BALL STATE
UNIVERSITY

Biosafety Manual

Prepared by

Office of Research Integrity

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IMPORTANT TELEPHONE NUMBERS

EMERGENCY TELEPHONE NUMBER

Fire, Police, Emergency Medical Service **911**

ASSISTANCE TELEPHONE NUMBERS

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1. INTRODUCTION

1.1. Purpose

The purpose of this Manual is to establish the process for compliance with the following documents for Ball State University:

- *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) April, 2019,*
- *Biosafety in Microbiological and Biomedical Laboratories (BMBL), and*
- Occupational Safety and Health Administration Bloodborne Pathogens Standard, 29 CFR 1910.1030.

1.2. Scope

Ball State University is actively committed to preserving the health and safety of its students, staff, and faculty, and to protecting the environment and the community. It is recognized that use of potentially pathogenic microorganisms, other biological materials, and organisms containing recombinant or synthetic nucleic acid molecules is necessary in many University research and teaching laboratories. To ensure the safe handling of these organisms, the University requires compliance with the *NIH Guidelines* and with the recommendations in *BMBL*. Compliance with other applicable federal, state, and local regulations is also required.

2. AUTHORITY AND RESPONSIBILITY

2.1. BSU Office of Research Integrity

BSU Office of Research Integrity (ORI Biosafety) will be responsible for:

- Preparing the Biosafety Manual and reviewing as needed or at least annually;
- Making known the availability of the Biosafety Manual to each faculty member who works with biological materials;
- Investigating accidents involving biological materials and toxins, infectious agents, and recombinant or synthetic nucleic acid molecules;
- Tracking the certification of Biological Safety Cabinets (BSC's);
- Assisting and advising on the collection and disposal of biological waste when appropriate;
- Providing biosafety training;
- Conducting laboratory inspections;
- Assisting investigators with risk assessments and risk mitigation including recommending or requiring safety equipment and PPE as necessary;
- Administering all elements of the biosafety program;
- Assist faculty with protocol submissions to the IBC and review IBC submissions; and
- Reporting any significant problems with or violations of the NIH Guidelines and any significant research-related accidents or illness to the appropriate institutional official and to the NIH Office of Science Policy (OSP) immediately and within 30 days, as required.

2.2. Departments

Departments shall be responsible for:

- Ensuring the compliance of the Principal Investigators; and
- Providing appropriate work space for the research being performed.

2.3. Principal Investigators (PI)

For the purpose of this Manual, a Principal Investigator is defined as a person having ultimate responsibility for biological work being conducted and the oversight of personnel involved in said work at Ball State University. In addition to traditional Principal Investigators, this definition includes clinical laboratory directors, professors of teaching laboratories, and anyone else holding an equivalent position involved in biological work at Ball State University.

Principal Investigators shall be responsible for:

- Direct and primary responsibility for the safe operation of the laboratory;
- Assessing the risks of their experiments;
- Ensuring the safe operation of their laboratory;
- Notifying ORI Biosafety in the event of an injury or illness that occurs in the laboratory;
- Training laboratory personnel in safe lab-specific work practices;
- Ensuring they are providing necessary personal protective equipment;
- Complying with this Biosafety Manual as well as all applicable state and federal regulations and guidelines; and
- Registering the following experiments with the IBC :
    - Experiments involving recombinant or synthetic nucleic acid molecules, including transgenic plants and animals;
    - Work with infectious agents;
    - Experiments involving the use of human blood or other potentially infectious materials, such as unfixed human tissues, human cell lines, and certain body fluids; and animal and plant pathogens; and
    - Biological materials at BL2 or higher or the use of rDNA molecules that are intended to be used as part of teaching laboratories.

2.4. Institutional Biosafety Committee (IBC)

The IBC, as Administered by ORI Biosafety shall be responsible for:

- Reviewing recombinant or synthetic nucleic acid molecules research conducted at or sponsored by the University for compliance with the NIH Guidelines, and approve those research projects that are found to conform with the NIH Guidelines;
- Reviewing research involving infectious agents conducted at or sponsored by the University for compliance with the guidelines in Biosafety in Microbiological and Biomedical Laboratories (BMBL), and approving those research projects that are found to conform with the recommendations in BMBL;
- Notifying the PI of the results of the IBC's review and approval;
- Assist the ORI Associate Director with reporting any significant problems with or violations of the NIH Guidelines and any significant research-related accidents or illness to the appropriate institutional official and to the NIH Office of Science Policy (OSP) immediately and within 30 days, as required; and

- Following the guidelines for membership defined by NIH.

2.5. Designated Medical Service Provider

The Designated Medical Service Provider for the BSU campus shall:

- Provide medical evaluation, as required by the OSHA Bloodborne Pathogens Standard (CFR 1910.1030), and as recommended in the BMBL and NIH Guidelines; and
- Provide vaccinations, as recommended or required.

2.6. Laboratory Personnel

The Laboratory Personnel shall:

- Comply with safety recommendations for the work being performed;
- As applicable, follow lab procedures as described in the approved IBC protocol;
- Complete all required training;
- Report all accidents or injuries to the PI; and
- When the following incidents occur, notify the PI and ORI Biosafety:
  - Experiments involving recombinant or synthetic nucleic acid molecules;
  - Work with biological materials; or
  - Experiments involving the use of human blood or other potentially infectious materials, such as unfixed human tissues, human cell lines, and certain body fluids; and animal and plant pathogens.

3. PROGRAM ELEMENTS

3.1 Emergency Procedures

3.1.1. Biological Spills

Spill kit materials and written procedures are kept in each laboratory where work with microorganisms is conducted. Basic equipment includes concentrated disinfectant (such as chlorine bleach), absorbent material, latex or nitrile gloves, autoclave bags, sharps container, and forceps or other mechanical device to pick up broken glass. Do NOT handle broken glass with hands.

3.1.2. General Biological Spill Clean-Up Guidelines

- Wear gloves, protective eyewear and a lab coat.
- Use forceps or other mechanical means to pick up broken glass and discard into sharps container.
- Cover spilled material with paper towels.
- Add diluted disinfectant in sufficient quantity to ensure effective microbial inactivation, let sit 15 minutes.
- Dispose of towels in waste container.
- Wipe spill area with diluted disinfectant. Discard of clean-up materials in waste container.
- Wash hands with soap and water when finished.
- Report all spills to ORI Biosafety.

3.1.3. Specific Biological Spill Clean-Up Guidelines

3.1.3.1. Spill of BSL-1 material

- Wearing gloves and a lab coat, pick up broken glass with forceps and place in sharps container.
- Absorb the spill with paper towels or other absorbent material.
- Add diluted disinfectant in sufficient quantity to ensure decontamination, let sit for 15 minutes.
- Discard these materials into waste container.
- Wipe the spill area with the appropriate dilution of a disinfectant effective against the organism. Discard of clean-up materials in waste container.
- Autoclave all gloves and other materials worn to clean up the spill.
- Wash hands with soap and water.
- Report all spills to ORI Biosafety.

3.1.3.2. Spill of Human Blood

- Wear gloves, face protection and lab coat to clean up spill.
- If broken glass is present, use forceps to pick up and place in sharps container.
- Absorb blood with paper towels and add diluted disinfectant in sufficient quantity to ensure decontamination, let sit for 15 minutes.
- Using a detergent solution, clean the spill site of all visible blood.

- Discard all materials into trash container.
- Autoclave all gloves and other materials worn to clean up the spill.
- Wash hands with soap and water.
- Report all spills to ORI Biosafety.
- If an injury has occurred, complete an Occupational Injury/Illness Report and seek medical evaluation.

3.1.3.3. Spill of BSL-2 Material

- Keep other workers out of the area to prevent spreading of spill material.
- Post warning sign (Appendix D), if needed.
- Remove contaminated clothing and put in a biohazard bag for decontamination later.
- Wash hands and any exposed skin and inform the PI of the spill. Contact ORI Biosafety for assistance, if needed.
- Wear gloves, face protection and lab coat to clean up spill.
- If broken glass is present, use forceps to pick up and place in sharps container.
- Absorb the spill with paper towels and add diluted disinfectant in sufficient quantity to ensure decontamination, let sit for 15 minutes.
- Discard all materials into waste container.
- Wipe the spill area with the appropriate dilution of a disinfectant effective against the organism. Discard of clean-up materials in waste container.
- Autoclave all gloves and other materials worn to clean up the spill.
- Wash hands with soap and water.
- Report all spills to ORI Biosafety.
- If an injury has occurred, complete an Occupational Injury/Illness Report and seek medical evaluation.

3.1.3.4. Spill of Recombinant or Synthetic DNA Material

- Keep other workers out of the area to prevent spreading of spill material.
- Post warning sign (Appendix D), if needed.
- Remove contaminated clothing and put in a biohazard bag for decontamination later.
- Wash hands and any exposed skin and inform the PI of the spill. Contact ORI Biosafety for assistance, if needed.
- Wear gloves, face protection and lab coat to clean up spill.
- If broken glass is present, use forceps to pick up and place in sharps container.
- Absorb the spill with paper towels and add diluted disinfectant in sufficient quantity to ensure decontamination, let sit for 15 minutes. For best results, use a fresh 10% vol/vol dilution of bleach.
- Discard all materials into waste container.
- Wipe the spill area with the appropriate dilution of a disinfectant effective against the organism. Discard of clean-up materials in waste container.

- Autoclave all gloves and other materials worn to clean up the spill.
- Wash hands with soap and water.
- Report all recombinant or synthetic DNA spills to the ORI Biosafety immediately.
- If an injury has occurred, complete an Occupational Injury/Illness Report and seek medical evaluation.

3.1.3.5. Spill of BSL-3 Material

- Stop work immediately.
- Avoid inhaling airborne material while quickly leaving the room. Notify others to leave. Close door, and post with warning sign (Appendix D).
- Prior to leaving the anteroom, remove contaminated clothing, turn exposed area inward, and place in a biohazard bag. Wash hands with soap and water.
- Notify the PI and ORI Biosafety immediately. Do not reenter the lab until given permission from ORI Biosafety. After hours and weekends call 911.
- Following instruction from the Biological Safety Officer, allow 30 minutes for aerosols to disperse before re-entering the laboratory to begin clean-up.
- If given authority to clean the spill, put on personal protective equipment (HEPA filtered respirator, gown, gloves, and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, sharps container, and paper towels).
- Contain the spill with absorbent paper towels or disposable pads. Carefully add appropriate disinfectant to the spill; avoid creating aerosols when pouring the disinfectant. Leave the room and allow 30 minutes for the disinfectant to inactivate the material.
- Pick up broken glass with forceps and discard in sharps container.
- Clean up liquid with paper towels and collect all contaminated materials into biohazard bag or container. Remove all spilled materials and decontaminate the area again with an appropriate disinfectant.
- Autoclave (or soak in 10% bleach solution) lab coat, gloves, and other protective equipment that was worn for clean-up.
- Wash hands thoroughly with soap and water.
- If an injury has occurred, complete an Occupational Injury/Illness Report and seek medical evaluation.

3.1.3.6. Spill in a Biological Safety Cabinet

- Leave the cabinet fan running.
- Wearing gloves and lab coat, spray or wipe cabinet walls, work surfaces, and equipment with an appropriate disinfectant such as 10% bleach or 70% ethanol. If necessary, flood work surface, as well as drain pans and catch basins below the work surface, with disinfectant. Allow at least 20 minutes contact time.
    - Contact ORI Biosafety if uncertain as to the proper

disinfectant to use

- Soak up the disinfectant and spill with paper towels, and drain catch basin into a container. Lift front exhaust grille and tray, and wipe all surfaces. Ensure that no paper towels or solid debris are blown into area below the grille.
- Surface disinfect all items that may have been splashed on before removing them from the cabinet.
- Discard all clean-up materials into biohazard waste container. Wash hands and exposed skin areas with soap and water.
- ORI Biosafety should be notified if the spill overflows into the interior of the cabinet. It may be necessary to do a more extensive decontamination of the cabinet.

3.1.3.7. Spill of Radioactive Biological Material

A spill involving both radioactive and biological materials requires emergency procedures that are different from the procedures used for either material alone. As a general rule, disinfect the microorganism using a chemical disinfectant, then dispose of all clean-up materials in a separate bag/container labeled to indicate that the radioisotope is mixed with a chemically disinfected microorganism. **Do not use bleach solutions as a disinfectant on materials that contain iodinated compounds because radioactive iodine gas may be released.** Be sure to use procedures to protect yourself from the radionuclide while disinfecting the biological material. Before any clean-up, consider the type of radionuclide, the characteristics of the microorganism, and the volume of the spill. Contact the BSU Radiation Safety Office for specific radioisotope clean-up procedures.

3.1.3.7.1. *Preparation for Clean-up*

- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave.
- Close door and post with warning sign.
- Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag.
- Wash all exposed skin with soap or hand washing antiseptic, followed by a three-minute water rinse.
- Inform the PI, ORI Biosafety, and Radiation Safety of the spill and monitor all exposed personnel for radiation.
- Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up materials (diluted disinfectant, autoclavable containers, forceps, paper towels, sharps container).
- Confirm with the Radiation Safety Officer that it is safe to enter the lab.

3.1.3.7.2. *Clean-up of Radioactive Biological Spill*

- Put on protective clothing (lab coat, face protection, gloves, and shoe covers). Depending on the nature of the spill, it may be advisable to wear a HEPA filtered

respirator instead of a surgical mask. In setting up your spill plan, contact ORI Biosafety for advice since the use of many types of respirators requires prior training, fit-testing, and medical approval.

- Pick up any sharp objects with forceps and put in sharps container labeled according to Radiation Safety guidelines.
- Cover the area with paper towels, and carefully pour diluted disinfectant around and into the spill. Avoid enlarging the contaminated area. Use additional disinfectant as it becomes diluted by the spill. Allow at least 20 minutes contact time. **Do not use bleach solutions on iodinated materials**; radioactive iodine gas may be released. Instead, use an alternative disinfectant such as an iodophor.
- Wipe surrounding areas where the spill may have splashed with disinfectant.
- Absorb the disinfectant and spill materials with additional paper towels, and place into an approved radioactive waste container. Keep separate from other radioactive waste. Do not autoclave radioactive isotope-contaminated biological waste unless approved by the Radiation Safety Officer.
- Disinfect contaminated protective clothing prior to disposal as radioactive waste.
- Place contaminated item(s) on absorbent paper and scan for radioactivity. If none is detected, dispose of these items as biohazard waste.
- If radioactive, spray with disinfectant and allow a 20-minute contact time. Wrap the item(s) inside the absorbent paper and dispose of as radioactive waste.
- Wash hands and exposed skin areas with soap and water, and monitor personnel and spill area for residual radioactive contamination. If skin contamination is detected, repeat decontamination procedures under the direction of the Radiation Safety Officer. If spill area has residual activity, determine if it is fixed or removable and handle it accordingly.

3.2. Injury Involving Biological Materials

Any individual who experiences an exposure or potential exposure while on campus, will be offered a medical consultation and advised of available treatments at the Ball State Student Health Center in the Amelia T. Wood building at 1500 W. Neely Avenue, Muncie, IN 47306.

Exposure or potential exposure involving biological materials can occur from any of the following:

- Contact with non-intact skin such as cuts, rashes, or abrasions;
- Contact with mucosal membranes-eyes, nose, and mouth; and
- Sharps puncturing or cutting the skin.

Should an exposure occur:

- If immediate threat to life call 911; otherwise
- Wash the exposed area for 15 minutes;
- Report the incident to your work supervisor immediately;
- Notify ORI Biosafety of the exposure;
- Follow campus specific procedures and proceed to the first floor of the Ball State Student Health Center. Medical personnel will evaluate you and you will be asked to complete an Occupational Injury/Illness Report.

Lab specific procedures may differ slightly and in such cases, these must be followed, while ensuring that the minimum above requirements are also met.

3.3. Introduction to Biohazardous Materials and Research

Laboratory research involving biological agents are subject to various federal and state regulations depending on the nature of the agents used and the experimental manipulations in which they will be employed. The following section of this Manual is intended to serve as a guide to the various federal and state agencies that govern biological research and their laws, regulations, and guidelines.

Principal Investigators are responsible for understanding the scope of their research program, identifying the regulations to which their work is subject, and complying with those regulations. ORI Biosafety is available to assist the Principal Investigator should guidance be needed in identifying and complying with those laws, regulations, and guidelines.

Principal Investigators should also note that many granting agencies require that grant recipients certify compliance with all relevant laws, regulations, and guidelines to which their research is subject. The scope of these regulations includes procedures and facilities involved in protecting laboratory workers, the public, and the environment from laboratory biological hazards.

3.3.1. Microorganisms

The National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) publish guidelines for work with infectious microorganisms. The publication, entitled Biosafety in Microbiological and Biomedical Laboratories (BMBL) recommends that work be done using one of four levels of containment: Biosafety Level 1 (BSL-1), BSL-2, BSL-3 and BSL-4 (see section 3.4). The *NIH Guidelines* (Appendix E1-3) classifies pathogenic agents into one of four risk groups according to specific criteria. Ball State University requires that all laboratories adhere to these NIH/CDC guidelines. Noteworthy, there are no BSL-3 or BSL-4 laboratories on the BSU campus.

3.3.2. Microorganisms Capable of Causing Infection in Humans

Investigators must register any project involving a pathogenic agent with the IBC and receive its approval before work has begun. Following receipt of the completed IBC Protocol Submission Form, the laboratory will be inspected by ORI Biosafety to ensure that it meets the containment requirements listed in *BMBL* for the agent being studied. If the lab meets the requirements, the work will be reviewed and approved or disapproved by the IBC.

3.3.3. Genetically Engineered Microorganisms

Work with all genetically engineered organisms must comply with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*. These guidelines classify recombinant or synthetic nucleic acid molecules experiments into four levels of containment (BSL-1, BSL-2, BSL-3, and BSL-4) based on the hazard of the microorganism and the procedures and quantities being used. Additionally, the United States Department of Agriculture (USDA) requires permits for field testing of genetically engineered plants. Ball State University requires that all laboratories follow and ensure compliance with these guidelines.

3.3.4. Registration Document

Each PI is responsible for submitting IBC protocols for all experiments involving biohazardous materials at BSL-2 or higher, biological toxins, and recombinant or synthetic nucleic acid molecules, including those exempt from *NIH Guidelines*. ORI Biosafety inspects all laboratories where BSL-2 or BSL-3 biocontainment is required, and all BSL-1 laboratories which are subject to the *NIH Guidelines* prior to protocol approval.

3.3.5. Review and Approval of Experiments

The IBC, which oversees recombinant and synthetic nucleic acid molecule research at Ball State University will review and approve the submitted protocol or amendment based on the submission status according to the NIH Guidelines, which are generally summarized below. More specific information about the categories and corresponding approval can be found with the Office of Research Integrity.

3.3.5.1. Experiments covered by the NIH Guidelines

Many experiments involving recombinant or synthetic nucleic acid

molecules require registration and approval by the IBC before work may be initiated.

Experiments that require IBC approval before initiation include those that involve:

- Risk Group 2, 3, 4, or Restricted Agents as host-vector systems, cloning DNA from Risk Group 2, 3, 4, or Restricted Agents into nonpathogenic prokaryotic or lower eukaryotic host-vector systems, infectious virus, or defective virus in the presence of helper virus in tissue culture;
- Whole plants or animals; and
- More than 10 liters of culture.

Experiments that must be registered at the time of initiation include those that involve:

- The formation of recombinant or synthetic nucleic acid molecules containing no more than 2/3 of the genome of any eukaryotic virus propagated in tissue culture, recombinant or synthetic nucleic acid molecules-modified whole plants, and/or recombinant or synthetic nucleic acid molecules-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-C, or III-D of the NIH Guidelines; and
- The generation of transgenic rodents that require BSL-1 containment.

3.3.5.2. Experiments exempt from the NIH Guidelines

Experiments exempt from the *NIH Guidelines*, although requiring registration with the IBC, may be initiated immediately. ORI Biosafety will review the registration and confirm that the work is classified correctly according to the *NIH Guidelines*. Exempt experiments are those that:

- Use synthetic nucleic acids that can neither replicate nor generate nucleic acids capable of replicating in any living cell; are not designed to integrate into DNA, and do not produce a toxin that is lethal for vertebrates at an LD50 of <100 ng/kg body weight;
- Use recombinant or synthetic DNA molecules that are not in organisms or viruses;
- Consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent;
- Consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well-established physiological means;
- Consist entirely of DNA from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species);
- Consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent;

- Do not present a significant risk to health or the environment as determined by the NIH Director, with the advice of the Recombinant DNA Advisory Committee (RAC), and following appropriate notice and opportunity for public comment;
- Contain less than one-half of any eukaryotic viral genome propagated in cell culture;
- Use E. coli K12, Saccharomyces cerevisiae, or Bacillus subtilis host-vector systems, unless genes from Risk Group 3 or 4 pathogens are cloned into these hosts; and
- Involve the purchase or transfer of transgenic rodents for experiments that require BSL-1 containment.
- Work with biohazardous materials at BL2 that does not utilize recombinant or synthetic nucleic acid molecules.

3.3.6. Human Blood, Unfixed Tissue, and Cell Culture

Please refer to the *Ball State University Bloodborne Pathogens Exposure Control Plan* for detailed information on handling human material.

Work with human material is regulated by the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard, 29 CFR 910.1030. Human blood, unfixed tissue, cell culture, and certain other body fluids are considered potentially infectious for bloodborne pathogens such as hepatitis B virus (HBV), hepatitis C virus (CV), and human immunodeficiency virus (HIV). All human clinical material shall be presumed infectious and handled using BSL-2 work practices. This concept is called Universal Precautions. Principal Investigators are responsible for registering their use of human materials so training and immunization can be provided as required by OSHA.

3.3.7. Select Agents

Select Agents are microorganisms and toxins that have potential for criminal misuse to cause harm. The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 restricts their possession and use, and requires the University to collect and maintain information on the location and use on campus of any select agents or toxins. Please contact ORI Biosafety if you currently possess or plan to acquire any of the agents listed in Appendix A and have not yet reported that fact. Failure to provide notice may result in civil and criminal liability for individual researchers and/or the University. If you have questions, you may contact ORI Biosafety or visit the federal Select Agent website www.selectagents.gov which provides links to select agent program information.

3.3.8. Non-Human Primate (NHP) Unfixed Tissue and Primary Cell Culture

Non-human primates and their tissues pose special zoonotic risks as many of their diseases are often transmissible to humans and can be a serious health hazard. Although there are a number of NHP viruses that can cause disease in humans, monkeys of the genus Macaca, or their unfixed tissues, can carry the virus Cercopithecine herpesvirus 1 (other terms used: Herpes B-virus, Herpesvirus simiae, or simply B-virus). B-virus is frequently carried by Rhesus and Cynomolgus macaques, as well as other macaques. It can cause fatal encephalitis in humans.

Prior to working with any NHP primary cell cultures or unfixed tissues, PIs must register their work, and lab personnel must be trained in the safety procedures required for handling and post-exposure procedures. Sharps use with these materials shall be eliminated or restricted.

3.4. Biosafety Containment Levels

Four levels of biosafety are defined in the publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, published by the CDC and NIH. The levels, designated in ascending order by degree of protection provided to personnel, the environment, and the community, are combinations of laboratory practices, safety equipment, and laboratory facilities (see Appendices E1-3). Most microbiological work at Ball State University is conducted at BSL-1 or BSL-2 containment. The Ball State University Biosafety Manual supersedes the information in the BMBL Appendices E1-3 and must be followed should information differ.

Below is a summary of each biosafety level. Detailed criteria for each level are described in Appendix E1-3.

3.4.1. Biosafety Level 1

Suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment or to protect the research materials. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science. Use personal protective equipment as appropriate, including lab coats and gloves. Use eye protection when splashing is likely.

Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

3.4.2. Biosafety Level 2

Practices, equipment, and facility design and construction are applicable to research, clinical, diagnostic, and teaching laboratories in which work is done with moderate- risk agents that are present in the community. Hepatitis B virus, HIV, salmonellae, and *Toxoplasma* spp. 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 shall 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 shall 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 such as a biological safety cabinet (BSC) or safety centrifuge cups. Personal protective equipment shall be used as appropriate, including lab coats and gloves. Eye protection shall be used when splashing is likely.

Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

3.4.3. Biosafety Level 2+ BSL3 Practices

Used to describe biocontainment within a Biosafety Level 2 laboratory but using specific Biosafety Level 3 practices. This is not intended to be used as a substitute for Biosafety Level 3 with any Risk Group 3 biohazards. The final determination of this biocontainment is based on a risk assessment of the research planned. The risk assessment and review by the IBC may determine that safety practices above BSL-2 are required, but the research does not warrant the more complex BSL-3 laboratory suite.

No inclusive list of BSL-2+ viral vectors, microorganisms, biohazards, or experimental designs exists and decisions on BSU research biocontainment is based on case-by-case risk assessment. The focus of BSL-2 + BSL-3 Practices is a reduction in exposure to aerosols and/or particularly hazardous agents that do not quite meet the definition of Risk Group 3 biohazards. Some examples of experiments that may fall under the above definition would be:

- Viral vectors that have inserts of oncogenes or other gene products that may be toxic, particularly if injections are involved;
- Specific multi-drug resistant BSL-2 bacteria;
- High concentrations of Risk Group 2 viruses represented as inhalation hazards; and
- Large volumes of viral vectors, i.e., greater than 10 liters.

3.4.4. 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 on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, researchers perform all laboratory manipulations in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. At this level, secondary barriers 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 laboratories at Ball State University.

3.4.5. Biosafety Level 4

Practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. There are no BSL-4 laboratories at Ball State University.

Summary of Recommended Biosafety Levels

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	• PPE: Laboratory coats; latex or nitrile disposable gloves; eye/face protection as needed	Laboratory bench and sink required. Autoclave available
2	• Agents associated with human disease • Routes of transmission include percutaneous injury, ingestion, and mucous membrane exposure	BSL-1 practices plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety Manual defining any needed waste decontamination or medical evaluation program	Primary barriers: • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials	BSL-1 plus: • Recommended negative differential pressure • Readily available eyewash
3	• Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequences	BSL-2 practices plus: • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum	Primary barriers: • Class I or II BSCs or other physical containment devices used for all open manipulation of agents • PPE: • Protective laboratory clothing; latex or nitrile disposable gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into laboratory
4	• Dangerous/exotic agents which pose high risk of life-threatening disease • Aerosol transmitted laboratory infections have occurred; or related agents with unknown risk of transmission	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers: • All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum • Decontamination systems • Other requirements outlined in the BMBL

3.5. Animal Facilities

Four standard biosafety levels are also described for activities involving infectious disease work with commonly used experimental animals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4, and provide increasing levels of protection to personnel and the environment.

One additional biosafety level, designated BSL-3-Agriculture (or BSL-3-Ag) addresses activities involving large or loose-housed animals and/or studies involving agents designated as High Consequence Pathogens by the USDA. BSL-3-Ag laboratories are designed so that the laboratory facility itself acts as a primary barrier to prevent release of infectious agents into the environment. More information on the design and operation of BSL-3-Ag facilities and USDA High Consequence Pathogens can be found in *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*.

3.5.1. Animal Biosafety Level 1 (ABSL-1)

Assigned for animal work that does not involve biological agents or involves well-characterized agents that are not known to cause disease in immunocompetent humans, and that are of minimal potential hazard to laboratory personnel and the environment.

3.5.1.1. ABSL-1 Facility Requirements

In addition to the facility requirements listed for BSL-1 laboratories, ABSL-1 laboratories must meet the following requirements:

- Animal facilities must be separated from areas that are open to unrestricted personnel traffic.
- External facility doors must be self-closing and self-locking.
- Doors to animal rooms must open inward, be self-closing, and kept closed when experimental animals are present.
- The animal care facility must be designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors, and ceilings) must be water-resistant.
- Windows are not recommended. Any windows must be resistant to breakage. Where possible, windows should be sealed.
- If floor drains are provided, the traps should always be filled with an appropriate disinfectant.
- Ventilation should be provided in accordance with the *Guide for Care and Use of Laboratory Animals*, latest edition. No recirculation of exhaust air may occur. It is recommended that animal rooms maintain negative pressure compared to adjoining hallways.
- The facility must have a hand washing sink.
- Cages are washed manually or in a cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- Emergency eyewash and shower must be readily available.

3.5.2. Animal Biosafety Level 2 (ABSL-2)

Assigned for animal work with those agents associated with human disease that pose moderate hazards to personnel and the environment. ABSL-2 builds on the

practices, procedures, containment equipment, and facility requirements of ABSL-1.

3.5.2.1. ABSL-2 Facility Requirements

In addition to the facility requirements listed for BSL-2 and ABSL-1 laboratories, ABSL-2 laboratories must meet the following requirements:

- Access to the facility is limited with secure locked doors.
- Ventilation should be provided in accordance with criteria from *Guide for Care and Use of Laboratory Animals, latest edition*. The direction of airflow in the animal care facility is inward; animal rooms should maintain negative pressure compared to adjoining hallways.
- An autoclave should be available in the animal care facility to decontaminate infectious waste.
- A hand washing sink must be in the animal room where infected animals are housed or manipulated, as well as elsewhere in the facility.

Facility standards and practices for invertebrate vectors of disease and hosts are not specifically addressed in this section. Refer to the *Arthropod Containment Guidelines* for containment requirements for experimentally infected arthropod vectors of disease.

3.5.3. Animal Biosafety Level 3 (ABSL-3)

Assigned to animal work involving indigenous or exotic agents that present the potential of aerosol transmission and of causing serious or potentially lethal disease. ABSL-3 builds on the practices, procedures, containment equipment, and facility requirements of ABSL-2.

3.6. Clinical Laboratories

Clinical laboratories, especially those in health care facilities, receive clinical specimens with requests for a variety of diagnostic and clinical support services. Typically, the infectious nature of clinical material is unknown, and specimens are often submitted with a broad request for microbiological examination for multiple agents (e.g., sputa submitted for "routine," acid-fast, and fungal cultures). *It is the responsibility of the Laboratory Director to establish standard procedures in the laboratory that realistically address the issue of the infective hazard of clinical specimens*.

Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and serological identification of isolates can be done safely at BSL-2, the recommended level for work with bloodborne pathogens such as HBV and HIV. The containment elements described in BSL-2 are consistent with the OSHA standard*, "Occupational Exposure to Bloodborne Pathogens."* This requires the use of specific precautions with **all** clinical specimens of blood or other potentially infectious material (Universal or Standard Precautions). Additionally, other recommendations specific for clinical laboratories may be obtained from the Clinical Laboratory Standards Institute.

3.7. Laboratory Attire and Personal Protective Equipment

- Shoes must cover the entire foot. Open toed shoes and sandals are inappropriate footwear in laboratories. Fabric and athletic shoes offer little or no protection from biological spills. Leather shoes with slip-resistant soles are recommended.
- Street clothing is to be chosen so as to minimize exposed skin below the neck. Long pants are required. Avoid rolled up sleeves. Shorts (including cargo shorts), capris, miniskirts, tank tops, sleeveless shirts and midriff-length shirts are inappropriate clothing in laboratories.
- Laboratory coats are designed to be fluid resistant and help protect the user from accidental splashes and spills of biological material. Laboratory coats are required whenever working with biological material.
- Gloves must be worn whenever handling or working with biological material. Latex and nitrile gloves are typically appropriate for work in biological laboratories. It is important to keep in mind what chemicals will be used alongside biologicals and chose gloves appropriate for the task that will be performed. Gloves should be chosen that are the appropriate size to help minimize the risk for incident.
- Safety glasses or goggles are required whenever there is a risk of splashing.
- Additional PPE, including a face shield, a face mask, or an N95 respirator may be required depending on the agent in use and the planned manipulations. These requirements will be outlined in any approved IBC protocols.

3.8. Use of Biohazard Labels

Biohazard labels are labels incorporating the universal biohazard symbol. They are stating that the item is either contaminated with biohazardous material or contains biohazardous material. Biohazard labels must be red or red-orange with biohazard symbol and the word “biohazard” in a contrasting color. Biohazard labels are required to be used in the following situations:

- On the outside door of laboratories where biological material is stored or manipulated;
- Bags/containers of biological waste;
- Bags/containers of contaminated laundry;
- Refrigerators and freezers used to store biological material;
    - Refrigerators and freezers in common storage rooms should have individual emergency contact information on each.
- Bag/containers used to store, dispose of, transport, or ship biological material; and
- Contaminated equipment to be serviced or shipped.

3.9. Biological Safety in Teaching Laboratories

Teaching laboratories are frequently used for laboratory classes, demonstrations, and lectures. Because hazards are present in these areas, the following rules and guidelines are provided to ensure that students and instructors are safe and compliant. These guidelines are intended to assist in outlining the requirements for use of personal protective equipment (PPE), the use of appropriate street clothing, and prohibition of food and drinks in laboratories. The classroom instructor is responsible for ensuring that participants adhere to these rules and guidelines.

3.9.1. Food and Drinks

- No food or drinks are permitted in teaching laboratories at any time.

3.9.2. Personal Protective Equipment (PPE)

3.9.2.1. When hazards are present or used in the laboratory:

- The instructor and students must utilize the appropriate PPE for the experiments.
- PPE is always selected and used based on the hazards presented and risk of exposure (i.e. when biological materials are present and nearby on bench tops, biological safety cabinets, etc. or laboratory procedures are being performed).
- Safety eyewear (goggles, safety glasses) must be used to prevent injury or exposure of the eyes.
- Protective clothing (lab coats) must be worn to prevent contamination of the body and street clothes. Protective clothing must be left in the lab or locker at the end of the period.
- Appropriate chemically resistant gloves must be used for handling chemicals to prevent contamination of the hands.

3.9.2.2. When biological demonstrations are being performed for observational purposes:

- The instructor and audience must be equipped with the appropriate PPE to protect them from the hazards associated with the demonstration.
- Instructors must not deviate from the established procedures or adjust quantities of materials during the demonstration without prior approval.

3.9.2.3. ***When hazards are not present in the laboratory*** *(i.e. when all chemicals, biological, or radiological materials are secured in closed containers (chemical cabinets, biological freezers or incubators) and bench surfaces are clean and/or decontaminated:*

- PPE is unnecessary and does not need to be utilized.

3.9.3. Street Clothes

Street clothing and footwear appropriate for laboratory work must be worn by the instructor and students for all activities (including lectures, lab sessions, and demonstrations) because some lectures are followed by lab sessions in the same course.

Street clothing should be chosen so as to minimize exposed skin below the neck. Long pants are required. Avoid rolled up sleeves. Shorts (including cargo shorts),

capris, miniskirts, tank tops, sleeveless shirts and midriff-length shirts are inappropriate clothing in laboratories.

Shoes must cover the entire foot. Open-toed shoes and sandals are inappropriate footwear in laboratories. Fabric and athletic shoes offer little or no protection from chemical spills. Leather shoes with slip-resistant soles are recommended.

When PPE is utilized for laboratory sessions and demonstrations (not lectures) long hair must be restrained and jewelry/watches removed.

3.10. Decontamination

Sterilization, disinfection, and antisepsis are all forms of decontamination. **Sterilization** implies the killing of all living organisms. **Disinfection** refers to the use of antimicrobial agents on inanimate objects; its purpose is to destroy all non-spore forming organisms. **Antisepsis** is the application of a liquid antimicrobial chemical to living tissue.

3.10.1. Chemical Disinfectants

Chemical disinfectants are used to render a contaminated material safe for further handling, whether it is a material to be disposed of as waste, or a laboratory bench on which a spill has occurred. It is important to choose a disinfectant that has been proven effective against the organism being used. Chemical disinfectants are registered by the EPA under the following categories:

- Sterilizer or Sterilant - will destroy all microorganisms including bacterial and fungal spores on inanimate surfaces.
- Disinfectant - will destroy or irreversibly inactivate specific viruses, bacteria, and pathogenic fungi, but not bacterial spores.
- Hospital Disinfectant - agent shown to be effective against *S. aureus*, *S. choleresis* and P*. aeruginosa*. It may be effective against *M. tuberculosis*, pathogenic fungi or specifically named viruses.
- Antiseptic - agent formulated used on skin or tissue - not a disinfectant.

3.10.2. Disinfectants Commonly Used in the Laboratory

3.10.2.1. Iodophors

- Recommended dilution is 75 ppm, or approximately 4.5 ml/liter water.
- Effective against vegetative bacteria, fungi, and viruses.
- Effectiveness reduced by organic matter (but not as much as with hypochlorites).
- Stable in storage if kept cool and tightly covered.
- Built-in color indicator; if solution is brown or yellow, it is still active.
- Relatively harmless to humans.

3.10.2.2. Hypochlorites (bleach)

- Working dilution is 1:10 to 1:100 household bleach in water.

- Effective against vegetative bacteria, fungi, most viruses at 1:100 dilution.
- Effective against bacterial spores at 1:10 dilution.
- Very corrosive.
- Rapidly inactivated by organic matter.
- Solutions decompose rapidly; fresh solutions used must be made daily.

3.10.2.3. Alcohols (ethanol, isopropanol)

- The effective dilution is 70-85%.
- Effective against a broad spectrum of bacteria and many viruses.
- Fast acting.
- Leaves no residue.
- Non-corrosive.
- Not effective against bacterial spores.

3.10.3. Important Characteristics of Disinfectants

	Hypochorites "Bleach"	Iodoform "Wescodyne"	Ethyl Alcohol
Shelf-life > 1 week		X	X
Corrosive	X	X	
Residue	X	X	
Inactivation by Organic Matter	X	X	
Skin Irritant	X	X	
Respiratory Irritant	X		
Eye Irritant	X	X	X
Toxic	X	X	X

3.10.4. Dilution of Disinfectants

3.10.4.1. Chlorine compounds (Household Bleach)

Dilution in Water	% Available Chlorine	Available Chlorine (mg/l or ppm)
Not diluted	5.25	50,000
1/10	0.5	5,000

1/100	0.05	500

Bleach solutions decompose at room temperature and should be made fresh daily. However, if stored in tightly closed brown bottles, bleach solutions retain activity for 30 days. The use concentration is dependent on the organic load of the material to be decontaminated. Use a 1/100 solution to disinfect clean surfaces, and 1/10 solution to disinfect surfaces contaminated with a heavy organic load. To disinfect liquid biological waste before disposal, add concentrated bleach to a final concentration of 10% vol/vol.

3.10.4.2. Iodophor

Manufacturer's recommended dilution is 3 ounces (90 ml) into 5 gallons water, or approximately 4.5 ml/liter. For porous surfaces, use 6 ounces into 5 gallons water, or approximately 9 ml/liter.

3.10.4.3. Alcohols

Ethyl alcohol and isopropyl alcohol diluted to 70 - 85% in water are useful for surface disinfection of materials that may be corroded by a halogen or other chemical disinfectant.

3.11. Autoclaving Procedures for Biological Waste

Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable system available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents.

For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation.

3.11.1. Container Selection

3.11.1.1. Polypropylene bags

Commonly called biohazard or autoclave bags, these bags are able to withstand autoclaving and are tear resistant, but can be punctured or burst during autoclaving. Therefore, place bags in a rigid container such as a polypropylene or stainless steel pan during autoclaving. Bags are available in a variety of sizes, and some are printed with an indicator that changes color when processed.

Polypropylene bags are impermeable to steam, and for this reason should not be twisted and taped shut, but gathered loosely at the top and secured with a large rubber band or autoclave tape. This will create an opening through which steam can penetrate.

3.11.1.2. Polypropylene Containers and Pans

Polypropylene is a plastic capable of withstanding autoclaving, but resistant to heat transfer. Therefore, materials contained in a polypropylene pan will take longer to autoclave than the same materials in a stainless steel pan. To decrease the time required to sterilize material in these containers do the following:

- Remove the lid (if applicable).
- Turn the container on its side when possible.
- Select a container with the lowest sides and widest diameter possible for the autoclave.

3.11.1.3. Stainless Steel Containers and Pans

Stainless steel is an efficient conductor of heat and is less likely to increase sterilizing time, though is more expensive than polypropylene.

3.11.2. Preparation and Loading of Materials

- Fill liquid containers only half full.
- Loosen caps, or use vented closures.
- Always put bags of biological waste into autoclavable pans to catch spills.
- Position biohazard bags on their sides, with the bag neck taped loosely.
- Leave space between items to allow steam circulation.
- Household dishpans melt in the autoclave. Use autoclavable polypropylene or stainless steel pans.
- Add water to loads containing dry or absorbent material to facilitate proper steam generation and sterilization.

3.11.3. Cycle Selection

- Use liquid cycle when autoclaving liquids, to prevent contents from boiling over.
- Select fast exhaust cycle for glassware.
- Use fast exhaust and dry cycle for wrapped items.

3.11.4. Time Selection

- Bags of biological waste should be autoclaved for 60 minutes at 121°C and 15 psi to assure decontamination.
- Take into account the size of the articles to be autoclaved. A 2-liter flask containing 1 liter of liquid takes longer to sterilize than four 500 ml flasks each containing 250 ml of liquid.
- Material with a high insulating capacity (animal bedding, high-sided polyethylene containers) increases the time needed for the load to reach sterilizing temperatures.

3.11.5. Removing the Load Safely

CAUTION - AUTOCLAVES MAY CAUSE SERIOUS BURNS. TO PREVENT INJURY:

- Check that chamber pressure has returned to zero before opening door.
- Wear eye and face protection. Wear thermal protective gloves to handle materials.

- Stand behind door when opening it.
- Slowly open door only a crack. Beware rush of steam as a burn hazard is present.
- Keep face away from door as it opens. Escaping steam may burn face.
- Wait 5 minutes after opening door before removing liquids.
- Liquids removed too soon may boil up and out of container, burning operator.

It is the responsibility of the autoclave user to transport autoclaved waste to the regular trash or dumpster. Follow your departmental specific procedures.

3.12. Autoclave Monitoring and Validation

Autoclaves used to decontaminate laboratory waste should be tested periodically to assure effectiveness. As an institutional practice, ORI Biosafety advises semi-annually or quarterly QA testing of autoclaves. Two types of tests are used: 1) a chemical indicator that fuses when the temperature reaches 121°C, and 2) heat-resistant spores (*Bacillus stearothermophilis*) that are killed by exposure to 121°C for approximately 15 minutes. Both types of tests should be placed well down in the center of the bag or container of waste, at the point slowest to heat.

The chemical test should be used first to determine that the temperature in the center of the container reaches 121°C.

Ampules of heat-resistant spores should be used in subsequent test runs to determine the length of time necessary to achieve sterilization.

If you need assistance, contact ORI Biosafety.

3.13. Use and Disposal of Sharps

3.13.1. To prevent needlestick injuries:

- Avoid using needles whenever possible.
- Do not bend, break, or otherwise manipulate needles by hand.
- Do not recap needles by hand. Do not remove needles from syringes by hand.
- Immediately after use, discard needle and syringe (whether contaminated or not) into puncture resistant sharps containers.
- Never discard sharps into regular trash.
- Never discard sharps into bags of biological waste.
- Use care and caution when cleaning up after procedures that require the use of syringes and needles.
- Use extra care when two persons are working together.
  Locate sharps container between the workers when possible.
  Do not overfill sharps containers.
- Locate sharps containers in areas in which needles are commonly used.
- Make containers easily accessible.

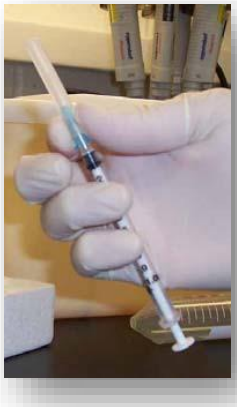
- Occasionally needles must be filled, recapped, and set aside for use later. In these cases, recapping may be performed by the one-handed scoop technique, or by placing the needle in a sterile conical tube.

3.13.2. In the event of a needlestick injury

- Follow procedures identified in section 3.2 of this document. Notify supervisor and go immediately to your Designated Medical Service Provider.

3.13.3. To dispose of sharps other than needles

- Do not handle broken glassware directly. Instead, remove it to a sharps container or other puncture-resistant container using a brush and dustpan, tongs or forceps.
- Discard razor blades and scalpel blades into sharps containers.



3.14. Biological Waste Disposal Procedures

3.14.1. Biological Waste

All biological waste from BSL-1, BSL-2, and BSL-3 laboratories must be decontaminated prior to disposal.

If you do not have access to an autoclave or the autoclave is not functioning, contact Environmental Health and Safety for pick-up.

Decontamination and disposal are the responsibility of the person/laboratory generating the waste.

Collect disposable, solid materials contaminated by an infectious agent, **excluding sharps, or broken or unbroken glass,** into autoclave-proof bags (bag must have biohazard symbol) within a sturdy container with biohazard symbol. When full, these bags are autoclaved, cooled, put into plain opaque household trash bags, and then placed in the building's dumpster. Please refer to Appendix B for Ball State University waste guidelines.

Decontaminate liquids containing a biological agent by adding a chemical disinfectant such as sodium hypochlorite (household bleach, 10% vol/vol) or an iodophor, **or** by autoclaving, then dispose of by pouring down the sink. It is not necessary (or advisable) to autoclave liquids that have been chemically disinfected a minimum of twenty minutes.

3.14.2. Reusable Labware

Lab personnel decontaminate Items such as culture flasks and centrifuge bottles before washing by one of two methods.

- Autoclave items in an autoclavable container.
- Chemically disinfect items by soaking in diluted disinfectant for one hour before washing.

3.14.3. Disposal of Blood Products and Body Fluids

All human blood and other potentially infectious materials (OPIM) should be handled using Universal Precautions under BSL-2 biocontainment. Refer to Appendix B for Ball State University specific waste guidelines and refer to the Ball State University Bloodborne Pathogens Exposure Control Plan for waste disposal guidelines.

Discard disposable items contaminated with human blood or body fluids (**excluding sharps and glassware)** into autoclavable biohazard containers or bags. Material must be packaged and decontaminated as BL2 biohazardous waste, refer to 3.14.1 or disposal procedures.

3.14.4. Disposal of Sharps and Disposable Glassware

Discard all needles, needle and syringe units, scalpels, and razor blades, **whether contaminated or not,** directly into rigid, labeled sharps containers. Do not recap, bend, remove or clip needles. Sharps containers must not be overfilled. Biohazardous sharps containers must be autoclaved as above.

Uncontaminated (no biological materials have been used) Pasteur pipettes and broken or unbroken glassware are discarded into containers specifically designed for broken glass disposal, or into heavy-duty cardboard boxes that are closeable. When boxes are full, tape closed and place in the building's dumpster.

Contaminated Pasteur pipettes and broken or unbroken glassware are treated in one of two ways:

- Discarded into approved sharps containers, or
- Decontaminated by autoclaving or chemical disinfection, then discarded into glass disposal boxes or bins.

Sharps contaminated with radioactive materials or hazardous chemicals must be discarded into separate sharps containers labeled with the name of the isotope or chemical. Contact ORI Biosafety or Radiation Safety for disposal information.

3.14.5. Multi-hazard or Mixed Waste

Avoid generating mixed waste if possible. Keep volume to minimum.

Do not autoclave mixed waste, i.e., chemical waste combined with biological waste.

When discarding waste containing an infectious agent and radioactive material, inactivate the infectious agent first, then dispose as radioactive waste. Seek advice from the Radiation Safety Officer (RSO) for your respective campus before beginning inactivation procedures.

When discarding waste containing an infectious agent and a hazardous chemical, inactivate the infectious agent first, then dispose as chemical waste. Seek advice before beginning inactivation procedures. Contact ORI Biosafety for assistance.

3.14.6. Disposal of Animal Tissues, Carcasses and Bedding

Disposal of animal carcasses/tissues is coordinated through the Animal Care Facility.

- Place animal carcasses/tissues into non-transparent bag. Double-bag when carcass contains zoonotic agent (transmissible from animals to humans).
- Place bag in freezer at Animal Care Facility or other designated location.

Disposal of animal carcasses/tissues that are contaminated with radioactive materials requires special handling. Disposal instructions are available by contacting Radiation Safety.

3.14.7. Disposal Containers

Each laboratory is responsible for purchasing containers for the disposal of biological waste. The following types of containers are available:

3.14.7.1. Sharps Containers

Sharps containers may be purchased from laboratory product distributors. They are available in various sizes, and must be puncture resistant, have a biohazard symbol, labeled as "sharps," and have a tightly closing lid. Do not purchase “needle-cutter” devices, which may produce aerosols when used.

3.14.7.2. Biohazard Autoclave Bags

May be purchased from various laboratory product distributors, such as Fisher Scientific, VWR, and Baxter. Be sure to select polypropylene bags that are able to withstand autoclaving. They should be placed inside a rigid container with lid while waste is being collected. The rigid container must have the biohazard symbol.

3.14.7.3. Glass Disposal Boxes

May be purchased from various laboratory product distributors. Alternatively, heavy-duty, closeable cardboard boxes may be used for disposal of broken glass. They should be lined with a clear plastic bag and the bottoms reinforced with tape. Glass disposal boxes are only to be used for the disposal of non-biohazard glass.

3.15. Laboratory Inspections and Corrective Action Procedures

Laboratory inspections are conducted to ensure that laboratories utilizing biological materials meet specific requirements and follow certain safety guidelines. Inspections are intended to promote a safe laboratory working environment and to ensure compliance with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, the Biosafety in Microbiological and Biomedical Laboratories, 5th ed. and the OSHA Bloodborne Pathogens Standard. Interactive inspections are conducted when the inspector makes observations and speaks with a laboratory designee to answer and discuss specific laboratory procedures and safety practices.

3.15.1. Annual Inspections

Annual Biological Safety Inspections are conducted for all laboratories utilizing biological materials for teaching or research purposes at BL1 or higher. Annual inspections are PI specific, thus more than one inspection may be conducted per room for shared spaces. Upon inspection of the laboratory, deficiencies will be documented and an inspection report sent to the PI. The inspection report will contain a description of the individual deficiencies as well as recommended or required corrective actions.

It is expected that all deficiencies be addressed and corrected as soon as possible. PIs will be given 2-3 weeks from the receipt of their inspection report to begin corrective action. A written verification of complete or partial correction is required. Corrective action can be emailed to ORI Biosafety email address or a hard copy can be sent via campus mail to ORI Biosafety, WQ 100B. ORI Biosafety is available to provide advice on how to address the deficiencies.

Imminent danger or egregious violations are cause to terminate laboratory operations immediately.

3.15.2. Corrective Action Procedures

3.15.2.1. Level 1

Failure to take sufficient corrective action by the end of the initial three-week corrective action period or review of the severity of remaining violations will determine if the process proceeds to Level 1. If very little or no progress has been made, a Level 1 response will be necessary and a re-inspection of the laboratory will be conducted if necessary. ORI Biosafety will follow up with the PI and/or send copies of the Level 1 re-inspection report to the PI.

ORI Biosafety will discuss the Level 1 re-inspection with the PI to agree upon corrective actions. The PI will be given an additional ten (10) business days to correct all violations or have a timeline developed for remediation of listed deficiencies. Written verification of corrected deficiencies must be submitted to ORI Biosafety within that time period. A follow-up inspection may be conducted to verify that all corrections have been made unless written verification is deemed sufficient.

3.15.2.2. Level 2

If written verification has not been submitted or has been deemed insufficient within the additional ten (10) day time period, a follow up inspection will be conducted by ORI Biosafety. The ORI Biological Safety Officer will send a letter and copies of inspections and any PI, lab manager, or lab supervisor responses to the PI, the IBC, and the Department Chair or Director. The letter will give the PI an additional five (5) business days to correct remaining violations and submit written verification.

Mandatory retraining of laboratory personnel will be considered if the violations reveal a lack of understanding or deliberate avoidance of biological safety guidelines.

3.15.2.3. Level 3

If written verification of completed corrective actions has not been submitted to ORI Biosafety by the end of the process through Level 2 (a total of 30 business days), ORI Biosafety will send a letter of non-compliance to the PI, the IBC, the Department Chair or Director, and the administrative head of the college, school, or unit. A re-inspection and follow-up inspection will be conducted as necessary.

Failure of the PI to submit verification of corrections will impact their ability to obtain approvals for permits and grant certifications requiring validation of compliance with applicable state and federal regulations. If the laboratory involves work with non-exempt recombinant or synthetic nucleic acid molecules an incident report of non-compliance will be sent to the NIH.

Extensions to provide corrective action may be requested in writing, at any stage of this process, from ORI Biosafety.

3.15.2.4. Level 4

If the steps taken in the previous action levels have not resulted in the submission of a written verification of completed corrective actions to ORI Biosafety within the established timeline then the laboratory will be deemed noncompliant. The Chair of the academic unit, where the laboratory is located, and the University Vice Provost for Research will be notified of the noncompliant laboratory and punitive action will be requested which may include prohibiting employee access to the laboratory until corrective action has been taken. The IBC may terminate approved protocols and place a hold on funding until appropriate action is taken.

3.15.3. IBC Approval Inspections

Protocol specific laboratory inspections will be conducted prior to protocol approval for protocols utilizing material that fall under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, all

BL2 or higher protocols, and protocols and research that have been otherwise required to be reviewed and approved by the IBC. Upon inspection of the laboratory any deficiencies must be corrected or addressed before approval of the proposed IBC protocol. Deficiencies will be documented and an inspection report sent to the PI. ORI Biosafety would be available to provide advice to the PI to address and correct any deficiencies in a timely manner for approval. An annual inspection may be accepted in place of a separate IBC inspection as deemed appropriate by ORI Biosafety.

3.16. Research Related Vaccinations

In certain cases, while working with biological agents that can cause disease in humans, it is advisable to offer vaccination for those agents. The need for research related vaccination will be dependent on the research description given in the Institutional Biosafety Committee (IBC) protocol. The IBC along with the ORI Biosafety for your respective campus will conduct a risk assessment to evaluate if the research warrants recommendation or requirement of a specific vaccine. In some cases vaccination would be a requirement of conducting proposed research. The identification of Ball State University employees who may become exposed to infectious biological agents and need vaccination is based on the IBC protocol and the Center for Disease Control and Prevention list of vaccine preventable diseases. The vaccine preventable diseases list is available for your review at https://www.cdc.gov/vaccines/vpd/vaccines-list.html

Medical evaluations and vaccinations at Ball State University will be performed by the Designated Medical Service Provider. Evaluation and vaccination is considered confidential. Some physical conditions may affect the ability of an individual to be vaccinated. The Designated Medical Service Provider for the campus may ask for a medical history to assist in determination of these conditions. If an employee elects to decline the vaccine, but continues to work under the protocol, they must sign the declination section of the Vaccination Acceptance/Declination form (Appendix C1-2). A copy of the form will be kept with the Designated Medical Service Provider.

Obtaining vaccines which have been recommended or required by ORI Biosafety and the IBC will be at no cost to employees of Ball State University. The IBC and ORI Biosafety may recommend or require vaccination of non-Ball State University employees who have been listed on IBC protocols. ORI will cover the expense of vaccinations for Ball State University employees. The cost of vaccination for non-Ball State University employees is the responsibility of the individual or Principal Investigator.

Contact ORI Biosafety to request consideration of a research related vaccination and to obtain necessary forms. Vaccination Acceptance/Declination forms are required to be filled out and signed by ORI Biosafety before a vaccine is obtained. For billing purposes, ORI Biosafety will be notified that personnel have completed recommended vaccination(s). Evaluation and vaccination is considered confidential and no personal medical information will be shared with staff or faculty at Ball State University.

3.17. Biosafety Equipment

3.17.1. Biosafety Cabinets (BSCs)

The BSC is designed to provide protection to the product, the user, and the environment when appropriate practices and procedures are followed. Three types of BSCs (Class I, II, III) and the horizontal laminar flow cabinet are described below.

The common element to all classes of BSCs is the high efficiency particulate air (HEPA) filter. This filter removes particles of 0.3 microns with an efficiency of 99.97%. However, it does not remove vapors or gases.

The BSC requires regular maintenance and certification and should be completed by an NSF 49 accredited vendor to assure that it protects you, your experiments, and the environment. Each cabinet must be certified when it is installed, each time it is moved or repaired, and at least annually. Annual certification is a requirement under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (Appendix G-II-C-4-j of the Guidelines). Individual departments or PIs are responsible for costs of certification and repairs or replacement of HEPA filters. Annual certification is verified by ORI Biosafety during annual inspections and before IBC protocol approvals.

3.17.1.1. Types of Biosafety Cabinets

- **Class I BSCs** protect personnel and the environment, but not research materials. They provide an inward flow of unfiltered air, similar to a chemical fume hood, which protects the worker from the material in the cabinet. The environment is protected by HEPA filtration of the exhaust air before it is discharged into the laboratory or ducted outside via the building exhaust.

- **Class II BSCs** (Types A1, A2, B1, B2) provide personnel, environment, and product protection. Air is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air within the cabinet provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air passes through the exhaust HEPA filter, it is contaminant-free (environmental protection), and may be recirculated back into the laboratory (Type A) or ducted out of the building (Type B).

- **Class III BSCs** (sometimes called Class III glove boxes) were designed for work with infectious agents that require BSL-4 containment, and provide maximum protection to the environment and the worker. The cabinet is gas-tight with a non-opening view window, and has rubber gloves attached to ports in the cabinet that allow for manipulation of materials

in the cabinet. Air is filtered through one HEPA filter as it enters the cabinet, and through 2 HEPA filters before it is exhausted to the outdoors. This type of cabinet provides the highest level of product, environmental, and personnel protection.

3.17.1.2. Installing or Relocating Biosafety Cabinets

After installing a BSC, work may not begin until the BSC has been certified and tested by an outside vendor to ensure proper functionality. Care should be taken when deciding on the initial placement or relocation of a BSC. BSCs should be placed away from doorways and high traffic areas. They should also be placed away from heating and cooling vents to help maintain proper airflow within the cabinet.

3.17.1.3. Disposal of Biosafety Cabinets

BSC must be space decontaminated before disposal. Contact ORI Biosafety prior to the decontamination and disposal of the cabinet.

3.17.1.4. Repairs of Biosafety Cabinets

Repairs may only be conducted by NSF-accredited technicians. If your BSC is in need of a repair, contact ORI Biosafety or your department administrator for assistance in locating a repair technician.

3.17.1.5. Operation of Class II Biological Safety Cabinets

- Turn on cabinet fan 15 minutes before beginning work.
- Disinfect the cabinet work surface with 70% ethanol or other disinfectant.
- Place supplies in the cabinet. Locate container inside the cabinet for disposal of pipettes (Movement of hands in and out of the cabinet to discard pipettes into an outside container disrupts the air barrier that maintains sterility inside the cabinet.).
- Work as far to the back (beyond the air split) of the BSC workspace as possible. Always use mechanical pipetting aids.
- Do not work in a BSC while a warning light or alarm is signaling.
- Locate liquid waste traps inside cabinet and use a hydrophobic filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container (such as a cardboard box) to prevent spilling. It is recommended that a secondary flask be utilized. See 3.17.6
- Wear gloves when there is potential for skin contact with infectious material.
- Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper air flow and the level of protection provided. Also, keep the front and rear grilles clear.

- When work is completed, remove equipment and supplies from the cabinet. Wipe the work area with 70% ethanol and allow cabinet to run for 15 minutes.
- Some BSCs are equipped with ultraviolet (UV) lights. However, if good procedures are followed, UV lights are not needed. If one is used, due to the limited penetrating ability of UV light the tube should be wiped with alcohol every two weeks, while turned off, to remove dust. UV radiation must not take the place of 70% ethanol for disinfection of the cabinet interior.
- The UV lamp must never be on while an operator is working in the cabinet.
- Minimize traffic around the BSC and avoid drafts from doors and air conditioning.
- Do not put your head inside the BSC. This compromises the sterility of the environment and, more importantly, could expose you to infectious pathogens.
- Do not tamper with the BSC or interfere with its designed function. It was engineered to operate optimally with no obstructions around the sash or grilles.
- Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current which prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence which disrupts the pattern of HEPA-filtered air supplied to the work surface. Therefore, the **use of open flames and gas burners is strongly discouraged in biosafety cabinets.** 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. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops can also be used.

3.17.2. Horizontal Laminar Flow "Clean Air Benches"

Horizontal laminar low benches are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user, providing only product protection. They can be used for certain clean activities, such as dust-free assembly of sterile equipment or electronic devices. However, they should never be used when handling cell culture materials or potentially infectious materials, or as a substitute for a BSC in research laboratories.

3.17.3. Centrifuge Containment

- Examine centrifuge tubes and bottles for cracks or stress marks before using them.

- Never overfill centrifuge tubes since leakage may occur when tubes are filled to capacity. Fill centrifuge tubes no more than 3/4 full.
- Centrifuge safety buckets and sealed rotors protect against release of aerosols.

3.17.4. Protection of Vacuum Lines

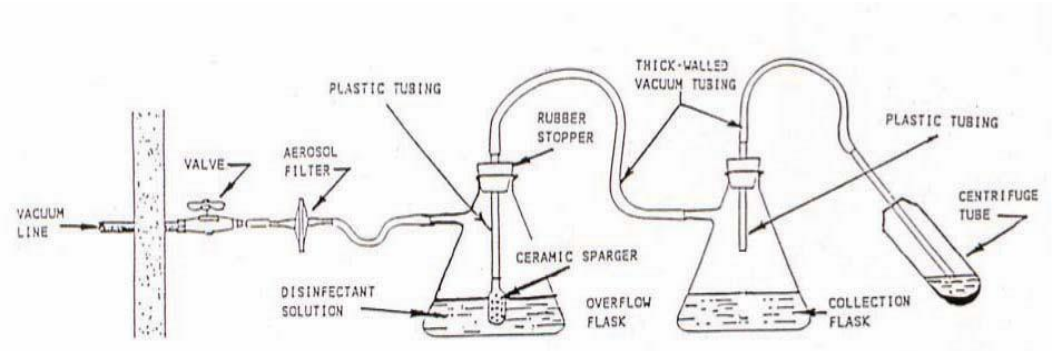
All central vacuum lines used to aspirate supernatants, tissue culture media, and other liquids that may contain microorganisms must be protected from contamination by the use of a collection flask and overflow flask. In addition, a hydrophobic vacuum line filter must be used.

3.17.5. Collection and Overflow Flasks

- Collection tubes should extend at least 2 inches below the sidearm of the flask.
- Locate the collection flask inside the biosafety cabinet instead of on the floor, so the liquid level can be seen easily and the flask emptied before it overflows. The second flask (overflow) may be located outside the cabinet.
- If a glass flask is used at floor level, place it in a sturdy cardboard box or plastic container to prevent breakage by accidental kicking.
- In BSL-2 and BSL-3 laboratories, the use of Nalgene flasks is recommended to reduce the risk of breakage.

3.17.6. Vacuum Line Filter

A hydrophobic filter will prevent fluid and aerosol contamination of central vacuum systems or vacuum pumps. The filter will also prevent microorganisms from being exhausted by a vacuum pump into the environment. Hydrophobic filters such as the Whatman HEPA-Vent Filter are available from several scientific supply companies (Fisher Scientific, catalog #09-744-79).



An alternative to this setup is a medical grade suction canister, which is an increasingly popular option.

It can be found at:
https://www.vitalitymedical.com/cardinal-health-medi-vac-guardian-suction-canister-65651212.html

3.18. Shipment of Biological Materials

3.18.1. General Information

Anyone who prepares or ships packages containing biological or infectious substances (human or animal pathogens) or biological substances containing recombinant or synthetic DNA, must attend a training class before providing a package for transport by commercial carrier. The U.S. Department of Transportation (DOT) and the International Air Transport Association (IATA) regulate shipment of human and animal pathogens. The regulations are complex and exacting. They require that researchers who prepare infectious materials for shipment receive periodic training (every 2 years). In addition, packages must be marked and labeled exactly as the regulations specify, and packaging materials must have been tested and certified to withstand certain durability and pressure tests. Cardboard boxes in which supplies have been received cannot be used to ship infectious materials. Recent events have led to greater scrutiny for compliance with these regulations. Training is also required when receiving and signing for packages containing infectious substances. Please contact ORI Biosafety for assistance with packaging and shipping biohazardous material and for information regarding required training.

3.18.2. Permits

Permits are required from the Centers for Disease Control and Prevention (CDC) to import or transport 1) any microorganism that causes disease in humans; 2) biological materials, such as blood and tissues, when known or suspected to contain an infectious agent; 3) live insects, such as mosquitoes, known or suspected of being infected with any disease transmissible to humans; and 4) any animal known or suspected of being infected with any disease transmissible to humans. Importation permits are issued only to the importer, who must be located in the U.S. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the U.S. Public Health Service Division of Quarantine and release by U.S. Customs. Transfers of previously imported material within the U.S. also require a permit. ORI Biosafety must be notified prior to submission of application for permit and are available to assist through the permitting process.

Application for the permit should be made at least 10 working days in advance of the anticipated shipment date. Further information and application forms may be obtained by calling the CDC at (404) 639-3235, or through the CDC website at http://www.cdc.gov/od/eaipp/

Permits are required from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for importation or domestic transport of agents infectious to livestock; and of biological reagents containing animal, particularly livestock, material (this includes tissue culture media containing growth stimulants of bovine origin such as calf serum). Further information and application forms may be obtained by calling the USDA/APHIS at (301) 734-4401, or http://www.aphis.usda.gov/animal_health/permits/.

Permits are also required from the USDA/APHIS for **interstate movement, importation, or release into the environment (i.e., field tests)** of genetically engineered organisms that are **plant pests**, or that contain portions (plasmids, DNA fragments, etc.) of **plant pests.** Application should be made at least 120 days in advance of the anticipated release or shipment date. ORI Biosafety must be notified prior to the submission of application for permit and are available to assist through the permitting process.

Information and application forms may be obtained by calling the USDA/APHIS at (301) 734-4401, or through the APHIS web site at:
http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/biotechnology.

Facility registration and completion of the CDC/USDA Form 2 are required by the CDC prior to transfer of **select agents and toxins** (42 CFR Part 73). If proposed research involves the use of any agents listed in Appendix A the PI must contact ORI Biosafety immediately to initiate the registration process. It is strictly forbidden to use, transport, or possess any of the agents listed without an active registration. Your ORI Biosafety will assist you with obtaining approval for research involving Select Agents and should be your first contact if your research designs include Select Agents.

A validated license is required by the Department of Commerce for **export** of certain microorganisms and toxins (listed in 15 CFR Part 774) to all destinations. Investigators wishing to ship these items may contact the Biosafety Officer in the Office of Research Integrity for assistance in meeting these requirements.

3.18.3. Packaging

Various carriers (FedEx, UPS, US Postal Service or others) have different requirements for packaging and labeling infectious substances. In addition, various agencies such as the International Air Transport Association (IATA), and the Department of Transportation (DOT) have developed guidelines and procedures to facilitate the safe shipment of infectious substances. Therefore, it is important to check with the carrier you have chosen to determine their specific requirements for shipping infectious agents. In addition to the materials listed above that require permits, the following materials are likely to require special packaging and/or labeling:

- Infectious Substance: a viable microorganism, or its toxin, which causes or may cause disease in humans. DOT requires shippers of infectious substances to attend training every 2 years.
- Diagnostic Specimen: any human or animal material including blood, tissue, and tissue fluids, shipped for the purpose of diagnosis.
- Biological Product: a product for human or veterinary use, such as vaccines and investigational new drugs.

The basic component of all shipping requirements, with various minor modifications, is triple packaging, as follows:

- A primary container that contains the specimen;
- A secondary container that contains the primary container and packaging capable of absorbing the specimen; and

- An outer rigid shipping container that contains the secondary container and other material.

3.18.4. Genetically Modified Microorganisms (GMOs)

The International Air Transport Association's Dangerous Goods Regulations (50th ed.) states that:

- GMOs of Category A agents must be shipped as Category A.
- GMOs of Category B agents must be shipped as Category B.
- If a GMO is not classified as Category A or B it would be classified as UN 3245 Category 9

3.18.5. Human Clinical Materials

The OSHA Bloodborne Pathogens Standard requires that all packages containing human blood and other potentially infectious materials be labeled with the universal biohazard symbol or color-coded. Various carriers may have additional requirements. For more information regarding OSHA Bloodborne Pathogens Standard and the handling of blood and OPIM refer to the Ball State University Bloodborne Pathogens Exposure Control Plan.

3.18.6. On-Campus Transport Between Laboratories or Buildings

When moving infectious substances between labs or buildings on campus, the following minimum procedures must be followed:

- Sample must be in sealed primary container. Utilize plastic containers whenever possible.
- Place primary container in sealed secondary container, with absorbent (paper towels) between primary and secondary container suitable for the volume transported.
- If dry ice is needed, the secondary container should be placed in an outer container, with the dry ice placed between the secondary and tertiary container (never place dry ice in a sealed container).
- Place biohazard label on outer container.

3.19. Dual Use Research of Concern (DURC)

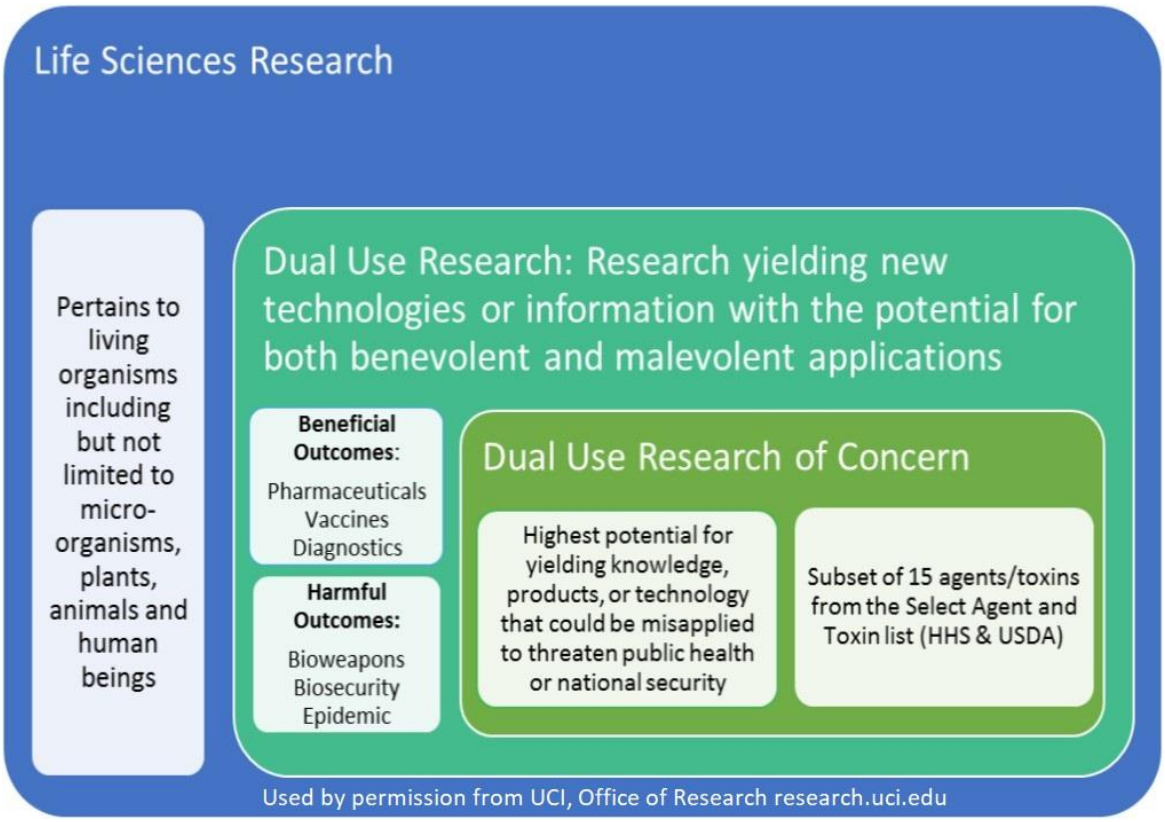
3.19.1. General Information

"Dual Use Research" is research conducted for legitimate purposes that generates knowledge, information, products or technologies that can be utilized for benevolent and harmful purposes. Much life sciences research could be considered dual use – that is, much research yields outputs with some potential to be misused.

The *March 2012 DURC Policy* and the *Policy for Institutional Oversight of Life Sciences DURC* define "dual use research of concern," as:

> *Life Sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural*

crops and other plants, animals, the environment, materiel, or national security.



3.19.2. Purpose

The purpose is to strengthen ongoing institutional review and oversight of certain life sciences research with high-consequence pathogens and toxins in order to identify potential DURC and mitigate risks where appropriate. The roles and responsibilities of United States (USG) funding agencies, research institutions, and life scientists, and provides requirements and performance standards for review of life sciences research, identification of potential DURC, and development and implementation of risk mitigation measures for DURC are fully described in the *United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern*. In so doing, the Policy seeks to preserve the benefits of life sciences DURC while minimizing the risk that the knowledge, information, products, or technologies generated from such research could be used in a manner that results in harm to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security.

3.19.3. Applicability

Consistent with the *March 2012 DURC Policy*, research that uses one or more of the agents or toxins listed in Section 3.19.3.1, and produces, aims to produce, or can be reasonably anticipated to produce one or more of the effects listed in Section 3.19.3.2 will be evaluated for DURC potential.

*3.19.3.1. Agents and Toxins**

- Avian influenza virus (highly pathogenic)
- *Bacillus anthracis*
- Botulinum neurotoxin
- *Burkholderia mallei*
- *Burkholderia pseudomallei*
- Ebola virus
- Foot-and-mouth disease virus
- *Francisella tularensis*
- Marburg virus
- Reconstructed 1918 Influenza virus
- Rinderpest virus
- Toxin-producing strains of *Clostridium botulinum*
- Variola major virus
- Variola minor virus
- *Yersinia pestis*

3.19.3.2. Categories of Experiments

- Enhances the harmful consequences of the agent or toxin
- Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical and/or agricultural justification
- Confers to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies
- Increases the stability, transmissibility, or the ability to disseminate the agent or toxin

- Alters the host range or tropism of the agent or toxin
- Enhances the susceptibility of a host population to the agent or toxin
- Generates or reconstitutes an eradicated or extinct agent or toxin listed in 6.2.1, above

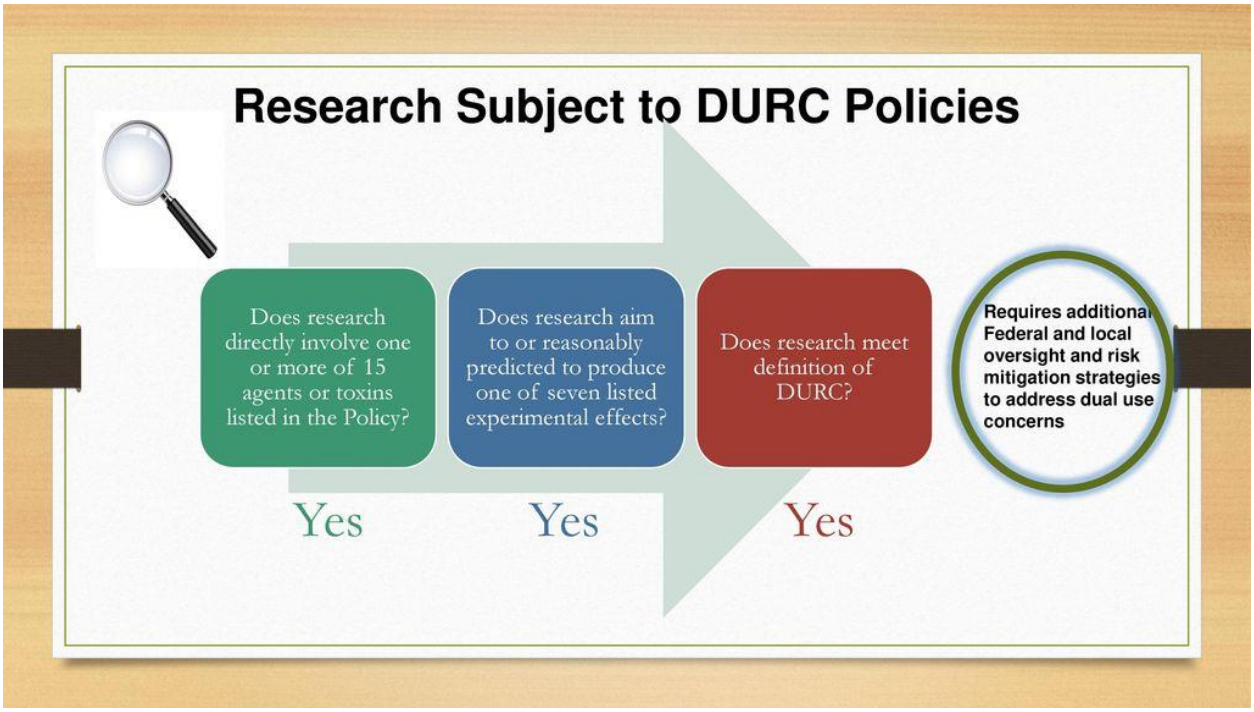
*The 15 agents and toxins listed in this Policy are subject to the select agent regulations (42 CFR Part 73, 7 CFR Part 331, and 9 CFR Part 121), which set forth the requirements for possession, use, and transfer of select agents and toxins, and have the potential to pose a severe threat to human, animal, or plant health, or to animal or plant products. It is important to note, however, that the Federal Select Agent Program does not oversee the implementation of this Policy or the March 2012 DURC Policy.

Note: Research involving any quantity of botulinum neurotoxin should be evaluated for DURC potential.

3.19.4. Compliance

Non-compliance may result in suspension, limitation, or termination of USG funding, or loss of future USG funding opportunities for the non-compliant USG-funded research project and of USG funds for other life sciences research at the institution, consistent with existing regulations and policies governing USG funded research, and may subject the institution to other potential penalties under applicable laws and regulations.

3.19.5. Oversight of DURC



3.20. Biosecurity

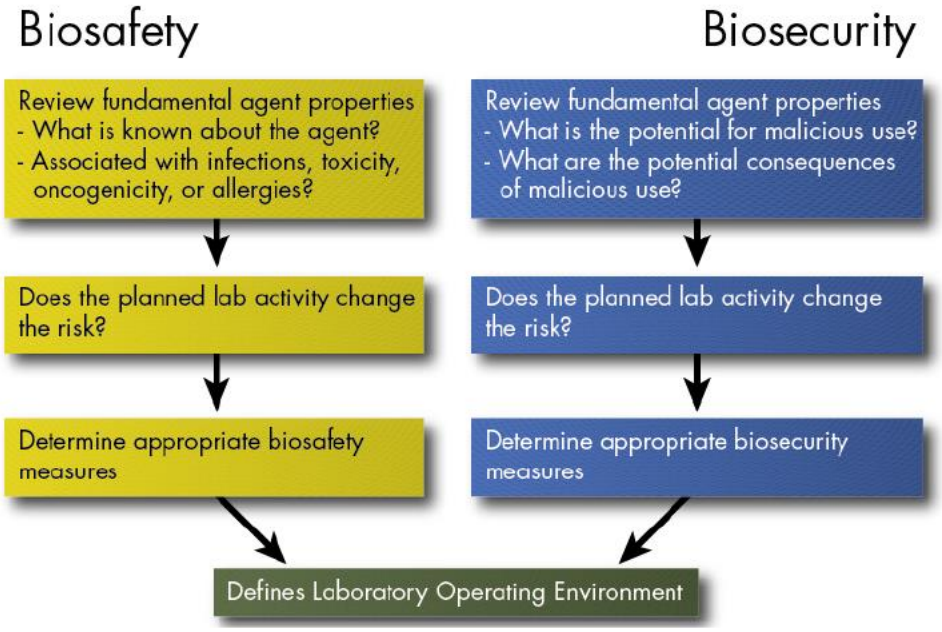
3.20.1. Biosecurity versus Biosafety

Biosafety is comprised of administrative policies, regulatory standards, work practices, safety equipment and PPE, lab design, and researcher compliance to minimize (or remove) risks of exposure to researchers and the environment.

Biosecurity involves assessment of risks and implementing institutional policies that aim to protect sites through the prevention of the theft or misuse of biological materials, lab equipment, and data.

An institutional Biosecurity Plan should address each topic listed below:

- Physical security
- Personnel security and access control
- Information security
- Material control and accountability
- Vulnerability assessments
- Emergency response plans

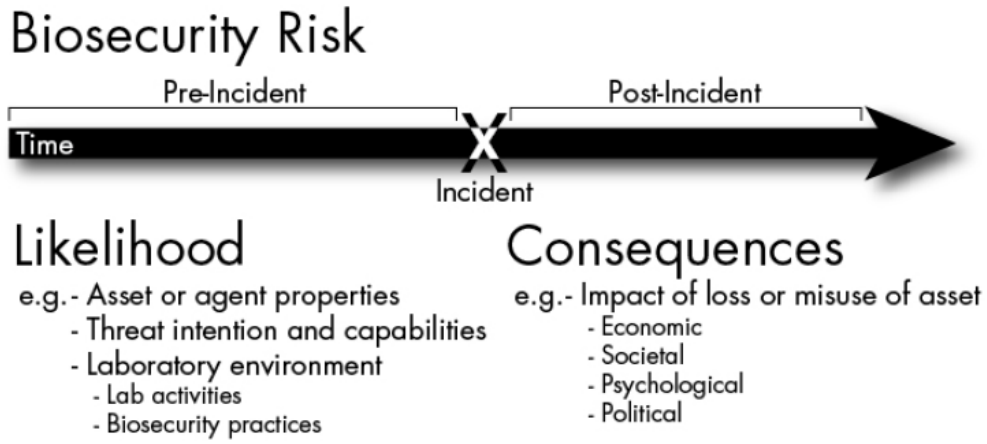


3.20.2. Biosecurity Risk Assessment

In assessing a biosecurity risk, the malicious intent is typically focused upon an item of value, or asset, within the laboratory. In a biosecurity risk assessment, it is critical to define what assets exist within the laboratory. Some of the assets which may exist within a biological institution include valuable biological materials (VBM), valuable laboratory materials (VLM), e.g. equipment, intellectual property, informational assets, and intangible assets (such as the institution's reputation). Once the assets are identified, a biosecurity risk can be defined as the likelihood that the asset can be acquired from a laboratory and the consequences of the loss of that asset (to include misuse of the asset following acquisition). There are many biosecurity risks based upon these assets in biological institutions, and depending

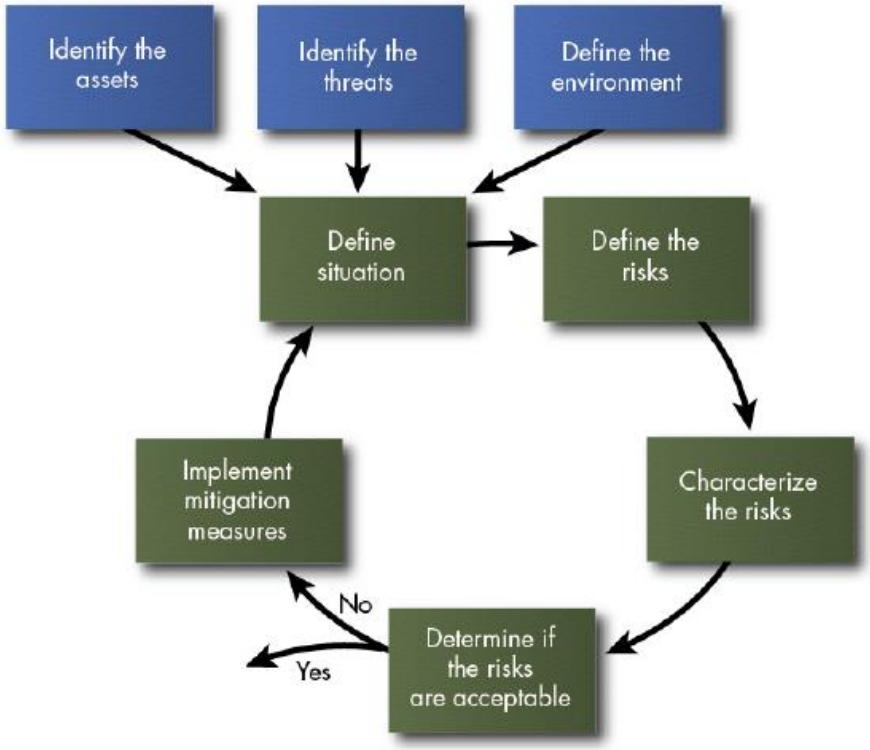
upon the situation and the asset, the risks may impact the researcher(s), the facility, the human and animal community, and the economy.

Unlike biosafety risks, biosecurity risks are often difficult to identify and characterize because they are dependent upon possible intent of the individual(s) interested in illicitly attaining and/or using the asset (threat).



The benefits of risk assessment in the laboratory extend beyond risk reduction and mitigation and can help provide the information related to the following:

- Effective allocation of resources to mitigate risks
- Identification of training needs and supervision
- Advance planning for renovation
- Evaluation of procedural changes
- Compliance with governmental regulations
- Justification for space and equipment needs
- Evaluation of emergency plans
- Planning for preventative maintenance
- Evaluation of exchanges and workflow with other laboratories/units



3.20.3. Vulnerability Assessment

A vulnerability assessment is done periodically, or more frequently as risks change at the institution, and is a systematic process to identify and measure the following:

- Potential threats to security
- The perceived impact of an event
- The effectiveness of how well current security measures would prevent an incident
- Potential solutions or protections
- Assessing the resources needed to implement the solutions or protections
- Obtaining upper administrative buy in for the resources

3.20.4. Inventory Audit and Discrepancies

Access to toxins (Exempt level Select Agent or otherwise) in storage equipment (freezers or incubators) is restricted to authorized personnel only. The lab research user must keep a record of the agent location, how it is used, inventory, transfers (external/internal), destruction and access control. Stocks of toxins are stored in a locked -20 or -80°C freezer. Information regarding all frozen stocks is periodically recorded electronically while information about the use of agent is recorded in a laboratory notebook which is kept locked within the laboratory.

Logs are kept of inventory in long term storage and are audited and reconciled at least annually with the physical inventory, preferably during the annual lab inspection performed by the BSU Biosafety Officer. If non-exempt Select Agent toxins are stored, CDC Form 1 Section 7C would be updated as needed, based on changes in inventory. Inventory audits must be signed and witnessed. Errors

will be marked with a single line drawn through the error and be accompanied by the initials of the person making the error. Inventory audit records are scanned and accessed from a secure location outside the facility. Original copies are filed in the research laboratory. The next inventory audit will also be compared with the previous one and examined for consistency. Discrepancies between audits will be noted and investigated. The use of photocopies, errors marked out so that original text is unreadable, white-out, etc will be considered evidence of alteration of the inventory record.

Upon discovery that the inventory records have been altered in any way or if any discrepancies are uncovered, ORI Biosafety should be contacted as soon as possible.

3.20.5. Biosafety versus Biosecurity Summary

As stated previously, a laboratory risk assessment should be a structured process to identify and manage the biorisks present within a biological laboratory. A risk assessment reviews all aspects of the work environment, including location, proposed work activities, personnel, storage, sample transfer and transport, destruction, access, and security, among others. This methodology should initially be focused on a qualitative risk assessment process. However, *it is important to note that the methodology discussed is also applicable to quantitative or semi-quantitative processes.* Determining when to use the qualitative method or the quantitative method is dependent upon the situation and how one prefers to view and communicate the risk assessment results. The basic risk assessment process can be initiated by the following steps.

1. Define the situation	What work is occurring?
2. Define the risks	What can go wrong?
3. Characterize the risks	How likely is it to happen? What are the consequences?
4. Determine if the risks are acceptable	Engage management and other key stakeholders
5. Implement risk mitigation measures	Ensure all risks are acceptable post implementation of mitigation measures

For a biosafety risk assessment, risk varies with:

- The properties of the biological agent, the at-risk hosts, and the specific laboratory
  processes, including any mitigation measures already in place.
- The severity of the consequences to a lab worker or to the environment if there is an exposure and infection.

For a biosecurity risk assessment, risk varies with:

- The likelihood of successful theft of an asset (including VBM) by an adversary (threat) in the institutional environment.
- The severity of the consequences of the theft based upon the properties of the asset stolen and the intent of adversary.

4. TRAINING AND RECORDKEEPING

4.1 Biological Safety Training

Biological safety training is required for personnel associated with work in biological laboratories, either for research or teaching purposes. The training ensures that personnel working in certain laboratories have been trained in basic biological safety principals before conducting work in laboratories at Ball State University. This training is intended to promote a safe laboratory working environment and to help ensure compliance with the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules and the Biosafety in Microbiological and Biomedical Laboratories, 5th ed. Biological safety training pertains to all IBC protocols and research that fall under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, all Biological Safety Level 2 or higher protocols, and teaching laboratories at Biological Safety Level 2. Professors and assistants in teaching laboratories at BL2 need to complete the biological safety training. Students are not required to complete biological safety training but should receive classroom and topic specific training from the instructor.

4.1.1. Training Types

Biological safety training may be completed as a classroom presentation or as an online module.

- **Classroom**
  Classroom training will be available for scheduling outside of the regular monthly schedule for a group of 5 or more participants. This includes students within certain degree programs.

- **Online**
  Biological safety training is available online through Canvas as an alternative to classroom training. The online training includes ORI Biosafety contact information in case of questions or if assistance is needed.

4.1.2. Training Frequency

Biological safety training is required one time. Additional training may be required as a remediation step through the annual inspection process or by the IBC in response to certain laboratory incidences.

4.2 Bloodborne Pathogens Training

Bloodborne Pathogens Training is offered upon hire and refresher training is required annually for any employee that falls under the scope of the OSHA Bloodborne Pathogens Standard. For more information please refer to the Ball State University Bloodborne Pathogens Exposure Control Plan.

4.3 Laboratory Specific Training

BSU Laboratory Safety training is required for all faculty, staff, and students that have the potential for exposure to hazardous compounds. It is available on Canvas as listed as CMTYLabSafety Community 2019.

Laboratory specific training is the responsibility of the PI or professor. Specific training must be conducted on special handling requirements and safety procedures of agents in the laboratory.

4.4 Biological Shipment Training

Any person shipping or receiving human or animal pathogens, non-exempt human material, or biologicals containing recombinant or synthetic DNA is required to complete DOT/IATA shipment training. This training is required every two (2) years. Training requirement is assessed during the annual biological safety laboratory inspection.

5. REFERENCES

- *Biosafety in Microbiological and Biomedical Laboratories* (5th edition)
- CDC Permit to Import or Transport Etiologic Agents
- Export Administration Regulations Commerce Control list (15 CFR Part 774)
- Ball State University Bloodborne Pathogens Exposure Control Plan
- National Select Agent Program
- *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules(April 2019)*
- NIH Office of Science Policy
- USDA/APHIS Permit to Import or Transport Livestock Pathogens

REVISIONS

October 2019

October 2020

APPENDIX A: HHS and USDA Select Agents & Toxins

The Federal Select Agent Program is jointly comprised of the Centers for Disease Control and Prevention/Division of Select Agents and Toxins and the Animal and Plant Health Inspection Services/ Agriculture Select Agent Services to regulate the possession, use, and transfer of biological agents listed in 7 C.F.R. Part 331, 9 C.F.R. Part 121, and 42 C.F.R. Part 73 (select agents and toxins). The FSAP administers the select agents and toxins regulations in close coordination with the Federal Bureau of Investigation's Criminal Justice Information Services (CJIS).

For biological agents and toxins determined by HHS to have the potential to pose a severe threat to public health and safety (select agents and toxins), the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (42 U.S.C. 262a) directs the promulgation of regulations to establish and enforce safety procedures for the possession and use of select agents and toxins, including measures to ensure proper training and appropriate skills to handle such agents and toxins. 42 U.S.C. § 262a(c)

For biological agents and toxins determined by USDA to have the potential to pose a severe threat to animal health or animal products (select agents and toxins), the Agricultural Bioterrorism Act of 2002 (7 U.S.C. 8401) directs the promulgation of regulations to establish and enforce safety procedures for the possession and use of such select agents and toxins, including measures to ensure proper training and appropriate skills to handle such select agents and toxins. (7 U.S.C. 8401(c))

If you have an intention to engage in research involving any of the Select Agents and Toxin below, please contact ORI Biosafety for initial instructions.

HHS SELECT AGENTS AND TOXINS

Abrin
Botulinum neurotoxins*
Botulinum neurotoxin producing species of *Clostridium**
Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$)
Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus[1]
Ebola virus*
*Francisella tularensis**
Lassa fever virus
Lujo virus
Marburg virus*
Monkeypox virus[1]
Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
Ricin
Rickettsia prowazekii
SARS-associated coronavirus (SARS-CoV) Saxitoxin
South American Haemorrhagic Fever viruses:
- Chapare
- Guanarito
- Junin
- Machupo
- Sabia

Staphylococcal enterotoxins A,B,C,D,E subtypes
T-2 toxin
Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses:
- Far Eastern subtype
- Siberian subtype
- Kyasanur Forest disease virus
- Omsk hemorrhagic fever virus

Variola major virus (Smallpox virus)*
Variola minor virus (Alastrim)*
*Yersinia pestis**

OVERLAP SELECT AGENTS AND TOXINS

Bacillus anthracis *
Bacillus anthracis Pasteur strain
Brucella abortus
Brucella melitensis
Brucella suis
*Burkholderia mallei**
*Burkholderia pseudomallei**
Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan equine encephalitis virus[1]

USDA SELECT AGENTS AND TOXINS

African horse sickness virus
African swine fever virus Avian influenza virus[1]
Classical swine fever virus
Foot-and-mouth disease virus*
Goat pox virus
Lumpy skin disease virus
Mycoplasma capricolum[1]
Mycoplasma mycoides[1]
Newcastle disease virus[1, 2]
Peste des petits ruminants virus
Rinderpest virus*
Sheep pox virus
Swine vesicular disease virus

USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS

Peronosclerospora philippinensis (Peronosclerospora sacchari)
Phoma glycinicola (formerly Pyrenochaeta glycines)
Ralstonia solanacearum
Rathayibacter toxicus
Sclerophthora ayssiae
Synchytrium endobioticum
Xanthomonas oryzae

*Denotes Tier 1 Agent

GENETIC ELEMENTS

Nucleic acids that can produce infectious forms of any of the select agent viruses,
Nucleic acids that encode for the functional forms of any select agent toxin, and
Genetically modified select agents and toxins

1 C = Cysteine residues are all present as disulfides, with the 1st and 3rd Cysteine, and the 2nd and 4th Cysteine forming specific disulfide bridges; The consensus sequence includes known toxins α-MI and α-GI (shown above) as well as α-GIA, Ac1.1a, α-CnIA, α-CnIB; X1 = any amino acid(s) or Des-X; X2 = Asparagine or Histidine; P = Proline; A = Alanine; G = Glycine; X3 = Arginine or Lysine; X4 = Asparagine, Histidine, Lysine, Arginine, Tyrosine, Phenylalanine or Tryptophan; X5 = Tyrosine, Phenylalanine, or Tryptophan; X6 = Serine, Threonine, Glutamate, Aspartate, Glutamine, or Asparagine; X7 = Any amino acid(s) or Des X and; "Des X" = "an amino acid does not have to be present at this position." For example, if a peptide sequence were XCCHPA then the related peptide CCHPA would be designated as Des-X.

2 A virulent Newcastle disease virus (avian paramyxovirus serotype 2) has an intracerebral pathogenicity index in day- old chicks (Gallus gallus) of 0.7 or greater or has an amino acid sequence at the fusion (F) protein cleavage site that is consistent with virulent strains of Newcastle disease virus. A failure to detect a cleavage site that is consistent with virulent strains does not confirm the absence of a virulent virus.

3 Select agents that meet any of the following criteria are excluded from the requirements of this part: Any low pathogenic strains of avian influenza virus, South American genotype of eastern equine encephalitis virus , west African clade of Monkeypox viruses, any strain of Newcastle disease virus which does not meet the criteria for virulent Newcastle disease virus, all subspecies Mycoplasma capricolum except subspecies capripneumoniae (contagious caprine pleuropneumonia), all subspecies Mycoplasma mycoides except subspecies mycoides small colony (Mmm SC) (contagious bovine pleuropneumonia), and any subtypes of Venezuelan equine encephalitis virus except for Subtypes IAB or IC, provided that the individual or entity can verify

that the agent is within the exclusion category. 9/10/13

APPENDIX B: Ball State University Specific Waste Handling Guidelines

Biological Waste Management in Research Laboratories Procedures for Ball State University

The biological waste management program for research laboratories at BSU is administered by Ball State University Environmental Health and Safety in accordance with state regulation 410 IAC 1-3 and other applicable regulations. The guidelines below are to be followed for the disposal of all research-related biological waste. However, ***if a research protocol includes specific waste disposal procedures or additional precautionary measures, the protocol procedures must be followed.***

Type of waste		**Waste disposal**
ALL research waste generated in the following lab categories: **BSL-1 , BSL-2 , and ABSL-2** ** Exception: All transgenic plants in non-exempt BSL-1 and BSL-2 labs must be rendered biologically inactive but not necessarily by autoclave.* *****ABL-1 waste will be required to be decontaminated on an as needed basis when ORI Biosafety has determined that it may be contaminated.***	**Examples**: all wild type and genetically modified microorganisms, cell and tissue culture, fluids, human tissues, fluids, gloves, paper towels, animal bedding, food, and water	Solid: Waste Procedure #1 Liquid: Waste Procedure #2
All animal carcasses and tissues: Transgenic, wild type, wild-caught, and experimentally infected vertebrate animals		Waste procedure #3
Biologically contaminated sharps		Waste procedure #4
Fixed tissues, in which fixing has facilitated inactivation of potentially infectious agents		Waste procedure #5
Human fluids not defined as bloodborne pathogens (vomit and urine) and all materials contaminated with these fluids where there is no visible blood.		Waste procedure #6

APPENDIX B (CONTINTUED) – Ball State University Waste Procedures

1. Non-sharps biohazard waste
    a. All untreated biohazard waste needs to be labeled with a biohazard symbol so that lab staff and others are aware that the waste is biohazardous and needs to be decontaminated before final disposal.
    b. Biohazard waste needs to be placed into bags that are rated for autoclave use, and marked with a biohazard sticker. Appropriate bags are available from vendors such as VWR (product #14220-012) or Fisher (product #01-826B).
    c. Before autoclaving:
        i. Loosely close the bag in a manner that will prevent waste materials from being released and still allow air exchange between the interior of the bag and the ambient environment.
        ii. Place autoclave tape over the biohazard sticker or other highly visible location on the bag.
    d. Before final disposal:
        i. Ensure the autoclave tape visibly indicates proper autoclaving has occurred.
        ii. Ensure that all biohazard markings on the waste clearly indicate that the waste has been treated via autoclave tape tagging. If autoclave tape was not placed over the biohazard sticker, clearly deface the label with black marker or remove the biohazard sticker from the bag.
        iii. Place the autoclaved bag into the non-hazardous waste stream in the building according to building requirements. If there is not an autoclave with sufficient capacity for the waste accessible to you by internal building routes, contact EHS for assistance.

2. Place liquid materials into a suitably sized vessel. Add an appropriate chemical disinfectant and allow adequate contact time for deactivation. Contact ORI Biosafety for agent-specific procedures. After decontamination, dispose of treated liquids down the drain with copious amounts of water to the sanitary sewer.

3. Place carcasses and tissues that may putrefy or decay with an objectionable odor into a red biohazard bag. If the biohazard bag is not opaque, put the carcass in an opaque bag first. Double bag the materials if necessary to avoid perforations in the outer bag. Seal the bag and place in freezer. **Contact the animal care facility for further instructions.**

4. Place sharps in a puncture-proof container – either a commercially available biohazard sharps container or a sturdy cardboard box or plastic container. Needles, scalpels, razor blades, and biologically contaminated glass are required to go into biohazard sharps containers. Serological Pipettes and plastic pipette tips can be placed in biohazard sharps containers, lined and labeled cardboard boxes or other plastic containers that can withstand autoclaving. Seal the container or box and attach a strip of autoclave tape. After autoclaving, mark out or remove any biohazard symbols or tags and place in the waste container located in the autoclave room or the building dumpster. If using a cardboard box, tape the seams before placing in dumpster. All sharps containers are to be disposed of when no more than 2/3 full and must have a lid that is closed and secured prior to disposal.

5. Place preserved specimens in an appropriate container with a lid that will seal. Seal the container and attach a completed *Waste Chemical Tag or Label*. Request pickup from BSU EHS.

6. Liquids can be absorbed, bagged in any regular trash bag, and placed in the building dumpster or disposed as liquid into the sanitary sewer. Solids should be bagged in regular trash bags and placed in the building dumpster.

APPENDIX C-1: Ball State University Employee Vaccination Acceptance/Declination Form

Employees who work in and around areas where infectious agents are handled may elect to receive vaccination with the appropriate vaccine, when such vaccines are available. There is NO CHARGE to the employee. To *decline* a vaccination, sign the declination (Section 3). New employees will not be given vaccination until authorization for employment is satisfactory.

- Fill in form and return this form to ORI Biosafety either in person or via email for signature.
- Call the Designated Medical Service Provider to schedule an appointment for vaccination.
- Take a copy of this form to the vaccination appointment.
- Keep a copy for your records.

Section 1. To Be Filled Out By ORI Biosafety

Research specific vaccine; vaccination requested (employees), or IBC advised

PI name: _____

Protocol Number: _____

Vaccine: ☐ Pneumococcal ☐ Vaccinia vaccine ☐ Meningococcal

☐ Rabies vaccine (Pre Exposure only) ☐ Other: _____

Biosafety Signature: _____

Section 2. Employee Vaccine Acceptance

_____	_____	_____
Worker (Print)	(Sign)	(Date)
_____	_____	_____
Principal Investigator (Print)	(Sign)	(Date)

Section 3. Employee Declination Section for Research Specific Vaccine

I understand that due to my occupational exposure to infectious agents I may be at risk for acquiring a laboratory acquired infection. I have been given the opportunity to be vaccinated with a vaccine appropriate to the research organism, at no charge to myself. However, I decline the vaccination listed above at this time. I understand that, unless I have been previously immunized with the vaccine listed, by declining this vaccine I continue to be at risk of acquiring a laboratory acquired infection. If in the future I continue to have occupational exposure to infectious agents and I want to be vaccinated, I can receive the vaccine at no charge to me.

_____	_____	_____
Worker Name and ID Number (Print)	(Sign)	Date

The Designated Medical Service Provider is authorized to bill ORI for vaccination costs associated with the vaccines in Section 1 only if a Biosafety Staff signature is present.

APPENDIX C-2: Ball State University Non-employee Vaccination Acknowledgement

As a condition of protocol approval the IBC and ORI Biosafety may recommend or require non-employees to be vaccinated with the appropriate vaccine, when such vaccines are available. Funding for these vaccines is the responsibility of the individual or Principal Investigator. ORI does not fund non-employee vaccines.

- Fill in form and return this form to ORI Biosafety, either in person or via email for biosafety signature.
- Call the Designated Medical Service Provider for to schedule an appointment for vaccination.
- Take a copy of this form to the vaccination appointment.
- Keep a copy for your records.

Section 1. To Be Filled Out By ORI Biosafety

Research specific vaccine; vaccination requested (employees), or IBC advised

PI name: _____

Protocol Number: _____

Vaccine: ☐ Pneumococcal ☐ Vaccinia vaccine ☐ Meningococcal

☐ Rabies vaccine (Pre Exposure only) ☐ Other: _____

Biosafety Staff Signature: _____

Section 2. Non-Employee Acknowledgement Section for Research Specific Vaccine

By signing this form I acknowledge that the IBC and ORI Biosafety have advised I receive the research vaccine(s) checked above.

_____	_____	_____
Worker Name and ID Number (Print)	(Sign)	(Date)

.

APPENDIX D: Spill Clean-up Sign

WARNING!

BIOHAZARDOUS SPILL

DO NOT ENTER

APPENDIX E-1: Biosafety Level (BSL-1)

The following is taken from the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, February 2009 Centers for Disease Control and Prevention and National Institutes of Health

This information has been provided from the BMBL. The Ball State University Biosafety Manual and any recommendations made by the BSU IBC or ORI Biosafety preempts recommendations that follow from the BMBL for Biosafety Level 1.

Biosafety Level 1

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science. The following standard practices, safety equipment, and facility requirements apply to BSL-1:

Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

   Precautions, including those listed below, must always be taken with sharp items. These include:

   a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped with 2 hands, removed from disposable syringes, or otherwise manipulated by hand before disposal.
   b. Used disposable needles and syringes must be carefully placed in conveniently located puncture- resistant containers used for sharps disposal.

c. Non disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

    a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

    b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required.

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self- identify to the institution's healthcare provider for appropriate counseling and guidance.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Special containment devices or equipment, such as BSCs, are not generally required.

2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.

3. Wear protective eyewear when conducting procedures that have the potential to create

splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:
   a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.

   b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

   c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

Laboratory Facilities (Secondary Barriers)

1. Laboratories should have doors for access control.

2. Laboratories must have a sink for hand washing.

3. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not appropriate.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

   a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

   b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. Laboratories windows that open to the exterior should be fitted with screens.

APPENDIX E-2: Biosafety Level 2 (BSL-2)

The following is taken from the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, February 2009 Centers for Disease Control and Prevention and National Institutes of Health

This information has been provided from the BMBL. The Ball State University Biosafety Manual and any recommendations made by the BSU IBC or ORI Biosafety preempts recommendations that follow from the BMBL for Biosafety Level 2.

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that:

- Laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures;
- Access to the laboratory is restricted when work is being conducted; and
- All procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

The following standard and special practices, safety equipment, and facility requirements apply to BSL-2:

Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

b. Used disposable needles and syringes must be carefully placed in conveniently located puncture- resistant containers used for sharps disposal.

c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

   a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

   b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required.

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

2. Laboratory personnel must be provided medical evaluation and offered appropriate immunizations for agents handled or potentially present in the laboratory.

3. Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.

4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.

6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

    a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

    b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation and treatment should be provided, and appropriate records maintained.

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:

    a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.

b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.

2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.

3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:

a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

Laboratory Facilities (Secondary Barriers)

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.

2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.

3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.

6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.

7. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

8. An eyewash station must be readily available.

9. There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.

10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

APPENDIX E-3: Biosafety Level 3 (BSL-3)

The following is taken from the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition, February 2009 Centers for Disease Control and Prevention and National Institutes of Health

This information has been provided from the BMBL. The Ball State University Biosafety Manual and any recommendations made by the BSU IBC or ORI Biosafety preempts recommendations that follow from the BMBL for Biosafety Level 3.

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs, other physical containment devices, or by personnel wearing appropriate personal protective equipment.

A BSL-3 laboratory has special engineering and design features. The following standard and special safety practices, equipment, and facility requirements apply to BSL-3:

Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:

    a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

    b. Used disposable needles and syringes must be carefully placed in conveniently

located puncture- resistant containers used for sharps disposal.

c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.

8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:

   a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.

   b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.

10. An effective integrated pest management program is required.

11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.

2. Laboratory personnel must be provided medical evaluation and offered appropriate

immunizations for agents handled or potentially present in the laboratory.

3. Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.

5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.

6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

7. Laboratory equipment should be routinely decontaminated, as well as after spills, splashes, or other potential contamination.

    a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

    b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation and treatment should be provided, and appropriate records maintained.

9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.

10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC **(**preferably Class II or Class III), or other physical containment devices.

2. Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated with appropriate disinfectant before being laundered. Clothing is changed when contaminated.

3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers should:
    a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.

    b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

    c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

5. Eye, face, and respiratory protection must be used in rooms containing infected animals.

Laboratory Facilities (Secondary Barriers)

1. Laboratory doors must be self-closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Access to the laboratory is restricted to entry by a series of two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.

2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.
3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.
    a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.

    b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.

    c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

4. Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shutdowns. Selection of the appropriate materials and methods used to

decontaminate the laboratory must be based on the risk assessment of the biological agents in use.

5. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.

    a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

    b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
6. All windows in the laboratory must be sealed.

7. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.

8. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.

9. An eyewash station must be readily available in the laboratory.

10. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from “clean” areas toward “potentially contaminated” areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.

    a. Laboratory personnel must be able to verify directional air flow. A visual monitoring device which confirms directional air flow must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.

    b. The laboratory exhaust air must not re-circulate to any other area of the building.

    c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.

11. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.

12. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

13. Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.

14. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.

15. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following: an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas-tight isolation dampers; decontamination ports; and/or bag- in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

16. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.