the area with disinfectant solution and allow to remain in contact for required time (10 minutes for 1:9 dilution of bleach, for others follow manufacturer's instructions).
- Spray and or wipe down the cabinet interior and any items inside the BSC with a towel dampened with disinfectant.
- Place the paper towels and gloves in a Zip-lock bag and contact EHS for disposal.
- Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination; consult BSO immediately at (ehs@memphis.edu), for guidance. If a spill such as this has occurred, do not turn the BSC off.
- Report incident to supervisor and Principal Investigator.

8.1.4 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. Then, follow this procedure:

- 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.
- Attempt to determine if the spill is contained in a closed cup, bucket or tray carrier, or within a closed rotor.
- If the spill is contained as described above, 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

(ehs@memphis.edu), for assistance.

- Obtain and place into the BSC containers suitable for holding tubes, broken glass or other containers while cleaning centrifuge components.
- 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.
- After proper decontamination, carriers, rotors etc. can be washed with a mild detergent according to the manufacturer's instructions.
- 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 (if you do not have any on hand, contact EHS) and request pickup of the bag using the EHS waste pickup form (See chapter 9).

8.1.5 Biological/Radioactive emergencies/spills

EHS must be notified and will assist in the cleanup of a biological/radioactive 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 (EHS). The infectious agent will be neutralized 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.

8.2 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:

- If the exposure creates a medical emergency, go to the closest emergency medical center or call 911.
- Wash the area with soap and water or flush eyes, nose or mouth with large amounts of water for 15 minutes.
- Students not employed by the university must report to the Student Health Center during business hours for personal exposures. During off hours, an outpatient facility may be used if necessary.
- All exposures must be reported to the immediate supervisor and Principal Investigator. The Principle Investigator is responsible for helping the employee in beginning the Workman's Compensation process and reporting the exposure. EHS personnel will instruct the supervisor and the employee on what to do.
- Principal Investigators are responsible for reporting exposure incidents to Biosafety Officer.
- The Biosafety Officer will perform a follow-up investigation of the incident and report findings to the Institutional Biosafety Committee (IBC).

8.3 Security incidents

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

Chapter 9 Biohazardous Waste Management

9.2 Biohazardous Waste Definition

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

- Pathological waste - all human unfixed tissues, organs and anatomical parts, other than intact skin, which emanate from surgeries, obstetrical procedures, dental procedures, autopsies and laboratories, including embalming waste. Such waste shall be exclusive of bulk formaldehyde and other preservative agents.
- Liquid or semi-liquid blood such as human blood, human blood components and/or products made from human blood (e.g., serum, plasma) and other potentially infectious materials, to include regulated human body fluids such as semen, vaginal secretions, cerebrospinal fluid, pleural fluid, pericardial fluid, synovial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids where it is difficult or impossible to differentiate between body fluids, can be discharged into the collection system of a publicly owned treatment works (POTW) within the generating facility. Breast milk, urine, and feces are not considered medical waste and can also be discharged into the (POTW).
- Contaminated items to include dressings, bandages, packing, gauze, sponges, wipes, personal protective equipment, cotton rolls and balls, etc., which cannot be laundered or disinfected and from which blood, blood components, or regulated body fluids drip freely, or that would release blood or regulated body fluids in a liquid or semi-liquid state if compressed or are caked with dried blood or regulated body fluids and all capable of releasing these materials during handling, not to include feminine products, enema bags, used condoms, or diapers.
 - Contaminated disposable, single-use gloves such as surgical or examination gloves shall not be washed or decontaminated for reuse and are to be handled as a contaminated item.
 - Protective coverings such as plastic wrap and aluminum foil used to cover equipment and environmental surfaces when removed following their contamination are considered a contaminated item.
 - All patient care items from hospital isolation rooms and end-stage renal dialysis units, or from patients with communicable diseases, which cannot be laundered and which are contaminated with regulated body fluids or blood or potential infectious material, must be considered a contaminated item.
 - Biological waste and discarded materials contaminated with blood, excretion, exudates,

or secretions from isolated animals known, or suspected, to be infected with communicable diseases.

- Microbiological waste which includes, but is not limited to cells and tissue cultures, culture medium or other solutions and stocks of infectious agents, organ cultures, culture dishes, devices used to transfer, inoculate and mix cultures, paper and cloth that has come in contact with specimens or cultures and discarded live or attenuated vaccines.
- Contaminated sharps which include, but is not limited to, any contaminated object that can penetrate the skin, e.g., hypodermic needles, intravenous tubing with needles attached, syringes with attached needles, razor blades used in surgery, scalpel blades, Pasteur pipettes, capillary tubes, broken glass from laboratories, and dental wires. Potentially breakable container(s) of blood, regulated body fluid, microbiological waste, or infectious material must be treated as contaminated sharps when disposed of. Sharps that have been used in human or animal patient care, treatment or for other medical procedures are included. Any waste produced in the course of physically altering a human being or animal including tattooing, ear piercing, or any other process where a foreign object is used to cut or pierce the skin. All waste generated in this manner meeting the definition of sharps must be handled accordingly.
- Veterinary waste to include any and all animal related waste (carcasses, body parts, bulk blood and blood products, bedding of animals, etc.) which meets the definition of any of the five categories delineated above and has been or is suspected to have been exposed to a zoonotic disease or pathogens known to cause human disease, or which has been exposed to human pathogens in research or the production of biological, must be handled as medical waste. All contaminated sharps and microbiological waste must be handled as medical waste.
- Trace contaminated chemotherapy waste that can be treated as medical/infectious waste. Bulk wastes such as full expired vials of chemotherapy drugs are considered to be chemical waste NOT biological waste and should be managed accordingly. Trace contaminated chemotherapy waste includes:
 - Masks,
 - Empty drug vials,
 - Gloves,
 - Gowns,
 - IV tubing,
 - Empty IV bags/bottles and
 - Spill clean-up materials.
- Spill/clean-up material collected during or resulting from the clean-up of a spill of regulated medical waste.
- Crime scene/accident/trauma clean-up waste generated by individuals or commercial entities hired to clean crime scenes or accidents that are saturated with human blood, sharps, or sharp objects contaminated with human blood.

- **Recombinant DNA.** All contaminated liquid, solid, carcasses, and animal wastes from research activities involving RG-2 agents requiring BSL-2 and ABSL-2 containment. All contaminated liquid, solid, carcasses, and animal wastes from research activities involving RG-3 agents requiring BSL-3 and ABSL-3 containment. **NOTE: Although RG1-associated DNA is not included in the definition of medical waste; all materials that have been in contact with recombinant DNA must be decontaminated prior to disposal in the sewer or trash. These items are not subject to the logging requirements described for autoclaves and chemical disinfection but they must still be rendered biologically inactive. This includes recombinant plants.**
- Additional wastes may be added at the discretion of the Institutional Biosafety Committee (IBC) and the EHS director.

9.3 Responsibilities

9.3.1 Principal Investigator or Faculty in Charge of Lab

The principal investigator or faculty in charge of the lab is responsible for:

- Identifying the biohazardous waste generated by their activity,
- Segregating biohazardous waste into the appropriate waste stream,
- Managing biohazardous waste in accordance with this protocol and
- Providing appropriate training to personnel under their supervision.

9.3.2 Biological Safety Officer (BSO)

The biological safety officer is responsible for:

- Providing training upon request for management of biohazardous waste and
- Inspecting laboratories to ensure compliance with this protocol.

9.3.3 Environmental Health and Safety (EHS)

EHS is responsible for:

- Picking up those biohazardous wastes requiring offsite treatment from laboratories in a timely manner,
- Final packaging, manifesting and shipping them for disposal and
- Recordkeeping for wastes shipped off-site in compliance with ADH regulations.

9.4 Disinfection

Any material or surface that comes in contact with an infectious (biohazardous) substance must be disinfected prior to reuse. Disinfection is accomplished by appropriate use of an EPA registered disinfectant used according to the label instructions for the agent being inactivated. The most familiar disinfectants that are registered with the EPA are bleach (active ingredient sodium hypochlorite) at a concentration of 10% (one part household bleach mixed with nine parts water, hitherto called 10% bleach), Cavicide and 70% isopropyl alcohol. However, the disinfectant chosen must be effective against the agent that is being inactivated. If assistance is needed with this, contact EHS.

9.5 Treatment, Storage and Disposal

Tennessee Department of Environment and Conservation requires that biohazardous waste be treated by chemical disinfection, incineration or steam sterilization before disposal. The University of Memphis requires that all biohazardous waste be treated with 10% bleach (liquids only), sterilized by autoclave or packaged for offsite contract incineration.

9.5.1 General Storage and Disposal Methods for Different Types of Waste

- Disposable sharps are deposited in a sharps container. All disposable sharps are treated offsite. 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 a clear autoclave bag lacking the biohazard symbol then 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 EHS. They are 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.

9.5.2 Chemical Treatment Standards

- Household bleach is 5.5 - 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 1:10 (v/v) dilution of bleach to liquid biological waste is required.
- A minimum contact time of sodium hypochlorite with liquid waste is 20 minutes. After 20 minutes of contact, disinfected liquid waste is poured into the sanitary sewer.
- Bleach should be stored between 50 and 75°F. 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).
- Any dilution of bleach has a shelf life of 5 days.

9.5.3 Autoclave Standards

All steam sterilizers should:

- 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;

- Record the temperature and time during each complete cycle to ensure the attainment of a temperature of 121 degrees Centigrade (250 degrees Fahrenheit) for 30 minutes or longer (refer to Appendix H 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 should be checked for calibration at least annually.)
- Have a gauge that indicates the pressure of each cycle.
- 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.
- Assure that treatment residues are disposed of in accordance with applicable State and Federal Requirements.

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). More information on the types of biological safety cabinets can be found in the University of Memphis Laboratory Safety Manual.

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. While the cost of certification of BSCs is covered by the University, costs for moving and decontamination might be borne by the Principal Investigator (PI). The Biosafety Officer will provide a list of designated, NSF-certified vendors from which to choose when laboratory staff is responsible for coordinating BSC services 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, pipeting 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 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 of the interior of the cabinet.
- 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*, 6th Edition and by *The World Health Organization Biosafety Manual*. Contact EHS for more information on safer alternatives.
- Open flames (i.e., Bunsen burners) are not permitted inside a biological safety cabinet.
- Dependence on ultraviolet light (UV) as the primary means of disinfection 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 Biohazardous Materials

Biohazardous materials are considered a hazardous material by both the US Department of Transportation and by the International Air Transport Association (IATA, which oversees air transport of hazardous materials). Like all hazardous material, special training is required to safely and legally ship biohazardous materials. Please see the University of Memphis Hazardous Materials Shipping plan to determine the type of training needed to ship biohazardous materials.

Biohazardous materials require special shipping containers. There are several vendors that sell the appropriate containers; consult with EHS before shipping biohazardous materials to make sure that the shipping containers that will be use meet the regulatory 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 University of Memphis material by University of Memphis 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 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 the University of Memphis Hazardous Materials Shipping Plan; most substances requiring transport at The University of Memphis are exempt from the DOT definition of infectious.

Substances not meeting the DOT definition of infectious, but requiring BSL-2 (or higher) containment or covered by The University of Memphis'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.

The University of Memphis 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.

Under NO circumstances may public transportation be used for transport of 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, primate and mammalian cell lines must be handled with standard precautions i.e. treated as though they are contaminated with infectious agents and should utilize BSL-2 practices.

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 EHS has adopted the following guidance:

- All cell and organ cultures of human origin, including well established cell lines, human bodily fluids, non-human primate and mammalian cells and tissues should be handled in accordance with the OSHA Bloodborne Pathogens Standard and under Biosafety Level 2 (BSL-2) containment.

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.

Appendix F NIH Oversight of Research Involving Recombinant DNA

The NIH Guidelines for Research Involving Recombinant DNA Molecules 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.

According to the Guidelines the Institution must establish an Institutional Biosafety Committee (IBC) to 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. In turn, the IBC 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, and set biosafety containment levels as required by the Guidelines for some experiments under III-D-4-b.

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 BSL-2 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 BSL-1-P and BSL-2-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 minute will partially temper is, 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.

<500 mL	500mL – 1L	1L – 2L	2L-4L	4L
30 minutes	40 minutes	45-55 minutes	55-60 minutes	60 minutes

For any additional questions regarding autoclave use and safety, contact EHS.