BALL STATE UNIVERSITY

Biological Safety (Biosafety) Manual

Prepared by the

Environmental Health and Safety Office

In cooperation with the

University Biosafety Committee

Version: November, 2017

Table of Contents

EMERGENCY CONTACT INFORMATION:

In case of any Emergency (Fire, security, medical, large spill, exposure):
Dial 911 or 765-285-1111 (BSU Police Dispatch)

In case of biohazard or chemical spills or exposure:
Call the EHS Office at 765-285-2807 (working hours)
765-285-5081 (work control)
765-289-1241 (BSU Operator)
765-499-3060 (cell phone); or,
765-285-1111 (BSU PD)

Be prepared to identify the biological agent or molecule involved – have the Biological Data Sheet with you!

If you need emergency medical treatment, call 911 or go to the nearest hospital:
IU Health Ball Memorial Hospital is located at 2401 W. University Ave. Muncie, Indiana

Non-emergency medical attention (Faculty, Researchers, Staff, or Students):

Ball State Health Clinic: 765-285-8431

Fall/Spring Hours: Monday, Thursday, Friday - 8:00 AM to 4:30 PM
Tuesday and Wednesday - 9:00 AM to 6:30 PM

Summer Hours: Monday through Friday - 7:30 AM to 3:30 PM

Evening, Weekend or Holidays
If you need non-emergency medical care when the Health Center is closed, the local options are:

MedExpress: 1313 W. McGalliard Avenue: 765-287-8460

US Healthworks: 3911 W. Clara Lane: 765-288-8800

Southway Urgent Care: 3807 S. Madison Street: 765-747-1164

Biological Safety Agency Contact Information:

National Institutes of Health: 301-496-4000
9000 Rockville Pike
Bethesda, Maryland 20892

Indiana State Health Department
2 North Meridian Street
Indianapolis, IN 46204
800-382-9480

Centers for Disease Control: 800-232-4636
1600 Clifton Road
Atlanta, GA 30329-4027

Introduction

The Ball State University (BSU) Biological Safety Manual (*Biosafety Manual*, or *Manual*) is intended to be a resource for information, guidelines, policies, and required procedures that will enable and encourage safe research and education while eliminating, or reducing, the potential for exposure to biohazards. The information presented here also reflects the requirements and guidelines of federal and state regulations, including those governing work with *Recombinant DNA and Synthetic nucleic acids* (rDNA/sNA). The most current version of the *BSU Biosafety Manual* will be maintained on the Environmental Health and Safety (EHS) Office website: http://cms.bsu.edu/about/administrativeoffices/riskmanagement/ehs/envhealth/labsafety

The BSU *Biosafety Manual* is applicable to all laboratory, research, teaching, and support activities that may involve biohazards--both on campus, and in the field or satellite locations.

Biohazards are microorganisms, microbial toxins, or other biological agents that can infect and/or cause disease in humans, animals, or plants. Biohazards include infectious agents of humans, animals and plants, toxins of biological origin, human-derived materials, recombinant DNA, and any materials potentially containing infectious agents or biological hazards. Biohazards may include bacteria, bacterial toxins, viruses, fungi, rickettsia, prions, protozoans, parasites, genetically modified organisms, allergens, cultured human or animal cells (and the potentially infectious agents these cells may contain), viroids, prions, and other infectious agents as outlined in applicable laws, regulations, or guidelines. In addition, biohazards include human blood, body fluid, tissues, and cell lines of human origin. Biohazards are often referred to as *infectious agents* or *etiological agents*.

According to the CDC (Centers for Disease Control)/NIH (National Institutes for Health document, *Biosafety in Microbiological and Biomedical Laboratories*, also known as the *BMBL*, the three primary hazardous characteristics associated with biological agents are:

- The capability of an agent to infect and cause disease in a susceptible human or animal host;
- The virulence of an agent as measured by the severity of disease; and,
- The availability of preventive measures and effective treatments for the disease.

This Manual requires that all researchers or faculty complete and submit a *Biological Safety Registration Document*, a copy of which is provided in **Appendix P** to this Manual. That document will be reviewed by the BSU Biosafety Committee and/or the Biosafety Officer to determine the level of oversight, training, medical monitoring, and containment necessary, and whether the research is subject to the NIH Guidelines. The pdf-fillable registration document may be obtained by contacting the Biosafety Officer, Office of Research Integrity, or the BSU Biosafety Committee.

All research registrations and protocols must be reviewed and approved by the BSU Biosafety Committee (UBC or IBC) prior to beginning work if they involve the use of any of the following:

- Agents that can infect and/or cause disease in humans, animals, or plants.
- Experimentally infected animals and those naturally harboring zoonotic infectious agents.

- Recombinant and synthetic nucleic acid molecules (rDNA/sNA).
- Genetically modified organisms.
- Transgenic plants and animals.
- Human cell lines and other materials of human origin.
- Select agents and toxins (whether or not "exempt" organisms or toxin levels).
- Potential exposure to bloodborne pathogens as defined by the Occupational Safety and Health Administration.
- Biohazardous waste.

This *Biological Safety Plan* will also serve as the basis for any research or activities involving recombinant DNA or synthetic nucleic acids (rDNA/sNA) that are under the jurisdiction of the National Institutes of Health (NIH). Should such NIH regulated activities occur or be planned at BSU, the *University Biosafety Committee* (UBC) formed under this Manual, will then be chartered to constitute the core of the required *Institutional Biosafety Committee* (IBC) for such research in compliance with NIH requirements (see following section). However, regardless of whether any research involving rDNA/sNA, or other activities involving potentially infectious agents is under the auspices of the NIH--such work is to be performed in accordance with the same protective procedures (work practices and personal protection) and facility requirements (containment and engineering controls) that are established in this Manual.

Biosafety encompasses the knowledge, techniques, equipment, and facilities necessary to prevent or minimize an exposure to, or release of, a biohazard. The mission of the EHS and the Biosafety Committee is to assure a safe and healthy environment for individuals working with biohazards and to protect the community and environment by preventing the release and exposure to biohazards (including working with recombinant DNA and/or synthetic nucleic acids).

University or Institutional Biosafety Committee

It is the expressed intent of the university that a *University Biosafety Committee* be established and maintained to oversee laboratory, research, and field activities involving all biological safety hazards--including any recombinant DNA and synthetic nucleic acid research that is not the recipient of funding from the NIH, or otherwise directly subject to NIH guidelines or authority. At such time that the university receives funding, or otherwise becomes subject to the NIH standards for rDNA/sNA research activities, the *University Biosafety Committee* will be converted to, and comprise (with additional membership), the *Institutional Biosafety Committee* with the roles, membership, and responsibilities as required by the *NIH Guidelines Involving Recombinant DNA and Synthetic Nucleic Acid Molecules* (NIH Guidelines). **Appendix Q** to this Manual includes Section IV-B-2, *Institutional Biosafety Committee*, from those NIH Guidelines. This Manual, the associated biohazard policies and procedures, and the administrative operations developed and followed by the BSU Biosafety Committee (UBC) are designed to conform to the NIH Guidelines. This will allow for the rapid transformation of the UBC and its policies to a formal IBC should work with rDNA/sNA be proposed that is subject to the NIH standards. At such time, the Committee membership will be expanded and it will

formally register with the NIH Institutional Biosafety Committee Registration Management System *(IBC-RMS)*. This system supports online submission of IBC registrations and annual registration updates to the NIH Office of Science Policy (OSP).

The Biosafety Committee (referring to either the University Biosafety Committee, or the Institutional Biosafety Committee, whichever exists at the time) serves as the review board of all research and educational activities involving biohazards. The BSU Biosafety Committee has the authority to require operational changes in the event of noncompliance with required conditions.

Example: BSL-2 Facility and Operations

Biosafety Oversight

Guidance documents from the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) form the basis for the biosafety practices included in this ***Biological Safety Manual***. There are additional guidance documents and regulations imposed by various funding agencies that individual Principal Investigators (PIs) must be aware of and may need to incorporate into their *Laboratory-Specific Biosafety Manual.* The *Laboratory-Specific Biosafety Manual* is a document that must be prepared, and available, for each laboratory working with biological hazards at BSU that provides specific procedures that are particular to that laboratory or research activity. Those specific procedures address any circumstances or containment methods that are not addressed in this BSU Biological Safety Manual, or where departures from these requirements have been approved by the Committee or the BSO.

Biosafety requirements must sometimes be followed to ensure the continuation of any grant funding from federal agencies and for health and safety purposes; but, more importantly, are critical to the health and safety of BSU researchers, students, and staff engaged in research or

educational training involving all biological agents, regardless of NIH oversight.

Beyond our general biosafety standards at BSU, the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (*NIH Guidelines*) detail procedures and practices for the containment and safe conduct of various forms of recombinant or synthetic nucleic acid research. The *NIH Guidelines:*

- Mandate the establishment of an Institutional Biosafety Committee for the review and oversight of biological research;
- Outline roles and responsibilities for biosafety; and
- Establish the practices, procedures, and conditions under which recombinant and synthetic nucleic acid activities must be conducted.

All institutions that receive NIH funding for *regulated* recombinant or synthetic nucleic acid molecules activities must comply with the *NIH Guidelines*. Researchers at institutions that are subject to the *NIH Guidelines* must comply with the requirements even if NIH does not fund the individual project. **Non-compliance with the *NIH Guidelines* may result in suspension, limitation, or termination of financial assistance** for the research project and of NIH funds for other recombinant or synthetic nucleic acid activities at BSU or the requirement for prior NIH approval of any and/or all recombinant or synthetic nucleic acid projects at BSU. As stated previously, however, regardless of funding sources, all research and educational efforts at BSU involving biological agents are expected to comply with the operational guidelines established in this Manual which conform to the technical NIH Guidelines.

The CDC/NIH manual, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), describes the appropriate measures and facilities for work with all microbial agents, including bacterial, viral, fungal, parasitic, rickettsial, and prion agents as well as toxins of biological origin. The BMBL has become the code of practice for biosafety and, therefore, the primary basis and supporting document for this manual and establishes the principles of biosafety, containment and risk assessment, for the research and lab setting. For containment, the fundamental supporting elements are sound microbiological practices with safety equipment and facility safeguards incorporated. Combining these elements of containment provides for protection of lab workers, the public and the environment from potential exposures to biohazardous materials. Performing risk assessments for all work involving biohazardous materials allows the researchers, the biosafety committee and the biosafety staff to determine containment requirements appropriate for their work (i.e. appropriate microbiological practices, safety equipment and facilities) that will reduce the potential for laboratory acquired infections (LAIs).

Related Regulatory Programs

The requirements described in the Occupational Safety and Health Administration's (OSHA) *Bloodborne Pathogens Standard* (29 CFR § 1910.1030), apply to work with human blood, tissue, organs, body fluids, and cell cultures. Special training, medical surveillance, procedures, and equipment that must be in place for protection against bloodborne pathogens, needle sticks, and other sharps injuries, are described in the BSU Exposure Control Plan. Bloodborne pathogen materials are designated RG-2 and the BMBL specifies BSL-2 containment practices for

bloodborne pathogen materials in compliance with the OSHA Bloodborne Pathogens Standard. The BSU Exposure Control Plan is available on the BSU EHS website: http://cms.bsu.edu/about/administrativeoffices/riskmanagement/ehs/public-health

Handling and disposal of biohazardous waste is also regulated by OSHA under the OSHA Bloodborne Pathogens regulation and by state and federal statutes. The procedures for biohazardous waste handling are described in the BSU Waste Management Manual. This, and other waste management information is available on the BSU EHS website: http://cms.bsu.edu/about/administrativeoffices/riskmanagement/ehs/envhealth/wastemgmt

The requirements for packaging and shipment of biohazards are provided in the Department of Transportation's *Hazardous Materials Regulations* 49 CFR § 171-180. In addition, permits may be required to ship biological materials. Please refer to the *CDC Etiological Agent Import Permit Program* and the Animal and Plant Health Inspection Service (APHIS) permit program. Information on shipping procedures that comply with these regulations is found in the section on *Transport of Biological Materials* in this Manual.

Specific requirements for handling biological toxins are found in the BMBL and OSHA's *Occupational Exposure to Hazardous Chemicals in Laboratories,* standard 29 CFR § 1910.1450. Information regarding BSU's radiation safety program is found in the BSU *Radiation Safety Manual*.

Teaching and research activities involving the use of animals are regulated by the United States Department of Agriculture (USDA*) Animal Welfare Act.* The Animal Welfare Act was signed into law in 1966. It is one of the laws in the United States that regulates the treatment of USDA-covered species in research, exhibition, transport, and by dealers. The USDA Animal Welfare Act covers all mammals used in research except rats of the genus *Rattus* and mice of the genus *Mus* that are bred for use in research. There are additional exceptions for agricultural research and teaching activities. In addition, the Institutional Animal Care and Use Committee (IACUC) oversees all research and teaching activities involving vertebrate animals.

The Public Health Service (PHS) Policy implements the Health Research Extension Act of 1985, which applies to all institutions receiving animal research funds from PHS organizations (such as the National Institutes of Health). This law applies to all vertebrate species. The Health Research Extension Act of 1985 provides the legislative mandate for the PHS Policy. It directs the Secretary of Health and Human Services to establish guidelines for the proper care and treatment of animals used in research and for the organization and operation of animal care committees.

Roles and Responsibilities

The biological safety program at BSU has developed from the University's commitment to address and comply with regulations and recommendations for biosafety, biosecurity, and the humane treatment of animals in research and teaching activities, as well as the health and safety of the students, staff, researchers, community, and environment. The University Biosafety Committee

and the EHS Office provide oversight of BSU's biological safety program.

Roles and responsibilities for biosafety and biosecurity are included in this section.

Ball State University

BSU has instituted and maintains this biosafety program for personnel who may potentially be exposed to biological hazards (biohazards) during the performance of their duties. The biosafety program is designed to achieve regulatory compliance and to provide a means for employees to be informed about and protected from biohazards. To maintain regulatory compliance and to protect personnel from biohazards, Ball State University must:

- Appoint a *Biological Safety Officer* for the institution (required for any BSL-3 work or if over 10 liters of biohazards are maintained. Currently, the Environmental Specialist in the BSU EHS Office serves as the Biological Safety Officer for all biosafety activities at BSU.
- Ensure appropriate training is provided to personnel conducting research with biohazards or recombinant or synthetic nucleic acid materials.
- Ensure that research conforms to the provisions of the *NIH Guidelines, BMBL, and this Biosafety Manual*.
- Establish and maintain a *University Biosafety Committee* (UBC) to include adequate expertise and provide training. The UBC is created to anticipate and preliminarily comprise the Institutional Biosafety Committee (IBC), develop necessary policies and procedures, all to be accomplished in accordance with the NIH Guidelines and the CDC's BMBL.
- Establish and maintain a health surveillance program for personnel.
- Implement policies for safe conduct of biological and recombinant or synthetic nucleic acid research.
- Report, when required, any significant problems, violations or significant research-related accidents or illnesses to the NIH Office of Biotechnology Activities within 30 days.

The *BSU Biosafety Manual* provides a compilation of required work practices, protocols, equipment, facilities, and systems to work safely with biological agents and recombinant DNA/synthetic nucleic acids at Ball State University. However, the *BSU Biosafety Manual* should not be considered the only source for health and safety concerns. The *NIH Guidelines* and BMBL are principal references. An example particular to BSU is the BSU *Chemical Hygiene Plan,* which is a required guidance document for safe work in all laboratories--including those working predominantly with biological agents. It is further required that the principal investigator and supervisory personnel will supplement this information with instruction and guidance regarding specific practices and procedures unique to the work being done in their areas, by (1) first **completing a Registration Document;** (2) **performing and documenting a Risk Assessment**; and (3) **completing a Lab-Specific Biosafety Manual** to make all relevant documentation available to laboratory users. Suggested templates or examples for each of these required activities re provided in **Appendices F, G**, **and P** of this Manual.

The Biosafety Manual provides university-wide safety guidelines, policies and procedures for the use and manipulation of biohazards. Although the implementation of these procedures is the responsibility of the Principal Investigator (PI) or clinical laboratory or practice supervisor, its

success depends largely on the combined efforts of faculty, laboratory supervisors, clinicians, and employees. Planning for and implementation of biological safety must be part of every laboratory activity in which biohazardous materials are used. It is also important that the content and procedures presented in this Manual be part of the educational process for all graduate and undergraduate students as part of their training--not only for their immediate safety, but to create a safety culture and institutionalize these practices in future research and other endeavors.

In general, the handling and manipulation of biological agents and toxins, as well as recombinant DNA molecules or synthetic nucleic acids, requires the use of various precautionary measures depending on the infectious agents, or material(s) involved. This manual will provide assistance in the evaluation, containment and control of biohazards. However, it is imperative that all parties involved or working with these materials seek additional advice and training when necessary.

It is important to note here that while information regarding Risk Group 3 and 4 agents and Biosafety Level 3 and 4 control measures are included in this *Biosafety Manual*, activities using such organisms and requiring corresponding control features are not currently performed at this university. Information regarding such organisms or activities is presented only to better define and delineate the agents or research that may be used or approved with our current physical capabilities and institutional controls.

The BSU Biosafety Manual will be reviewed at least biennially by EHS and the Biosafety Committee. The Biosafety Committee (either the UBC or IBC) overseeing these functions, and the Biological Safety Officer, are always available to address health and safety concerns from any party.

University Biosafety Committee

The Biosafety Committee is charged with the review, approval and oversight of biohazards in research and teaching activities, as well research involving recombinant or synthetic nucleic acid molecules – regardless of whether such research is formally governed by the NIH Guidelines. Responsibilities of the University Biosafety Committee (UBC) include assessment of facilities in collaboration with the Biosafety Officer, and developing procedures, practices, and training of research personnel, or taking other steps necessary to assure compliance with *NIH Guidelines, the BMBL,* and other pertinent standards and regulations.

To successfully carry out these responsibilities, the University Biosafety Committee is appointed to include sufficient knowledge and expertise in biomedical research practices and biosafety. The University Biosafety Committee has the authority to approve, require modifications to secure approval, disapprove, suspend or terminate research activities as required to assure compliance with applicable regulations and guidelines.

The University Biosafety Committee shall have the following membership:

1. At least three (3) representatives from the Biology Department, Medical Education, and/or

Chemistry Departments with knowledge, experience, and expertise in research involving biological hazards, rDNA/sNA molecules, or biosafety and containment practices;
2. A representative from the Office of Research Integrity (ORI);
3. The Biological Safety Officer (and/or EHS representative);
4. A representative of Facilities Planning and Management (FPM); and,
5. Other members as needed to fulfill the duties and function of the Committee, including permanent or pro tempore members offering specialized knowledge or skills.

The UBC will meet as necessary to review the Documents of Registration, research protocols, and for such other causes or at such times necessary to fulfill its responsibilities, or as otherwise established by the Committee. The Committee, or a designated Office, will maintain a listing of the active Registrants working with biohazards. The Committee or BSO will also maintain an up-to-date list of prospective members that would satisfy the requirements of Section IV-B-2, *Institutional Biosafety Committee*, of the NIH Guidelines in the event that a formal Institutional Biosafety Committee must be constituted. In that event, the membership of the UBC will form the core of the IBC which will then consume the functions of the UBC on a permanent basis.

Institutional (NIH) Biosafety Committee

A formal Institutional Biosafety Committee (IBC) must be established under the *NIH Guidelines* specifically for the review of research involving recombinant or synthetic nucleic acid molecules which meets certain criteria and is funded by the NIH. As a condition for NIH funding of recombinant or synthetic nucleic acid molecule research, institutions must ensure that all such research conducted at or sponsored by the institution, irrespective of the source of funding, complies with the NIH Guidelines.

An *Institutional Biosafety Committee* is a committee that: (i) meets the requirements for membership specified in Section IV-B-2, *Institutional Biosafety Committee* (IBC), and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Section IV-B-2, *Institutional Biosafety Committee (IBC)* (see **Appendix Q**). The purpose and role of the IBC is to:

- Ensure adequate containment of potentially hazardous biological agents;
- Add a level of expert review and monitoring of potentially hazardous experiments;
- Inform the public about experimental plans that have a potential to be hazardous;
- Provide a means of communication among researchers and healthcare providers about potentially hazardous protocols;
- Keep a record of meetings, providing sufficient detail to serve as a record of major points of discussion and the committee's rationale for particular decisions, documenting that the IBC has fulfilled its review and oversight responsibilities;
- Report any significant problems or violations of National Institutes of Health Office of Biotechnology Activities NIH OBA Guidelines and any significant research- related accident or illness to NIH/OBA;
- File an annual report with the NIH (if performing NIH regulated recombinant DNA/synthetic nucleic acid research and the "Institutional" Biosafety Committee has been constituted).

Should research be proposed, undertaken, or NIH grant monies be received by any BSU department or entity for research involving recombinant or synthetic nucleic acid molecules - that are not otherwise exempted under NIH guidelines - the University Biosafety Committee (UBC) will transition to become the university's IBC by adding additional members, as necessary, and register with the NIH for that purpose. **Appendix Q** to this Manual is a copy of Section IV-B-2, *Institutional Biosafety Committee*, from the NIH guidance which relates the membership, procedures, and functions of the IBC.

IBCs are sometimes assigned additional review responsibilities such as the following:

- Select agents and toxins
- Blood borne pathogens
- Xenotransplantation
- Stem cell research
- "Dual Use" research
- Nanotechnology

Such broader purview, however, is a matter of institutional discretion and will be determined following consideration and any action by the Biosafety Committee.

Biosafety Officer

The responsibilities of the Biological Safety Officer (BSO) include, but are not limited to, the following:

- Develop, implement, and maintain the university's biosafety program to address issues of biosafety and biosecurity.
- Perform and review the required risk assessment to determine appropriate biosafety level and personal protective equipment (PPE) for handling recombinant and synthetic nucleic acid molecules or biohazards.
- Advise researchers on proper waste disposal methods based on federal and state regulations.
- Assist researchers in the development of plans for preventing and handling accidental spills and personnel contamination.
- Investigate laboratory accidents involving biohazards and recombinant and synthetic nucleic acid molecules.
- Develop, implement, and maintain the university's program for select agents and toxins.
- Perform periodic inspections to ensure that laboratory standards are rigorously followed.
- Promote regulatory compliance and a safe laboratory environment.
- Provide advice on laboratory security.
- Coordinate (with the BSU EHS Office) the BSU Bloodborne Pathogen / Universal Precautions Program and conduct training for laboratory personnel with such exposure potential.
- Provide technical advice to principal investigators and the Biosafety Committee on research safety procedures.
- Provide training and resources for the safe use and practices for those working with

potential biohazards, and laboratory equipment.

- Interface with BSU Facilities Planning and Management regarding physical facilities.
- Report to the Biosafety Committee and the institution any significant problems, violations of the *NIH Guidelines*, and any significant research-related accidents or illnesses of which the Biological Safety Officer becomes aware.
- The Biosafety Officer has the authority to immediately halt research that he/she deem to be an immediate threat to safety of personnel, environment, or the community at large. The Biological Safety Officer must report such action to the Biosafety Committee immediately.

Unless, or until, another individual is appointed as the Biological Safety Officer by either the University, or the Institutional Biosafety Committee, this role will be fulfilled by the Environmental Specialist in the Environmental Health and Safety Office.

Principal Investigator

A scientist, trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling biohazards must be responsible for the conduct of work with any biohazards or materials. This individual should consult with the Biosafety Committee, Biological Safety Officer, or other health and safety professionals with regard to risk assessment.

Responsibilities of the principal investigator include:

- Accept direct responsibility for the health and safety of those working with biohazardous materials and/or select agents and toxins.
- Complete and submit the BSU ***Registration Document*** to the UBC, IBC (if constituted) and the Biological Safety Officer for those agents, molecules, or toxins with which research is being performed.
- Perform and document the required ***Risk Assessment*** for the biological agent(s) employed in the research or educational laboratory.
- Develop and maintain the required ***Laboratory-Specific Biosafety Manual*** designed to supplement this BSU *Biological Safety Manual* with specific information relative the laboratory wherein the biohazardous materials are stored, used, or exposed;
- Adhere to approved emergency plans for handling accidental spills and personnel contamination.
- Comply with permit and shipping requirements for recombinant or synthetic nucleic acid molecules, transgenic, or biohazards. This includes permits, material transfer agreements, and other documentation for international, interstate and intrastate transport of genetically modified and biohazardous material.
- Develop specific biosafety Standard Operating Procedures (SOPs) for animals and biohazards used in the laboratory.
- Ensure compliance by laboratory personnel with relevant regulations, guidelines, and policies.
- Ensure all appropriate personal protective equipment is provided and used.
- Ensure proper training, including refresher training, and instruction for laboratory personnel in safe practices and protocols, including, at a minimum, training in aseptic techniques and

characteristics of the material(s) used. These signed documents must remain easily accessible in the laboratory at all times.

- Ensure the integrity of the safety equipment (e.g., biological safety cabinets), maintain biological containment (e.g., purity and genotypic and phenotypic characteristics), and ensure correct procedures or conditions are followed to prevent a release of or exposure to recombinant or synthetic nucleic acid molecules and/or biohazards, select agents or toxins.
- Inform the laboratory staff of the BSU *Occupational Health and Safety Program*, possible symptoms of illness relating to materials used, and provisions for any precautionary medical practices advised or required, e.g., vaccinations or serum collection.
- Propose appropriate microbiological practices and laboratory techniques to be used for the research.
- Provide to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken. Instruct, train and supervise research personnel in:
    - Aseptic technique.
    - Laboratory practices and techniques required to ensure safety.
    - Procedures for dealing with spills or potential exposures to the agents described in the research.
    - Characteristics of the material(s) used.
    - Signs and symptoms biohazards.
    - NIH classification of work (if working with r/sNA molecules).
- Obtain University or Institutional Biosafety Committee approval prior to initiating or modifying any research involving use of recombinant or synthetic nucleic acid molecules and/or biohazards and maintain that approval through timely submission of annual reviews.
- Immediately report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety and any other university committees (e.g., University or Institutional Biosafety Committee, Institutional Review Board, Institutional Animal Care and Use Committee) that have reviewed and approved the research activity.
- Supervise laboratory staff to ensure that the required safety practices and techniques are employed. Correct work errors and conditions that may result in accidents, injuries, or the release of biohazards.

Principal investigators are also responsible for full compliance with the *NIH Guidelines* during the conduct of NIH-regulated recombinant or synthetic nucleic acid research.

- The PI will consult with the IBC to determine whether the recombinant or synthetic nucleic acid molecule research is subject to the NIH authority and the *NIH Guidelines*.
- Develop specific biosafety standard operating procedures for recombinant or synthetic nucleic acid molecules or biohazards used in the laboratory.
- Obtain Institutional Biosafety Committee approval before initiating recombinant or synthetic nucleic acid molecule research subject to the *NIH Guidelines*.
- Make the initial risk assessment and determination of biological containment levels in

accordance with the *NIH Guidelines* when registering research with the Biosafety Committee.

- Immediately report any significant problems, violations of the *NIH Guidelines*, or any significant research-related accidents and illnesses to the Biological Safety Officer, Biosafety Committee, NIH Office of Biotechnology Activities, and other authorities, as appropriate.
- Submit any subsequent changes (e.g., changes in the source of DNA or host-vector system) to the disclosure to the Biosafety Committee for review and approval or disapproval.

Office of Research Integrity

- Provides the necessary liaison between Principal Investigators, the University or Institutional Biosafety Committee, granting agencies, and regulatory agencies.
- Serves as the Office of Record for documentation involving the Institutional Biosafety Committee and provides administrative assistance to the Biosafety Committee.
- Provides all necessary documentation for PIs to comply with University submission requirements.
- Provides assistance to Principal Investigators and researchers regarding regulatory requirements, export/import controls, conflict of interest, select agents, etc.

BSU Health Center

The responsibilities of the BSU Health Center include, but are not limited to, the following:

- The BSU Health Center, in conjunction with the EHS Office, maintains a medical health surveillance program for individuals who are exposed to animals used for research or teaching purposes, and provides treatment or prophylactic measures for those with medical problems related to laboratory and/or animal exposures.
- Similar protocols will be employed for all ABSL-2 and ABSL-3 research where exposure to biological agents occurs or potential is likely. This may include review of medical questionnaires, physical examinations, vaccinations, or the recommendation of other prophylactic measures as needed for researchers, students, or others who may be exposed to biological agents.

Laboratory Personnel

The responsibilities of animal care and laboratory personnel include but are not limited to the following:

- Participate in appropriate training and instruction to ensure that they are adequately trained and fully understand the instructions. This includes taking refresher courses as applicable.
- Fully comprehend all biohazards and select agents and toxins being used in the lab and the potential risks associated with exposure, as well as fully understanding the associated emergency response procedures.
- Follow all laboratory practices, protocols, and comply with all applicable policies, procedures, and guidelines.
- Complete any necessary medical surveillance.

- Obtain necessary and recommended vaccinations, or submit declination forms as permitted.
- Report thefts, security incidents, accidents, spills, or contamination incidents to supervisor.

Responsible Official

A Responsible Official (RO) will be assigned should it become necessary as required under the Department of Health and Human Services (HHS) and USDA *Select Agent and Toxin regulations.* This individual's responsibilities can be found in the applicable regulations. Although this list is not intended to be complete, critical responsibilities include:

- Develop and implement safety, security, and emergency response plans.
- Allow only approved individuals to have access to select agents or toxins.
- Provide appropriate training for safety, security and emergency response.
- Transfer select agents or toxins only to registered individuals.
- Provide immediate notice of any theft, loss, or release of a select agent or toxin.
- Provide proper laboratory facilities to contain and dispose of select agents and toxins.
- Maintain complete records relating to select agents as defined in 42 CFR 73.15.
- Report the identification of a select agent or toxin as a result of diagnosis, verification, or proficiency testing.
- Submit changes in the registration information by promptly notifying the CDC or Animal and Plant Health Inspection (APHIS)/USDA in writing. This includes modifications to the list of individuals that have been approved to work or access select agents, changes in work locations, and changes in protocols or objectives of the studies.
- Conduct regular inspections, at least annually, of the laboratory where select agents or toxins are stored or used to ensure compliance with all procedures and protocols of this safety plan. The results of these inspections must be documented and any deficiencies must be corrected and documented.

The RO for the Ball State University Select Agent Program will be listed below at such time that such a requirement becomes applicable:

Name: _____ Title: _____

Alternate: _____ Title: _____

Other Organizations/Departments

Other committees, including the Institutional Review Board, Laboratory Safety and Security Committee, Institutional Animal Care and Use Committee, Radiation Safety Committee, and the Department of Public Safety must consult and coordinate with the Biosafety Committee and Environmental Health and Safety Office on any proposals or activities under their purview which involve the use of, or potential exposure to, biohazards. All University Colleges, Schools, and Departments must also consult and coordinate with the IBC and EHS on such activities or potential exposure to biohazards.

Visitors, Vendors, and Contractors

Contractors must ensure that appropriate Personal Protective Equipment (PPE) is available for their own workers. All visitors, vendors, and contractors must:

- Comply with all security requirements and procedures.
- Be accompanied by a Department of Justice approved person at all times while in areas with select agents or toxins.
- Use Personal Protective Equipment (PPE) provided for them by the laboratory or animal handling room.

Information for Researchers

Agent Lists

The biological agents found in **Appendix D** have been listed according to the most appropriate Biological Safety Level to be used. The lists presented there are based upon the most recent information available in the *Risk Group Classifications for Infectious Agents* section of the *American Biological Safety Association* (ABSA) website. Every attempt to provide accurate classification for the agents listed has been made. As this is not a complete list of infectious agents, and these classifications are subject to change, please refer to the following ABSA website: https://my.absa.org/tiki-index.php?page=Riskgroups for classification of other agents, and the most up-to-date information for those listed.

Please note that Biological Safety Levels are not inherent to an agent but are performance recommendations and should be chosen only after a complete *Risk Assessment* is completed. A proper risk assessment takes into account the characteristics of the agent involved, the activities to be performed, and the environment in which the work will be completed. Therefore, certain agents may be used at different Biological Safety Levels depending upon the circumstances. For instance, human clinical samples from HIV-positive patients may be safely handled at BSL-2. Growth of HIV in culture should be performed under BSL-3 containment. Biological Safety Levels may be higher or lower than what is given below for a particular agent depending upon the circumstances of its use, and the containment facilities available and procedures approved for such use.

The Biohazard Committee and Biological Safety Officer (BSO) review all projects involving recombinant DNA, infectious disease agents, and agents of concern and will assist you in the risk assessment process. Once the Biosafety Committee (IBC) and/or the BSO assigns a Biological Safety Level, it must be adhered to unless new information to warrant a change, in most cases from peer-reviewed literature, is provided. The IBC and/or BSO will review the literature and make an adjustment, if warranted.

Project Registration: Ball State University Biosafety Committee

Many research projects involve work with potentially hazardous biological agents, known infectious disease agents, or biological materials that may or may not be specifically regulated by the federal or state government. Granting agencies commonly require that the university monitor the use of biological hazards, infectious disease agents, and recombinant DNA/synthetic nucleic acids in order for them to release funds to investigators. Therefore, BSU has developed a registration system to ensure that all biological materials are correctly identified, handled properly, and disposed appropriately.

The University or Institutional Biosafety Committee reviews all registrations. Approval by the Committee, in writing, is required before ordering or working with any new agents or toxins. Please contact the Committee or BSO for more information.

A fillable *Registration Document* is available on the EH&S website at: http://BSU.edu/Research/Compliance/Environmental-Health-and-Safety/Biological-Safety/. A copy of the registration form (jpg: non-fillable) is included as **Appendix P** of this Manual.

The Biohazard Committee administers four (4) registration programs for research and training projects.

Biological Agent (BA) Registration

Use of the following materials requires that the Principal Investigator or faculty complete and submit the biohazard registration document for approval by the Committee or the Biological Safety Officer.

Agents characterized at Biosafety Level 1 require registration. These will be expedited and administratively approved barring any complications.

Agents to be used at Biosafety Level 2 (BSL-2) or Biosafety Level 3 (BSL-3):

1. All human, animal, or plant pathogens that require BSL-2 or BSL-3 containment and handling (see earlier section: *Agents List*) must be registered. Please note that BSL-4 agents may not be stored or used at BSU.
2. Unknown human and animal pathogens must be registered. These are considered BSL-2 Until otherwise identified.
3. Cell lines or cultures that:
    a. have been immortalized with a virus (such as EBV or a retrovirus),
    b. are known to be tumorigenic in primates (including humans), or
    c. are primary human tumor cells.

    These are considered BSL-2 (or higher in many cases).
4. Human blood or other tissues, when used in research, must be registered.

5 Human blood or other tissue known to be HIV positive, or known to contain any human disease causing agent, may require higher containment depending upon the Committee or BSO evaluation.

Recombinant DNA / Synthetic Nucleic Acid (R-DNA) Registration

All rDNA projects that involve a recombinant organism (this excludes projects that involve DNA only, i.e. PCR) require registration with the Biosafety Committee. A subset of rDNA projects requires review and approval from the Institutional Biosafety Committee (IBC). The BSU IBC oversees all research projects and issues involving rDNA at BSU. Use of the following requires that the principal investigator complete and submit an rDNA registration document:

1. All rDNA projects, including the growth of recombinant bacteria for probe isolation (plasmid or phage preparations) require registration. Projects must be registered regardless of where the material came from or who originally constructed it.
2. Projects that are otherwise exempt from the NIH Guidelines must also be registered.
3. The development of transgenic animals and plants requires registration.

The following is provided as guidance regarding those research or experimental activities that are either covered by, or exempted from, the NIH guidelines:

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:

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

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

1. The formation of recombinant DNA molecules containing no more than 2/3 of the genome of any eukaryotic virus propagated in tissue culture;
2. Recombinant DNA-modified whole plants, and/or recombinant DNA-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-C, or III-D of the Guidelines; or
3. The generation of transgenic rodents that require BSL1 containment.

Experiments exempt from the NIH Guidelines:

Experiments exempt from the NIH Guidelines, although still requiring registration with the IBC,

may be initiated immediately. The BSO or Committee will review the registration and confirm that the work is classified correctly according to the NIH Guidelines. Exempt experiments are those that:

1. 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;
2. Use rDNA molecules that are not in organisms or viruses;
3. 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;
4. 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;
5. Consist entirely of DNA from an 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);
6. 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;
7. 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;
8. Contain less than one-half of any eukaryotic viral genome propagated in cell culture;
9. 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; or
10. Involve the purchase or transfer of transgenic rodents for experiments that require BSL1 containment.

R-DNA projects are performed at BSL-1, BSL-2, BSL-3 or the corresponding levels for whole plant (BSL-1P, BSL-2P, BSL-3P) or whole animal (BSL-1N, BSL-2N, BSL-3N) work. The Biological Safety Officer, in conjunction with the Biosafety Committee, will make the final determination.

Acute Toxins (AT) Registration

The use and storage of chemicals with a **mammalian $_{LD50}$ of < 100 µg/kg** require registration. For a partial list, see **Appendix D**. Acute toxins may only be ordered following written approval by the BSO or IBC.

Select Agents and Regulated Biological Materials

Select Agents are microorganisms and toxins that have potential for use by terrorists. 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 the BSO immediately if you currently possess or plan to acquire any of the agents listed in **Appendix E** 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 the BSO, or visit the federal Select Agent web site at www.selectagents.gov , which provides links to select agent program information.

Agents, such as plant pathogens or exotic microorganisms, which are regulated by federal or state agencies (CDC, HHS, DOC, USDA/APHIS, EPA, FDA, DPI, etc.) shall be registered with the Biological Safety Officer by submission of the biological agent Registration Document. All permits for transport, transfer, import or export of these regulated agents are the responsibility of the Principal Investigator. The BSU Responsible Official (RO) will be responsible for all Select Agent permits (see below). Please allow a minimum of 6 weeks to obtain new federal permits.

The agents and toxins listed in **Appendix E** are classified by the Federal government as *Select Agents*. Any possession, use, transfer or shipment of these materials is strictly controlled by regulation.

Researchers considering work with any of these materials must first contact the BSU Responsible Official for the approvals, permits, clearances and other necessary paperwork. Be aware that government clearance can take as much as 6 months to complete in advance of any project.

Failure to comply with these Federal Regulations is punishable by both fines and imprisonment.

Project Amendment

Changes to an existing *Registration Document* can be done on an amendment unless said changes result in a dramatic change to the overall project or the containment level.

Typical changes that qualify as a project amendment, not requiring a new Registration may include:

- Addition or deletion of new personnel;
- Addition or deletion of new agents (same containment level);
- Re-examination of the laboratory and agent Risk Assessment – if no changes to the RG or BSL classifications become necessary as a result;
- Minor modifications to the Laboratory-specific Biosafety Manual; and,
- Minor modifications to the protocol or procedures (final decision to be made by the IBC)

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 infectious hazard of clinical specimens.

Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and identification of isolates can be done safely at Biosafety Level 2, the recommended level for work with bloodborne pathogens such as hepatitis B virus and HIV. The containment elements described in Biosafety Level 2 are consistent with the *Occupational Exposure to Bloodborne Pathogens Standard* from the Occupational Safety and Health Administration (OSHA), which requires the use of specific precautions with all clinical specimens of blood or other potentially infectious material (Universal Precautions). Additionally, other recommendations specific for clinical laboratories may be obtained from the National Committee for Clinical Laboratory Standards.

Biosafety Level 2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as biological safety cabinets (Class I or II) should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. Biological safety cabinets should also be used for the initial processing of clinical specimens when the nature of the test requested or other information is suggestive that an agent readily transmissible by infectious aerosols is likely to be present (e.g., M. tuberculosis), or when the use of a biological safety cabinet (Class II) is indicated to protect the integrity of the specimen.

The segregation of clinical laboratory functions and limiting or restricting access to such areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented.

Biosafety and Recombinant DNA Technology

In the past several years, recombinant DNA/synthetic nucleic acid has become widely used in many fields of research. The National Institutes of Health (NIH) has established regulations on the use and containment of recombinant DNA materials in the laboratory. Regulations require persons conducting such research to file a registration form with the University or Institutional Biosafety Committee (UBC or IBC) which must approve the protocols related to recombinant DNA molecules.

As a condition for funding of recombinant DNA research, BSU must ensure that research conducted at or sponsored by BSU, irrespective of the source of funding, complies with the most current NIH *Guidelines for Research Involving Recombinant DNA Molecules.* At BSU, the responsibility for ensuring that recombinant DNA activities comply with all applicable guidelines rests with the university and the Institutional Biosafety Committee (IBC) acting on its behalf.

Guidelines for Working with Genetically Modified Animals

The Environmental Protection Agency (EPA) has specific guidelines for containment measures for transgenic animals including but not limited to *mice, rats, invertebrates and fruit flies*. Under the Genetically Modified Organisms (Contained use) Regulations, 2001, S.I. No. 73 of 2001, there is a statutory requirement for users of Genetically Modified animals to apply the principles of Good Animal House Practice.

NIH Recombinant DNA Review Categories

All recombinant DNA (rDNA) research proposals require the PI to make an initial determination of the required level of physical and biological containment. For that reason, the NIH has developed six categories (**III-A** to **III-F**) addressing different types of rDNA research. These categories are included in the BSU **Registration Document**.

If the proposed research falls within section **III-A** of the NIH Guidelines, the experiment is considered a "Major Action". This includes experiments involving human gene transfer experiments. As a result, the experiment cannot be initiated without submission of relevant information to the Office of Recombinant DNA Activities at NIH. In addition, the proposal has to be published in the Federal Register for 15 days, it needs to be reviewed by the NIH Recombinant DNA Advisory Committee (RAC), and specific approval by the NIH has to be obtained. The containment conditions for such an experiment will be recommended by the RAC and set by the NIH at the time of approval. The proposal requires IBC approval before initiation.

If the proposed research falls within section **III-B**, the research cannot be initiated without submission of relevant information on the proposed experiment to NIH/ Office of Biotechnology Activities (OBA) (For exceptions see the guidelines). Experiments covered in III-B include the cloning of toxic molecules. The containment conditions for such experiments will be determined by NIH/OBA in consultation with ad hoc experts. Such experiments require Institutional Biosafety Committee approval before initiation. Please refer to the guidelines for more specifics.

In section **III-C**, experiments with human subjects are covered. These experiments require IBC and IRB (Institutional Review Board) approval and NIH/OBA registration before initiation.

Section **III-D**, the next category, covers whole animal or plant experiments as well as projects involving DNA from Risk Group 2, 3 or 4 agents. Prior to the initiation of an experiment that falls into Section **III-D**, the PI must submit a *Registration Document for Recombinant DNA Research* to the Institutional Biosafety Committee. The IBC reviews and approves all experiments in this category prior to initiation.

Section **III-E** experiments require the filing of a *Registration Document for Recombinant DNA Research* with the IBC at the time the experiment is initiated. The IBC reviews and approves all such proposals, but Institutional Biosafety Committee review and approval prior to initiation of the experiment is not required.

Section **III-F** experiments are exempt from the NIH Guidelines however, they must still be registered with the IBC who will verify the exempt status of the registration.

Review Process Overview

Once your registration document has been submitted, a representative from the IBC or the Biosafety Officer will screen it and may contact you for more information about your research

or for fine-tuning of your registration document before it is turned over to the full committee. The Biosafety Officer, sometimes along with members of the EHS Office, will meet with you as necessary to conduct a brief inspection of the proposed laboratory location for the research and discuss the required *Risk Assessment* and *Laboratory-Specific Biosafety Manual* pertinent to your laboratory space and the project itself. The registration document is then distributed to the IBC for review. [Tentative: Since the Committee normally meets towards the end of each month to review projects, registrations must be submitted by mid-month at the latest, in order to be considered that month. The committee will then review it and report back to you. They may request additional information or changes to the registration before approval. The entire review process usually takes 6 to 8 weeks].

Responsibilities of the Principal Investigator (PI) for Recombinant DNA Research

The Principal Investigator is responsible for full compliance with the NIH Guidelines in the conduct of recombinant DNA research. Please refer to the most recent edition of the NIH *Guidelines for Research Involving Recombinant DNA Molecules* for more information.

General Responsibilities

As part of this general responsibility, the Principal Investigator shall:

1. Initiate or modify no recombinant DNA research which requires IBC approval prior to initiation until that research or the proposed modification thereof has been approved by the IBC and has met all other requirements of the NIH Guidelines;
2. Determine whether experiments are covered by Section III-E, *Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation*, and that the appropriate procedures are followed;
3. Report any significant problems, violations of the NIH Guidelines, or any significant research-related accidents and illnesses to the Biological Safety Officer, the Institutional Biosafety Committee, NIH, and other appropriate authorities (if applicable) within 30 days;
4. Report any new information bearing on the NIH Guidelines to the Institutional Biosafety Committee and to NIH;
5. Be adequately trained in good microbiological techniques;
6. Adhere to IBC-approved emergency plans for handling accidental spills and personnel contamination; and
7. Comply with shipping requirements for recombinant DNA molecules. Contact the Biosafety Committee or BSO for more information.

Submissions by the Principal Investigator to the NIH/OBA

The Principal Investigator shall:

1. Submit information to NIH/OBA for certification of new host-vector systems;
2. Petition NIH/OBA, with notice to the IBC, for proposed exemptions to the NIH Guidelines;
3. Petition NIH/OBA, with concurrence of the IBC, for approval to conduct experiments specified in Sections III-A-1, Major Actions Under the NIH Guidelines, and III-B, Experiments that Require NIH/OBA and IBC Approval Before Initiation;

4. Petition NIH/OBA for determination of containment for experiments requiring case-by-case review; and
5. Petition NIH/OBA for determination of containment for experiments not covered by the NIH Guidelines.

Submissions by the Principal Investigator to the Institutional Biosafety Committee

The Principal Investigator shall:

1. Make an initial determination of the required levels of physical and biological containment in accordance with the NIH Guidelines and the **BSU Registration Document**;
2. Select appropriate microbiological practices and laboratory techniques to be used for the research;
3. Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system) to the IBC for review and approval or disapproval;
4. Provide a completed **Risk Assessment** and **Laboratory-specific Biosafety Plan** for the project or activities; and,
5. Remain in communication with the IBC throughout the duration of the project.

Responsibilities of the Principal Investigator Prior to Initiating Research

The Principal Investigator shall:

1. Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;
2. Instruct and train laboratory staff in the:
    a. Practices and techniques required to ensure safety, and
    b. Procedures for dealing with accidents; and
3. Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

Responsibilities of the Principal Investigator During the Conduct of the Research

The Principal Investigator shall:

1. Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;
2. Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer, the IBC, NIH/OBA, and other appropriate authorities (if applicable);
3. Correct work errors and conditions that may result in the release of recombinant DNA materials;
4. Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics);
5. Comply with annual data reporting and adverse event reporting requirements for NIH- and FDA- approved human gene transfer experiments.

Risk Assessment

Risk assessment is a process used to examine the various factors associated with a procedure involving biological materials in order to identify the hazardous characteristics of the material, the activities that can result in a person's exposure to an infectious agent, the likelihood that exposure will cause a laboratory acquired infection, and the probable consequences of an infection. The information identified by risk assessment will provide a guide for the selection of biosafety levels, microbiological practices, safety equipment, and facility safeguards that can prevent laboratory acquired inspections and reduce the risk of environmental contamination. Please refer to **Appendix F** for a form to assist with, and document, the required performance of Risk Assessments.

The factors to consider in a risk assessment therefore include both the:

1. Intrinsic hazards of the agents; and,
2. Laboratory procedures.

Agent Hazards

- Capability to infect and cause disease in a susceptible host
- Virulence as measured by the severity of disease
- Availability of preventive measures and effective treatments for the disease
- Probable routes of transmission of laboratory infection
  - The predominant routes of transmission in the laboratory include mucous membrane exposure, parenteral inoculation, ingestion and inhalation of infectious aerosols.
- Infective dose
- Stability in the environment
- Host range
- Its endemic nature
- Reports of laboratory acquired infections
- Origin of the agent: Non-indigenous agents are of special concern because of the potential to introduce risk of transmission, or spread of human and animal or infectious diseases from foreign countries to the United States.
- Genetically modified agents involve the same consideration for risk assessment as for wild-type organisms.

Risk Groups: Classification of Infectious Agents on the Basis of Hazard Presented

Risk groups (RG) are a method used by the World Health Organization (WHO) and by the National Institutes of Health (NIH) to classify human etiological agents based on hazard to both the individual and to the community. There are four risk groups. These correlate to but are not necessarily equivalent to biosafety levels. Determining the risk group of a biological agent can be part of the biosafety risk assessment and helps in assigning the correct biosafety level for

containment. In general, RG-2 agents are handled at BSL- 2, and RG-3 agents at BSL-3, etc. However, the use of certain RG-2 agents in large quantities might require BSL-3 conditions, while some RG-3 agents may be safely manipulated at a BSL-2 under certain specified conditions. The acceptability of such deviations is dependent on the outcome of the Risk Assessment and alternate procedures, as approved by EHS, must be detailed in the Laboratory-Specific Biosafety Manual. The basic Risk Group Classifications are summarized in **Table 1**:

Table 1. Risk Group (RG) Classifications			
RG-1	**RG-2**	**RG-3**	**RG-4**
Agents not associated with disease in healthy adult humans.	Agents associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).

Basis for the Classification of Biohazardous Agents by Risk Group

Examples of RG-1 agents include microorganisms like Escherichia coli-K12 or Saccharomyces cerevisiae. A list of Risk Group 2, 3 and 4 agents can be found in **Appendix D**. It is important to note, however, that no list is all inclusive. Also, those agents not listed in RG-2, RG-3 or RG-4 are not automatically classified in RG-1. Those unlisted agents need to be subjected to a risk assessment based on the known and potential properties of the agents.

Hazards of Genetically-Modified Agents

When conducting a risk assessment of genetically modified agents, consideration of the same factors used in risk assessment of the wild-type organism should be done. However, it is important to address the possibility that the genetic modification could alter (i.e., increase or decrease) the pathogenicity of the agent or affect its susceptibility to antibiotics or other treatments. Sometimes, important information may not be available for a newly engineered agent and the risk assessment may be difficult or incomplete. In these cases, due diligence should be practiced and the biosafety level assignment should be made conservatively. Once more information is available another risk assessment should be completed.

Hazards of Cell Cultures

Human and animal cells and tissues have the potential to harbor latent infectious agents and personnel who handle these materials are at risk for possible exposure. For additional information and requirements for working with human cell cultures please refer to the BSU Exposure Control Plan and to the following section of this manual: *Guidelines for Working with Tissue Culture/Cell Lines*.

Laboratory Procedure Hazards

1. ***Parenteral inoculations:*** Injection of potentially hazardous materials can occur by a needle, other contaminated sharp or by bites from infected animals or arthropod vectors.
2. ***Spills and splashes into skin and mucous membranes:*** Mucous membranes include the eyes, nose and mouth.
3. ***Ingestion through mouth pipetting***
4. ***Animal bites and scratches***
5. ***Inhalation exposures to infectious aerosols:*** Aerosols, or respirable sized particles, are extremely hazardous because they are generated in many lab procedures and are usually undetected. The creation of infectious aerosols places the person carrying out the procedure and others in the laboratory at risk. Any procedure that breaks the surface tension of a liquid will produce aerosols. Pipetting, blenders, non self-contained centrifuges, sonicators and vortex mixers all produce aerosols. Procedures and equipment that create aerosols also create larger droplets that rapidly settle out of the air. These droplets can settle on surfaces and therefore contaminate gloved hands, work spaces and mucous membranes.

Information identified in risk assessments should provide guidance for the selection of the biosafety level (BSL) for work and establish the four primary controls necessary for safe work:

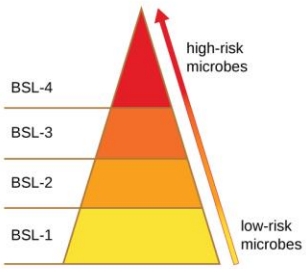
1. Work Place Practices
2. Personal Protective Equipment (PPE)
3. Administrative Controls
4. Engineering Controls

PIs can utilize the form in **Appendix F** to perform their program/project risk assessment. Pathogen safety data sheets are available in the BMBL and through Public Health Canada (see the Quick Reference Guide for link information).

Biological Safety

CDC and NIH have established four levels of biosafety, based on the degree of hazard associated with a microbial agent, to describe the combination of laboratory practices and techniques, safety equipment, and facilities needed to protect against exposure. The BMBL outlines these four (4) different biological safety levels that are appropriate for the operations performed in a laboratory, the documented or suspected routes of transmission of the biological agent, and the laboratory function or activity. The four biosafety levels (BSLs) require successively more stringent practices and facilities as work moves from the least restrictive, BSL-1, to work with the highest hazard level of BSL-4. Exposure to biohazards may be prevented or limited by establishing and following the appropriate biosafety level practices and conditions. The requirements for each laboratory biosafety level can be found in the BMBL. An illustrative table of the hazard levels appears in **Table 2** below:

Table 2: Illustration and Brief Summary of Biosafety Levels

Biosafety Levels			
Biological Safety Levels	**Description**	**Examples**	**CDC Classification**
BSL-4	Microbes are dangerous and exotic, posing a high risk of aerosol-transmitted infections, which are frequently fatal without treatment or vaccines. Few labs are at this level.	Ebola and Marburg viruses	
BSL-3	Microbes are indigenous or exotic and cause serious or potentially lethal diseases through respiratory transmission.	*Mycobacterium tuberculosis*	
BSL-2	Microbes are typically indigenous and are associated with diseases of varying severity. They pose moderate risk to workers and the environment.	*Staphylococcus aureus*	
BSL-1	Microbes are not known to cause disease in healthy hosts and pose minimal risk to workers and the environment.	Nonpathogenic strains of *Escherichia coli*	

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Currently four biosafety levels (1- 4) define the levels of containment necessary to protect personnel and the environment. A biosafety level 1 (BSL-1) is the least restrictive, while biosafety level 4 (BSL-4) requires a special containment laboratory or facility, which is not available at BSU. Since most of the research at BSU is conducted at biosafety levels 1 and 2 with few experiments at BSL-3, this manual will mainly focus on these three biosafety levels. For more information on Biosafety Level 4 requirements refer to the appropriate literature or contact the Biological Safety Officer. **Table 3** below summarizes the different biosafety level requirements that are presented in greater detail on the pages that follow:

Table 3: Biological Safety Levels			
BSL-1	**BSL-2**	**BSL-3**	**BSL-4**
Required 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.	Required for work involving agents that pose moderate hazards to personnel and the environment.	Required for 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 the inhalation route of exposure.	Required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. **Note: No research with biohazards at BSL-4 is currently permitted in BSU facilities.**

Biosafety Levels

As described and illustrated above, four biosafety levels (BSLs) are established which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity.

The recommended BSLs for the organisms represent those conditions under which the agent can ordinarily be safely handled. The Principal Investigator (PI), Laboratory Supervisor, or Clinic Director is specifically and primarily responsible for assessing risks and for appropriately applying the recommended biosafety levels.

His/her knowledge and judgment are critical in assessing risks and appropriately applying these recommendations. The recommended biosafety level represents those conditions under which the agent can ordinarily be safely handled. Generally, work with known agents should be conducted at the biosafety level recommended. When specific information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent practices may be specified with consideration of the Risk Assessment and approval from the BSO and/or IBC. Special characteristics of the agents used, the training and experience of personnel, and the nature or function of the laboratory may further influence the director in applying these

recommendations.

The following **Table 4** summarizes the major features (and differences) of the several Biosafety Levels that have been discussed which are then further presented in greater detail on the pages that follow.

Table 4: Summary of Recommended Biosafety Levels for Infectious Agents

Summary of Recommended Biosafety Levels for Infectious Agents

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	None required	Open bench top Sink for hand washing
2	Associated with human disease, hazards: percutaneous injury, ingestion, mucous membrane exposure	**BSL -1 practice plus:** • Limited access • Biohazard warning signs • "Sharps" precautions Biosafety manual defining any needed waste decontamination or medical surveillance	**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; PPE; laboratory coats; gloves; face protection as needed	**BSL -1 facility plus:** • Autoclave available • Single pass air with no recirculation • Hard, smooth, impervious floor, walls, and countertops • Hand washing sink at front of lab
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	**BSL -2 practice plus:** • Controlled access • Decontamination of all waste • Decontamination of lab clothing before laundering • Baseline serum Decontamination of all effluent	**Primary barriers** = Class I or II BSCs or other physical containment devices for all open manipulations of agents; PPE; protective lab clothing; gloves; respiratory protection as needed	**BSL -2 facility plus:** • Physical separation from access corridors • Self-closing, double- door access • Exhausted air not recirculated • Negative airflow into laboratory (single pass) • No floor drains • Hard, smooth, impervious ceilings
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	**BSL -3 practice 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 facility plus:** • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamination systems Many other requirements outlined in the text

The Biosafety Level 1 Laboratory

Biosafety Level 1 practices, safety equipment, and facilities are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other facilities in which work is done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. *Bacillus subtilis*, *Naegleria gruberi*, and infectious canine hepatitis virus are representative of those microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Vaccine strains which have undergone multiple in vivo passages should not be considered avirulent simply because they are vaccine strains.

Biosafety Level 1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required nor generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science.

The following standard and special practices, safety equipment, and facilities apply to the handling of agents assigned to **Biosafety Level 1:**

BSL-1: Laboratory Design and Facilities

The facilities required in a Biosafety Level 1 laboratory include the following:

Doors: Doors are required for access control. They should be kept locked when no one is present in the laboratory.

Sink: A sink must be available and supplied for handwashing (i.e., stocked with soap and paper towels).

Easily cleanable: The lab must be designed in a way that allows it to be cleaned easily. Carpets and rugs are not allowed. Spaces between benches, cabinets and equipment must be accessible for cleaning.

Furniture: Furniture in the lab must be appropriate for the anticipated use. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis and other chemicals. Chairs used in conjunction with lab work must be covered with a non-porous material that can be easily cleaned and disinfected.

Windows: If the lab has windows that can be opened to the outdoors, they must be fitted with screens.

BSL-1: Standard Microbiological Practices

The following standard microbiological practices must be used in a BSL-1 lab:

Controlled access: The lab supervisor must ensure that access to the laboratory is controlled. When procedures are in progress the lab door should be shut and when no one is present in the lab, the doors should be locked. Anyone requesting access to the laboratory should be questioned as to their purpose and identification should be provided.

Handwashing: Hands must be washed with soap and water after handling potentially infectious materials. Hands should be washed before leaving the laboratory and before touching common use surfaces (i.e, computers, telephones, etc.).

Eating, drinking, handling contact lenses and applying cosmetics: Eating, drinking, contact lens handling and cosmetic application must be done outside of the laboratory. Food and beverages for human consumption must be stored outside of the laboratory area in refrigerators or cabinets designated for that purpose.

Pipetting: Mechanical pipetting devices must be available and used. Mouth pipetting is prohibited.

Safe sharps practices: All policies regarding the safe use of sharps must be followed.
See the *Recommended Work Practices- Sharps* in **Appendix R**.

Minimize splashes and aerosols: Essentially all laboratory procedures involve steps which

create aerosols. All procedures should be completed in a manner which minimizes the creation of both splashes and aerosols. This can be done by using centrifuges with safety features (i.e., sealed cups and rotors), mechanical pipettors, conducting work inside of a biological safety cabinet, etc.

Decontaminate work surfaces: Work surfaces must be decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant should be used. If bench paper or plastic backed absorbents are used, they should be discarded and the space beneath decontaminated.

Proper decontamination and transport of waste: All cultures, stocks and other biohazardous materials must be properly decontaminated before disposal. If you will be transporting waste out of the laboratory (e.g., down the hall, to another floor of the building, etc.) to be decontaminated you must ensure that the waste is placed in a leak-proof container and is secured. Please refer to the Section of this manual: *Biohazardous Waste*, and the BSU *Waste Management Plan* for additional information regarding to the proper decontamination of biohazardous waste.

Door signage: All laboratory doors must have an *Admittance to Authorized Personnel Only* label. This label contains appropriate contact information for general and emergency entrance to the lab. Additionally, the lab entrance must be labeled with the universal biohazard symbol when infectious agents are in use.

Pest management program: A pest management program is managed through BSU Landscape Services. They should be contacted at the first sign of a problem.

Training: In addition to the completion of EHS required training courses, the principal investigator must ensure that all lab personnel receive site-specific training. This training should include information specific to their job duties, precautions to prevent exposures, and exposure response procedures. In addition, lab personnel should be given information about immune competence and conditions that could predispose them to infection, as appropriate. See Appendix K for a checklist to assist with and document this training.

BSL-1: Special Practices

There are no special practices required in a BSL-1 lab. The standard microbiological practices described above will normally suffice with proper training, procedures, and techniques.

BSL-1: Safety Equipment

The following **Personal protective equipment (PPE)** must be used in a BSL-1 lab:

Laboratory coats, gowns, or uniform use is recommended for potential BSL-1 exposures and is commonly required under BSU laboratory safety standards where hazardous chemicals are present.

Splash goggles must be worn when there is the potential for splashes of microorganisms or

other hazardous materials. Personnel who wear contact lenses should wear safety glasses or other eye protection at all times while in the laboratory.

Gloves must be worn as protection from hazardous materials. If latex gloves are used, alternatives should be made available. Gloves must be changed when contaminated, when the integrity has been compromised, or when necessary. Disposable gloves should be disposed of with other contaminated waste and must not be washed or reused. Powdered gloves are not permissible. Hands must be washed after removing gloves, and before leaving the laboratory.

The Biosafety Level 2 Laboratory

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in four major ways.

1. Laboratory personnel must have specific training in handling pathogenic agents and should be directed by competent scientists.
2. Access to the laboratory must be limited when work is being conducted.
3. Extreme precautions must be taken with contaminated sharp items.
4. Certain procedures in which infectious aerosols or splashes may be created must be conducted in biological safety cabinets or other physical containment equipment.

Biosafety Level 2 practices, equipment, and facilities are applicable to clinical, diagnostic, teaching, and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Salmonellae, clinical samples of Hepatitis B, and *Toxoplasma* spp. are representative of microorganisms assigned to this containment level. Biosafety Level 2 is appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the 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 precaution with contaminated needles or sharp instruments must be emphasized. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or devices such as a BSC or safety centrifuge cups. Other primary barriers should be used, as appropriate, such as splash shields, face protection, gowns, and gloves. Secondary barriers such as hand washing and waste decontamination facilities must be available to reduce potential environmental contamination.

The following standard and special practices, safety equipment, and facilities apply to the use of agents assigned to **Biosafety Level 2:**

BSL-2: Laboratory Design and Facilities

The facilities required in a biosafety level two laboratory include the following:

Doors: Self-closing doors are required for access control. They must be closed when work is in progress inside the lab and they should be kept locked when no one is present in the laboratory.

Sink: A sink must be available and supplied for handwashing (i.e., stocked with soap and paper towels). It should be located near the exit door or at the transition from the "dirty" laboratory space to the "clean" laboratory area. The sink should be foot-pedal operated.

Easily cleaned: The lab must be designed in a way that allows it to be cleaned easily. Carpets and rugs are not allowed. Spaces between benches, cabinets and equipment must be accessible for cleaning.

Furniture: Furniture in the lab must be appropriate for the anticipated use. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis and other chemicals. Chairs used in conjunction with lab work must be covered with a non-porous material that can be easily cleaned and is relatively resistant to disinfectant agents.

Windows: If the lab has windows that can be opened to the outdoors, they must be fitted with screens.

Biological safety cabinets: Biological safety cabinets (BSC) must be installed in a manner so that changes in room air do not interfere with the operation of the cabinet. They should be located

away from doors, windows that can be opened, high traffic areas, and other areas that could cause disruptions in the airflow of the cabinet. They must be tested and certified at least annually and whenever they are relocated or serviced. BSCs should be operated in accordance with the manufacturer's recommendations. See the following section of this manual for additional information: *Safety Equipment- Biological Safety Cabinets*.

Vacuum lines: Vacuum lines must be protected by High Efficiency Particulate air (HEPA) filters.

Eyewash stations: An eyewash station must be readily available.

Airflow: Ventilation systems must allow for inward flow of air without recirculation to spaces outside of the laboratory.

Waste decontamination: A method for decontaminating lab wastes (i.e., autoclave, incineration, etc.) must be available. It is the responsibility of the generating department to decontaminate all solid non-sharps biohazardous waste and all liquid biohazardous waste. EHS is responsible for the removal and proper treatment of sharps waste. See the BSU Waste Management Plan for additional information.

BSL-2: Standard Microbiological Practices

The following standard microbiological practices must be used in the BSL-2 lab:

Controlled access: The lab supervisor must ensure that access to the laboratory is controlled. When procedures are in progress the lab door should be shut and when no one is present in the lab, the doors should be locked. Anyone requesting access to the laboratory should be questioned as to their purpose and identification should be provided.

Handwashing: Hands must be washed with soap and water after handling potentially infectious materials. Hands should be washed before leaving the laboratory and before touching common use surfaces (i.e, computers, telephones, etc.).

Eating, drinking, handling contact lenses and applying cosmetics: Eating, drinking, contact lens handling and cosmetic application must be done outside of the laboratory. Food and beverages for human consumption must be stored outside of the laboratory area in refrigerators or cabinets designated for that purpose.

Pipetting: Mechanical pipetting devices must be available and used. Mouth pipetting is prohibited.

Safe sharps practices: All policies regarding the safe use of sharps must be followed. See the following section of this manual for additional information: *Recommended Work Practices- Sharps*.

Minimize splashes and aerosols: Essentially all laboratory procedures involve steps which

create aerosols. All procedures should be completed in a manner which minimizes the creation of both splashes and aerosols. This can be done by using centrifuges with safety features (i.e., sealed cups and rotors), mechanical pipettors, conducting work inside of a biological safety cabinet, etc.

Decontaminate work surfaces: Work surfaces must be decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant should be used. If bench paper or plastic backed absorbents are used, they should be discarded and the space beneath decontaminated.

Proper decontamination and transport of waste: All cultures, stocks, and other biohazardous materials must be decontaminated before disposal. If you will be transporting waste out of the laboratory (e.g., down the hall, to another floor of the building, etc.) to be decontaminated you must ensure that the waste is placed in a leak-proof container and is secured. Please refer to the following section of this manual: *Biohazardous Waste*, and the BSU Waste Management Plan for additional information regarding to the proper decontamination of biohazardous waste.

Door signage: All laboratory doors must have an *Admittance to Authorized Personnel Only* label. This label contains appropriate contact information for general and emergency entrance to the lab. Additionally, the lab entrance must be labeled with Biosafety Level 2 door sign. Both of these labels should be obtained from EHS.

Pest management program: A pest management program is managed through BSU Landscape Services. They should be contacted at the first sign of a problem.

Training: In addition to the completion of EHS required training courses, the principal investigator must ensure that all lab personnel receive site-specific training. This training should include information specific to their job duties, precautions to prevent exposures, and exposure response procedures. In addition, lab personnel should be given information about immune competence and conditions that could predispose them to infection, as appropriate. See **Appendix O** for a checklist to assist with and document this training.

BSL-2: Special Practices

The following special practices must be utilized in a BSL-2 lab:

Laboratory entrance: Before entering the laboratory, all people must be made aware of the potential hazards. They must also meet all entry and exit requirements (e.g., donning and doffing of personal protective equipment, immunization requirements, handwashing, etc.).

Medical surveillance: All laboratories using human-derived materials or cell lines must participate in the Bloodborne Pathogens Program. See the BSU Exposure Control Plan for additional information. For the use of other agents, medical surveillance and immunizations will be provided as appropriate. Occupational Health as well as EHS should be contacted for assistance.

Laboratory specific biosafety manual: Each laboratory must supplement this biosafety

manual with information that is specific for the individual laboratory. Supplemental information may include: specific PPE practices and location of supplies, laboratory specific training requirements, laboratory specific waste handling practices and autoclave procedures, safe operation and decontamination of lab specific equipment, proper use of disinfectants specific for the lab (appropriate concentration, contact time and shelf life), etc.

Training: Lab personnel must demonstrate proficiency in microbiological practices before handling BSL-2 agents. It is the responsibility of the PI to ensure that proficiency has been demonstrated.

Containers for potentially infectious materials: Containers used to collect, handle, process, store, or transport within a facility, potentially infectious materials must be durable, leak-proof and have a lid. The containers must be properly labeled with the contents and a biohazard symbol.

Decontamination of laboratory equipment: Lab equipment must be decontaminated routinely. It must also be decontaminated after spills, splashes or when potentially contaminated. All spills must be cleaned by personnel who are properly trained and have the proper equipment to handle infectious materials. All BSL-2 labs should have a biological spill kit available. See the following section of this manual for spill cleanup procedures and spill kit contents: *Biohazard Spill Cleanup Procedures*. All equipment must be decontaminated before being repaired, maintained, or removed from the laboratory. When any of these is to occur, lab personnel must complete an **Equipment Release Form** and attach it to the piece of equipment. See **Appendix H** for an example of the form.

Exposure incidents: Exposure response procedures should be posted in an easily accessible location in the laboratory. All lab personnel should be made aware of the proper procedures to follow in the event of a possible exposure to potentially infectious materials. See Appendix F for exposure response procedures.

Non-research related animals and plants in the laboratory Animals and plants not associated with the work being done are not allowed in the laboratory.

Aerosol generating procedures: All procedures that may result in the generation of potentially infectious aerosols (pipetting, mixing, vortexing, etc.) must be conducted within a biological safety cabinet or other approved containment devices.

BSL-2: Safety Equipment

The following safety equipment must be available and used in a BSL-2 lab. If not physically available in the research or educational laboratory itself, the *Laboratory-specific Biosafety Manual* must describe their location, the means of transporting the biological agent or toxin, and other protective measures at the location of the necessary equipment or devices:

Biological safety cabinets (BSC): A biological safety cabinet, or a combination of PPE and other containment devices (as approved by the biological safety officer) must be used when there is the potential for the creation of infectious aerosols or splashes. This includes, but is not

limited to: pipetting, centrifuging, mixing, sonicating, blending, shaking, opening containers, intranasal inoculation of animals, and harvesting tissues. A BSC must also be used when handling large volumes or high concentrations of potentially infectious materials. Centrifugation of these materials may be done outside of the BSC if sealed rotors or centrifuge safety cups are used. See the following section of this manual for additional information: ***Safety Equipment-Biological Safety Cabinets*.**

Personal protective equipment (PPE): The use of laboratory coats, gowns or uniforms is required when handling biohazardous materials. Splash goggles and face protection must be used when there is the potential for splashes of microorganisms or other hazardous materials. Personnel who wear contact lenses should wear safety glasses or other eye protection at all times while in the laboratory. Gloves must be worn as protection from hazardous materials. Two pairs should be worn as appropriate. If latex gloves are used, alternatives should be made available. Gloves must be changed when contaminated, when the integrity has been compromised, or when necessary. Disposable gloves must not be washed or reused. Hands must be washed after removing gloves, and before leaving the laboratory. All protective equipment must be removed before leaving the laboratory. Used disposable PPE should be disposed of with other contaminated waste. Reusable PPE (i.e., goggles) should be appropriately decontaminated before reuse. Reusable laboratory clothing should be laundered through BSU Laundry. It must not be taken home. If visibly contaminated, laundry should be placed in a biohazard bag before being placed with other items to go to laundry. *See Laundry.*
Animal rooms: Eye, face and respiratory protection should be used as appropriate in rooms containing infected animals.

Biological Safety Level 3 Laboratories

Currently, no facilities are available at BSU for BSL-3 activities and no such research is approved to be conducted. However, as a description of the required procedures and features of BSL-3 work can aid in illustrating the features that are necessary to BSL-1 and BSL-2 research or educational activities, the following is provided. Also, it is possible, with IBC and EHS approval and oversight, that RG-3 organism research or activities may be undertaken in the future utilizing modified BSL-2 facilities and procedures, subject to specified enhanced primary and secondary containment conditions, if a risk assessment demonstrates the feasibility.

The containment laboratory- Biosafety level 3 (BSL-3) is designed for work with agents that may cause serious or potentially lethal disease via inhalation. A BSL-3 lab may also be used when working with large volumes or high concentrations of Risk Group 2 microorganisms that pose an increased risk of aerosol spread.

Biosafety Level 3 practices, safety equipment, and facilities 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. Hepatitis B. (propagation of high concentrations only), *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and

exposure to infectious aerosols.

At Biosafety Level 3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and a specialized ventilation system that minimizes the release of infectious aerosols from the laboratory.

The following are the required features and practices when involved in BSL-3 activities:

BSL-3: Laboratory Design and Facilities

The facilities required in a biosafety level three laboratory include the following:

Location: The laboratory should be separated from areas that have unrestricted traffic flow within a building.

Doors: A series of two self-closing doors are required for access control. They must be closed when work is in progress inside the lab and they should be kept locked when no one is present in the laboratory.

Sink: A hands-free or automatic sink must be available and supplied for handwashing (i.e., stocked with soap and paper towels). It should be located near the exit door. If the lab is separated

into multiple labs, each area must have a sink available and supplied for handwashing.

Easily cleaned and decontaminated: The lab must be designed in a way that allows it to be cleaned and decontaminated easily. Carpets and rugs are not allowed. Seams, floors, walls and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed for whole room decontamination. Floors must be slip resistant, impervious to liquids and resistant to chemicals. Walls and ceilings should have a smooth, sealed finish to allow for decontamination. Whole lab decontamination should be considered when gross contamination has occurred, when there is a change in lab usage, for renovations, and for maintenance shutdowns.

Furniture: Furniture in the lab must be appropriate for the anticipated use. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis and other chemicals. Chairs used in conjunction with lab work must be covered with a non-porous material that can be easily cleaned and disinfected.

Windows: If the lab has windows they must be sealed.

Biological safety cabinets: Biological safety cabinets (BSC) must be installed in a manner so that changes in room air do not interfere with the operation of the cabinet. They should be located away from doors, windows that can be opened, high traffic areas, and other areas that could cause disruptions in the airflow of the cabinet. They must be tested and certified at least annually and whenever relocated or serviced. BSCs should be operated in accordance with the manufacturer's recommendations. Refer to the following section of this manual for additional information: *Safety Equipment- Biological Safety Cabinets*.

Vacuum lines: Vacuum lines must be protected by High Efficiency Particulate Air (HEPA) filters. Filters must be replaced as needed. Liquid disinfectant trap may be required.

Eyewash stations: An eyewash station must be readily available.

Airflow: A ducted ventilation system that provides directional airflow from "clean" areas to "potentially contaminated" ones is required. The lab must be designed so that under failure conditions that airflow will not be reversed. A means of visual verification of airflow must be available. Audible alarms should be considered. Exhaust air should be dispersed away from occupied building areas and from air intakes (or must be HEPA filtered) and cannot be recirculated to other areas of the building.

Biological safety cabinet exhaust air: The HEPA filtered exhaust air from a Class II BSC can be re-circulated within the laboratory as long as the cabinet is certified annually and operated according to the manufacturer's recommendations. The cabinet can also be connected to the building exhaust. Class III cabinets must be directly connected to the building exhaust. Air supply must be provided in a way that does not allow for positive pressurization of the cabinet.

Waste decontamination: A method for decontaminating lab wastes (i.e., autoclave, incineration, etc.) must be available. It is the responsibility of the generating department to decontaminate all solid non-sharps biohazardous waste and all liquid biohazardous waste. EHS is responsible for the removal and proper treatment of sharps waste. See the *Biohazardous Waste*

Management Section of this Plan for additional information.

Aerosol producing equipment: Equipment that may produce infectious aerosols (e.g., centrifuges, blenders, sonicators, etc.) must be used in containment devices that HEPA filter the exhaust air before being released to the laboratory. The HEPA filters must be tested or changed at least annually.

Equipment decontamination: The facility must be designed so that large pieces of equipment can be decontaminated before being removed from the laboratory.

Facility verification: The facility design, operational parameters and procedures must be verified and documented before initial operation. Annual facility re-verification is required.

BSL-3: Standard Microbiological Practices

The following standard microbiological practices must be used in the BSL-3 lab:

Controlled access: The lab supervisor must ensure that access to the laboratory is controlled. When procedures are in progress the lab door should be shut and when no one is present in the lab the doors should be locked. Anyone requesting access to the laboratory should be questioned as to their purpose and identification should be provided.

Handwashing: Hands must be washed with soap and water after handling potentially infectious materials. Hands should be washed before leaving the laboratory and before touching common use surfaces (i.e, computers, telephones, etc.).

Eating, drinking, handling contact lenses and applying cosmetics: Eating, drinking, contact lens handling and cosmetic application must be done outside of the laboratory. Food and beverages for human consumption must be stored outside of the laboratory area in refrigerators or cabinets designated for that purpose.

Pipetting: Mechanical pipetting devices must be available and used. Mouth pipetting is prohibited.

Safe sharps practices: All policies regarding the safe use of sharps must be followed. See the following section of this manual for additional information: *Recommended Work Practices- Sharps*.

Minimize splashes and aerosols: Essentially all laboratory procedures involve steps which create aerosols. All procedures should be completed in a manner which minimizes the creation of both splashes and aerosols. This can be done by using centrifuges with safety features (i.e., sealed cups and rotors), mechanical pipetting devices, conducting work inside of a biological safety cabinet, etc.

Decontaminate work surfaces: Work surfaces must be decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant should be used.

Proper decontamination and transport of waste: All cultures, stocks, and other

biohazardous materials must be decontaminated before disposal. If you will be transporting waste out of the laboratory (e.g., down the hall, to another floor of the building, etc.) to be decontaminated you must ensure that the waste is placed in a leak-proof container and is secured. Please refer to the following section of this manual: *Biohazardous Waste*, and the BSU Biohazardous Waste Management Plan for additional information regarding to the proper decontamination of biohazardous waste.

Door signage: All laboratory doors must have an "Admittance to Authorized Personnel Only" label. This label contains appropriate contact information for general and emergency entrance to the lab. Additionally, the lab entrance must be labeled with Biosafety Level 3 door sign. Both of these labels should be obtained from EHS.

Pest management program: A pest management program is managed through EHS. They should be contacted at the first sign of a problem.

Training: In addition to the completion of EHS required training courses, the principal investigator must ensure that all lab personnel receive site-specific training. This training should include information specific to their job duties, precautions to prevent exposures, and exposure response procedures. In addition, lab personnel should be given information about immune competence and conditions that could predispose them to infection, as appropriate.

BSL-3: Special Practices

The following special practices must be utilized in a BSL-3 lab:

Laboratory entrance: Before entering the laboratory, all people must be made aware of the potential hazards. They must also meet all entry and exit requirements (e.g., donning and doffing of personal protective equipment, immunization requirements, handwashing, etc.).

Medical surveillance: All laboratories using human-derived materials or cell lines must participate in the Bloodborne Pathogens Program. See the following section of this manual for additional information: Medical
Surveillance. For the use of other agents, medical surveillance and immunizations will be provided as appropriate. Occupational Health as well as EHS should be contacted for assistance.

Laboratory specific biosafety manual: Each laboratory must supplement this biosafety manual with information that is specific for the individual laboratory. Supplemental information may include: specific PPE practices and location of supplies, laboratory specific training requirements, laboratory specific waste handling practices and autoclave procedures, safe operation and decontamination of lab specific equipment, proper use of disinfectants specific for the lab (appropriate concentration, contact time and shelf life), etc.

Training: Lab personnel must demonstrate proficiency in standard and special microbiological practices before handling BSL-3 agents. It is the responsibility of the PI to ensure that proficiency has been demonstrated.

Containers for potentially infectious materials: Containers used to collect, handle, process, store, or transport within a facility, potentially infectious materials must be durable, leak-proof with a lid. The containers must be properly labeled with the contents and a biohazard symbol.

Decontamination of laboratory equipment: Lab equipment must be decontaminated routinely. It must also be decontaminated after spills, splashes or when potentially contaminated. All spills must be cleaned by personnel who are properly trained and have the proper equipment to handle infectious materials. All BSL-3 labs should have a biological spill kit available. See the following section of this manual for spill cleanup procedures and spill kit contents: *Biohazard Spill Cleanup Procedures*. All equipment must be decontaminated before being repaired, maintained, or removed from the laboratory. When any of these is to occur lab personnel must complete an Equipment Release Form and attach it to the piece of equipment. See Appendix E for an example of the form.

Exposure incidents: Exposure response procedures should be posted in an easily accessible location in the laboratory. All lab personnel should be made aware of the proper procedures to follow in the event of a possible exposure to potentially infectious materials. See Appendix F for exposure response procedures.

Non-research related animals and plants in the laboratory Animals and plants not associated with the work being done are not allowed in the laboratory.

Manipulating infectious materials: All procedures that involve the manipulation of infectious materials must be conducted within a biological safety cabinet, or other approved containment devices. Work involving open vessels cannot be conducted on the open bench. If a procedure cannot be conducted in a BSC, a combination of PPE and other containment devices can be used if approved by the Biological Safety Officer.

BSL-3: Safety Equipment

The following safety equipment must be used in a BSL-3 lab:

Biological safety cabinets (BSC): A biological safety cabinet must be used whenever working with infectious materials. Other physical containment devices may be used with the approval of the Biological Safety Officer.

Personal protective equipment (PPE): The use of laboratory coats, gowns or uniforms with a solid front is required when in the laboratory. Work with certain agents may require that street clothes be removed and dedicated lab clothing be worn. Protective clothing cannot be worn outside of the lab. Splash goggles and face protection must be used when there is the potential for splashes of microorganisms or other hazardous materials. Personnel who wear contact lenses should wear safety glasses or other eye protection at all times while in the laboratory. Gloves must be worn as protection from hazardous materials. Two pairs should be worn as appropriate. If latex gloves are used, alternatives should be made available. Gloves must be changed when contaminated, when the integrity has been compromised, or when necessary. Disposable gloves must not be washed or reused. Hands must be washed after removing gloves, and before leaving the laboratory. All protective equipment must be removed before leaving the laboratory. Used

disposable PPE should be disposed of with other contaminated waste. Reusable PPE (i.e., goggles) should be appropriately decontaminated before reuse. Reusable laboratory clothing must be decontaminated before being laundered and must be laundered at the University. It must not be taken home. If visibly contaminated, laundry should be placed in a biohazard bag before be placed with other items to go to laundry.

Animal rooms: Eye, face and respiratory protection should be used in rooms containing infected animals. This is suitable for work involving animals that are infected with agents assigned to Risk Group 2.

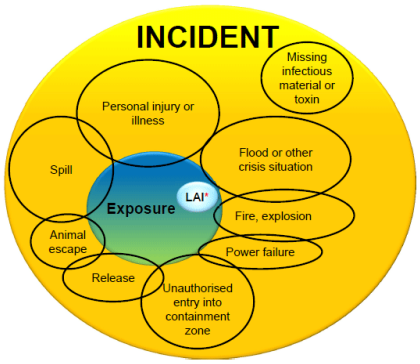
Biological Safety Level 4 Laboratories

Biosafety Level 4 practices, safety equipment, and facilities 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. Additionally, agents with a close or identical antigenic relationship to Biosafety Level 4 agents should also be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at Biosafety Level 4.

The primary hazards to personnel working with Biosafety Level 4 agents are respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals pose a high risk of exposure and infection to laboratory personnel, the community, and the environment.

The laboratory worker's complete isolation of aerosolized infectious materials is accomplished primarily by working in a Class III BSC or a full-body, air-supplied positive-pressure personnel suit. The Biosafety Level 4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation and waste management systems to prevent release of viable agents to the environment.

General Laboratory Practices



**Laboratory Acquired Infection*

Basic Laboratory Practice and Techniques

Biological safety is the application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or biohazards. Biosafety defines the containment conditions under which infectious agents can be safely manipulated. The objective of containment is to confine biohazards and to reduce the potential exposure of the laboratory worker, persons outside of the laboratory, and the environment to potentially infectious agents. It can be accomplished through the following means:

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infectious materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. The director or person in charge of the laboratory is responsible for providing or arranging for appropriate training of personnel.

Each laboratory must develop or adopt a biosafety or operations manual (*Site-specific Laboratory Biosafety Manual*) that identifies the hazard that will or may be encountered, and which specifies practices and procedures designed to minimize or eliminate risks. In particular, any practices more restrictive or deviating from the procedures established in this BSU Biosafety Manual are to be related and explained or justified in the *Site-specific Laboratory Biosafety Manual*. Personnel should be advised of special hazards and should be required to read and to follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must direct laboratory activities.

When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed. The laboratory director is responsible for selecting additional safety practices, which must be in keeping with the hazard associated with the agent or procedure.

Laboratory personnel, safety practices, and techniques must be supplemented by appropriate facility design and engineering features, safety equipment, and management practices.

Safety Equipment (Primary Barrier)

Safety equipment includes biological safety cabinets (BSCs), enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. Three types of BSCs (Class I, II, III) used in microbiological laboratories are described in Section 2. Open-fronted Class I and Class II BSCs are primary barriers which offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. The Class II BSC also provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet. The gas-tight Class III BSC provides the highest attainable level of protection to personnel and the

environment.

An example of another primary barrier is the safety centrifuge cup, an enclosed container designed to prevent aerosols from being released during centrifugation. To minimize this hazard, containment controls such as BSC's or centrifuge cups must be used for handling infectious agents that can be transmitted through the aerosol route of exposure.

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

Facility Design (Secondary Barrier)

The design of the facility is important in providing a barrier to protect persons working inside and outside of the laboratory within the facility, and to protect persons or animals in the community from infectious agents that may be accidentally released from the laboratory. Laboratory management is responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in Biosafety Level 1 and 2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities.

As the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features could include specialized ventilation systems to assure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules for isolation of the laboratory. Design engineers for laboratories may refer to specific ventilation recommendations as found in the Applications Handbook for Heating, Ventilation, and Air-conditioning (HVAC) published by the *American Society of Heating, Refrigerating, and Air-conditioning Engineers* (ASHRAE).

The following information applies to all laboratories housing or using biological materials. Information for specific biosafety levels will follow.

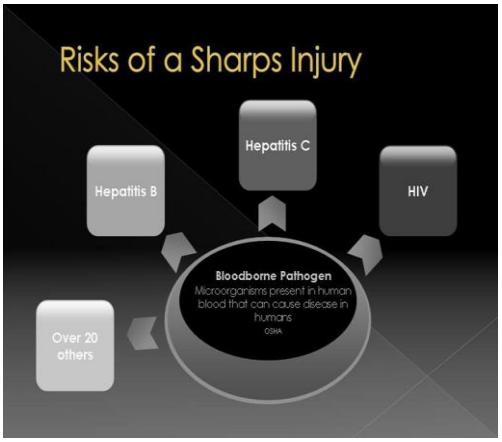
Routes of Infection (Exposure)

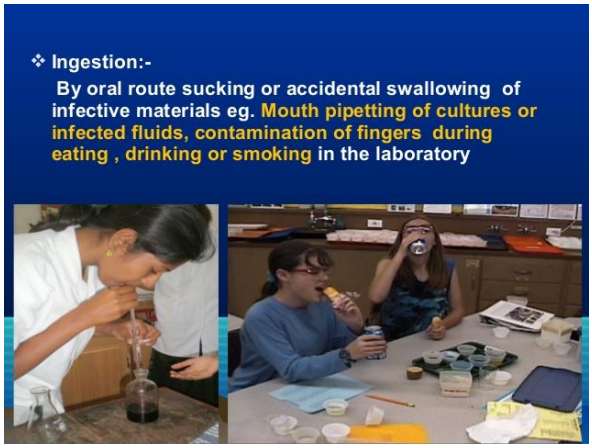
An infection occurs when disease-causing microorganisms enter the human body in sufficient numbers and by a particular route to overcome the body's defense system. The following routes of exposure (**Table 5**) have been reported for laboratory-acquired infections (LAIs):

TABLE 5: FIVE ROUTES OF LABORATORY-ACQUIRED INFECTIONS

(*MMWR Supplements 2012/61[01]; 1-101*):

Source of Infection Exposure	% of Occurrences
1. Parental inoculations with syringe needles or other contaminated sharps; 2. Spills or splashes onto skin or mucous membranes; 3. Ingestion or exposure through mouth; 4. Pipetting or touching mouth or eyes with fingers or contaminated objects;	20% of total
5. Infectious aerosols (and droplets) directly, through hand contamination, or fomite cross-contamination	80 % of total

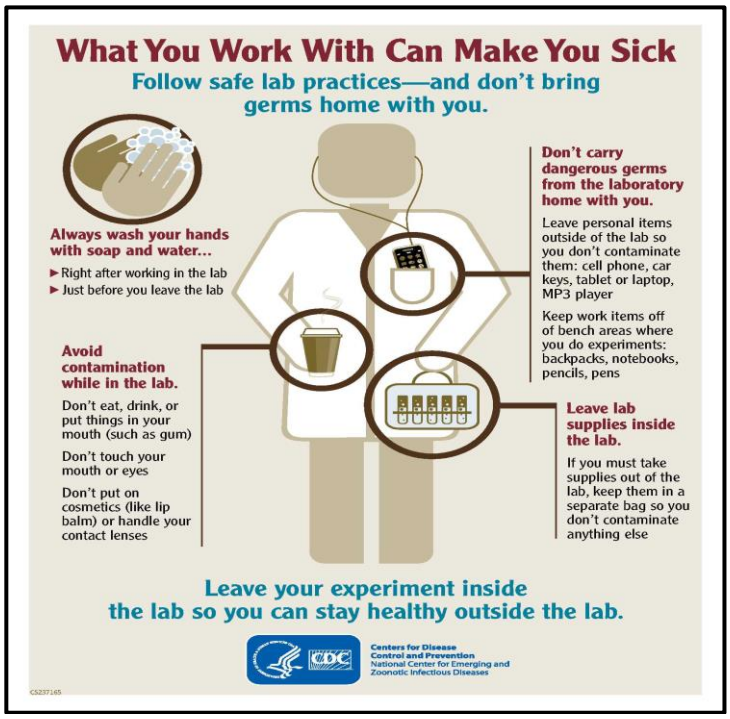




Once aerosolized, infectious agents can remain airborne for hours or days. Based on studies of LAIs, it has been found that one effective means of intervention is ensuring that all staff and students are made aware of the specific symptoms and etiology of the infectious agents, toxins, or molecules to which they may be exposed. This allows earlier identification and verification of an infection or intoxication and more timely treatment of the affliction.

Fomites: An often overlooked aspect of infection control is the role of fomites in transmission of infectious agents or cross-contamination of biological or chemical agents. Fomites are any nonliving object or substance capable of carrying infectious organisms, such as viruses or bacteria, and hence transferring them from one individual to another. Bench tops, door knobs, phones, skin cells, hair, clothing, and bedding are common reservoirs of contamination. Many organisms can survive on inanimate surfaces for minutes, hours, days, or even weeks and

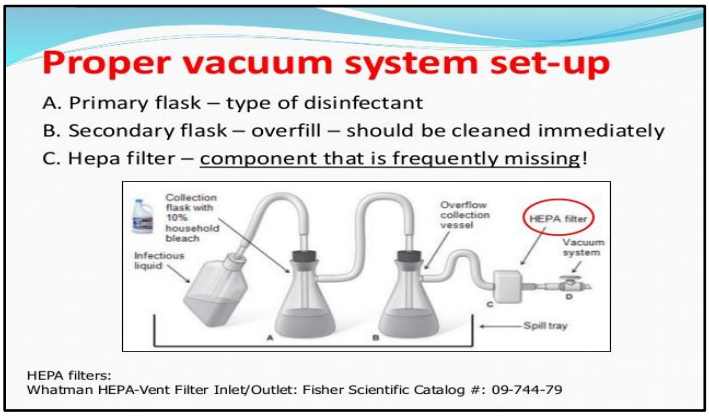
remain viable in sufficient numbers to either constitute an infective dose or colonize tissues.



Most of the laboratory-acquired infections reported in the literature point to accidents while actually working with some type of infectious agent. These include spills, splashes and accidents involving needles or other sharp objects. The general laboratory procedures outlined in this manual address those issues and provide for guidance in handling infectious or potentially infectious materials.

Aerosol Management

Aerosol formation has the potential to contaminate work surfaces, exposed skin and garments, and air. Bioaerosols are microorganisms dispersed or spread in the form of aerosols or droplets. They are in either dry or liquid forms, typically 5µm in diameter. Due to their small size, aerosols do not settle quickly and can remain airborne for long periods of time. If inhaled, bioaerosols can be carried to the alveoli, potentially causing infection. Thus, aerosols can result in topical, oral, and respiratory exposures for workers. Manipulation of a biological sample has the potential for releasing a portion of the sample in microdroplet form to the air and work surfaces which is the most common source of Laboratory-acquired Infections. Accordingly, work practices and equipment must be calculated to minimize or eliminate the formation or release of aerosols in the laboratory space



through the use of proper procedures and containment equipment as will be discussed in this section of the Manual.

One way to view the potential for release of aerosols from a given sample is to consider the amount of energy that is used to manipulate the sample. High-energy techniques such as homogenization have the potential to release aerosols of the sample if not properly contained. However, even low energy procedures such as removing screw caps and pouring or stirring of liquid medium can release aerosols of the sample. Some other procedures that can generate aerosolized biohazards are washing down animal rooms, laboratory dishwashing, transferring liquids, and separating blood serum. The results of a study investigating the formation of aerosols during common laboratory procedures are shown in **Table 6** below:

Table 6: Aerosols Created by Common Laboratory Procedures

Technique or Incident Being Performed	Average Colonies Recovered from Air During Operation
Pipetting 10 ml culture into 1,000 ml broth	2.4
Drop of culture falling 12 in. (30 cm) onto:	
Stainless steel	49.0
Painted wood	43.0
Hand towel wet with 5% phenol	4.0
Re-suspending centrifuged cells with pipette	4.5
Blowing out last drop from pipette	3.8
Shattering tube during centrifuging	1183.0
Inserting hot loop into broth culture	8.7
Streaking agar plates	0.2
Withdrawing syringe and needle from vaccine bottle	16.0
Injecting 10 guinea pigs	16.0
Making dilutions with syringe and needle	2.3
Using syringe/needle for intranasal inoculation of mice	27.0
Harvesting allontoic fluid from 5 eggs	5.6

Source: *Oregon Health and Science University*

These findings emphasize the importance of adhering to standard microbiological techniques, which minimize the total amount of energy to which a given sample is subjected during manipulation. Good work practices for some common laboratory procedures are provided here.

In a clinical setting, sputum and other specimens submitted for culture may contain unsuspected microorganisms, such as mycobacterium, which are highly infectious by the airborne route. If generation of an aerosol is likely to occur during the processing of these specimens, the use of a biosafety cabinet is recommended or required. Aerosols can be generated by many common laboratory techniques and can represent a significant source of laboratory-acquired infections. Almost any handling of liquids or of dry powders is likely to

generate aerosols and droplets. Examples of procedures generating aerosols from laboratory equipment include, but are not limited to: high speed blending, grinding, filtering, mixing, sonicating, agitation, flaming, pipetting, opening vials of lyophilized cultures, shaking and centrifuging.

The careful performance of certain laboratory procedures must be emphasized because some procedures can generate small particle aerosols. Those procedures, when used with highly infectious microorganisms or toxins should be confined to biosafety cabinets or hoods. To minimize aerosol production, pipettes should be drained gently with the tip against the inner wall of the receiving tube or vessel. Do not expel infectious materials forcibly from pipettes, and never bubble air through a suspension of infectious agents in open containers. It is recommended that these procedures be conducted in a biosafety cabinet when Biosafety Level 3 precautions are being used. The equipment should be selected for features designed to contain infectious liquids or aerosols.

Centrifuges with sealed buckets, safety trunnion cups, or sealed heads are effective in preventing escape of liquids and aerosols. In addition, sealed centrifuge tubes or bottles should be used. If fluid should escape from a cup or rotor during high-speed operation, the potential for extensive contamination and multiple infections is great. Centrifuge tubes, bottles, rotors and safety trunnion cups should be visually inspected on a regular basis to ensure that leakage does not occur during operational procedures. HEPA filters should be installed between the chamber and the vacuum pump of the ultracentrifuge.

Improper technique in the flaming of inoculating loops can also result in the spread of infectious agents. Spatter and release of droplets or aerosols can be prevented by heating the shaft until the sample has been heat-dried before flaming the loop itself. Spatter can also be controlled effectively by using a die-arm burner or electric microincinerator. The process of flaming can be avoided by using sterile, disposable loops.

Access

When procedures with the biohazard are in progress, or potential exposure exists, the lab door should be shut, and when no one is present in the lab, the doors must be locked. Anyone requesting access to the laboratory should be questioned as to their purpose and identification should be provided. Additional security precautions are required when dealing with Select Agents.

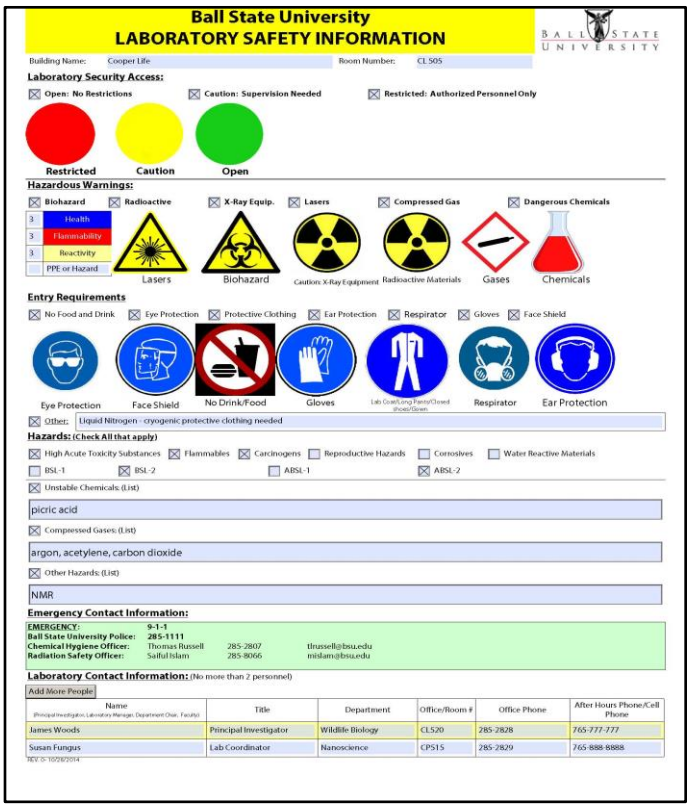
Biohazard Warning Signage

Biohazard signs and information should be posted at the main entrance door(s) to laboratories and animal rooms harboring such hazards. Signage for BSL-1 or higher labs must include the following additional information:

- Biosafety level
- Supervisor's or other responsible person's name
- Telephone number

- Restrictions on laboratory access
- PPE required for entry when biohazardous work is in progress or exposed.

The following BSU laboratory door hazard sign can provide all of the required information:



Ball State University
LABORATORY SAFETY INFORMATION

Building Name: Cooper Life | Room Number: CL505

Laboratory Security Access:

☒ Open: No Restrictions ☒ Caution: Supervision Needed ☒ Restricted: Authorized Personnel Only

Restricted | Caution | Open

Hazardous Warnings:

☒ Biohazard ☒ Radioactive ☒ X-Ray Equip. ☒ Lasers ☒ Compressed Gas ☒ Dangerous Chemicals

3 Health
3 Flammability
3 Reactivity
PPE or Hazard

Lasers | Biohazard | Caution: X-Ray Equipment | Radioactive Materials | Gases | Chemicals

Entry Requirements

☒ No Food and Drink ☒ Eye Protection ☒ Protective Clothing ☒ Ear Protection ☒ Respirator ☒ Gloves ☒ Face Shield

Eye Protection | Face Shield | No Drink/Food | Gloves | Lab Coat/Long Pants/Closed Shoes/Shoes | Respirator | Ear Protection

☒ Other: Liquid Nitrogen - cryogenic protective clothing needed

Hazards: (Check All that apply)

☒ High Acute Toxicity Substances ☒ Flammables ☒ Carcinogens ☐ Reproductive Hazards ☐ Corrosives ☐ Water Reactive Materials

☐ BSL-1 ☒ BSL-2 ☐ ABSL-1 ☒ ABSL-2

☒ Unstable Chemicals (List)

picric acid

☒ Compressed Gases: (List)

argon, acetylene, carbon dioxide

☒ Other Hazards: (List)

NMR

Emergency Contact Information:

EMERGENCY:	**9-1-1**		
Ball State University Police:	**285-1111**		
Chemical Hygiene Officer:	Thomas Russell	285-2807	tlrussell@bsu.edu
Radiation Safety Officer:	Saiful Islam	285-8066	mislam@bsu.edu

Laboratory Contact Information: (No more than 2 personnel)

Add More People

Name (Principal Investigator, Laboratory Manager, Department Chair, Faculty)	Title	Department	Office/Room #	Office Phone	After Hours Phone/Cell Phone
James Woods	Principal Investigator	Wildlife Biology	CL520	285-2828	765-777-777
Susan Fungus	Lab Coordinator	Nanoscience	CPS15	285-2829	765-888-8888

REV 5- 10/28/2014

A biohazard label is required for all areas or equipment in which RG-2 or higher agents are handled or stored or where BSL-2 or higher procedures are required, including on equipment such as refrigerators, freezers, biological safety cabinets, incubators, and transport containers.

An example of acceptable signage is illustrated below which is the BSU Designated Hazard area sign:

DANGER

DESIGNATED AREA

✓	Check Hazard (All that apply)
	Carcinogen
	Reproductive Toxin
	High Acute Toxin
	Level: Biosafety

AUTHORIZED PERSONNEL ONLY
-- when material in use or exposed –
BSU EHS Office

In this instance, the Biohazard symbol would be checked and the BSL appropriate for the laboratory would be marked under: **Level:** □

Food and Beverages in the Laboratory

In order to reduce potential exposures and to ensure compliance with prudent laboratory operations, regulations, and other best management practices, BSU prohibits the storage and consumption of food and drink in all designated laboratory space. The only exception is for food and beverages used in research and teaching projects. These materials must be labeled, *Not for Human Consumption*.

In order to prevent potential exposure to hazardous materials:

- Do not eat, drink, smoke, chew gum, apply cosmetics, or take medicine in laboratories where hazardous materials or infectious agents are handled or stored;
- Do not handle contact lenses in the laboratory area;
- Do not store food, beverages, cups, or other drinking and eating utensils in areas where hazardous or potentially infectious materials are handled or stored;
- Do not use glassware for laboratory operations to prepare or consume food or beverages;
- Do not use laboratory refrigerators, ice chests, cold rooms, and ovens for food storage or preparation;
- Do not use laboratory water sources or deionized laboratory water for drinking water.

Important: Food and beverages must never be stored in any laboratory refrigerator in which chemicals, biological, or radioactive materials are kept unless they have been labeled, *Not for Human Consumption.*

Personal Hygiene and Precautions

Attention to personal behavior and hygienic practices is critical to maintaining a safe laboratory where biohazards may be exposed. The required containment and exposure precautions are easily defeated by improper and unsafe work practices and lack of attention to personal hygiene. While many of these practices and precautions have been mentioned previously, they bear repeating or expansion here and apply to researchers, faculty, graduate and research assistants, staff, and all students:

1. Wash your hands thoroughly with soap and water when you arrive at the lab and again before you leave. This is preferable to use of any antibacterial soaps or lotions;
2. Absolutely no food, drinks, chewing gum, or smoking is allowed in the laboratory. Do not put anything in your mouth such as pencils, pens, labels, or fingers;
3. Minimize touching your face, mouth, eyes, or exposed skin when in the laboratory and before washing hands;
4. Wear the designated protective apparel (PPE) in the lab and do not wear it to other non-lab areas;
5. Avoid excessively loose fitting items of clothing. Wear appropriate shoes (sandals or open-toed shoes are not allowed) in the laboratory;

6. Long hair must be restrained, and exposed or loose jewelry removed;
7. Disposable gloves should fit properly, be non-latex material, and non-powdered;
8. Hands should be thoroughly washed whenever changing gloves and gloves should be changed at least every 2 hours if worn continuously;
9. Gloves should always be removed using the techniques demonstrated in Universal Precautions training;
10. Glove wearers should remain aware that while gloves can provide protection to wearers if they are not compromised, they can still transfer infectious agents between surfaces;
11. Keep your workspace free of all unnecessary materials. Backpacks, purses, and coats should be placed in lockers or other locations not likely to be contaminated. Place needed items on the floor near your feet, but not in the aisle;
12. Lab coats or other protective apparel should be stored in a plastic bag or other receptacle if it must be removed from the laboratory between uses;
13. Disinfect work areas before and after use with 70% ethanol, fresh 10% bleach, or another EPA approved disinfectant. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, and especially after spills, splashes, or other contamination. Keep the surfaces moist with the disinfectant for the required contact time;
14. Label all containers and other vessels clearly with the contents, including the identity of the infectious or genetic material;
15. Replace caps on reagents, solution bottles, and bacterial cultures. Do not open Petri dishes or other receptacles in the lab unless necessary to the course of study or research;
16. Inoculating loops and needles should be flame sterilized in a bunsen burner or other thermal device before you lay them down;
17. Turn off Bunsen burners and de-energize equipment when not in use;
18. Treat all microorganisms as potential pathogens. Use appropriate care and do not take cultures out of the laboratory without authorization, proper containment, and identification of the biohazard being transported;
19. Wear properly fitting disposable gloves when working with potentially infectious microbes or samples. If you are working with a sample that may contain a pathogen, then be extremely careful to use good bacteriological technique;
20. If also working with chemicals, be sure the gloves in use are chemically resistant to the particular chemical;
21. Sterilize equipment and materials necessary for research integrity;
22. Never pipette by mouth. Use a pipetting aid or adjustable volume pipettors. [In the distant past, some lab personnel were taught to mouth pipette. This practice has been known to result in many laboratory-acquired infections. With the availability of mechanical pipetting devices, mouth pipetting is strictly prohibited.]
23. Consider everything to be a biohazard. Do not pour anything down the sink. Autoclave liquids and broth cultures to sterilize them before discarding;
24. Dispose of all solid waste material in an autoclave bag and autoclave it properly before discarding in the regular trash. Before disposing in a solid waste container, place the autoclaved bag in an opaque outer trash bag;
25. Alternatively, place the contaminated material or waste in a biohazard bag (red bag) or infectious waste box (biohazard symbol) for handling and disposal as infectious waste;

26. Familiarize yourself with the location of safety equipment in the lab (e.g., eye-wash station, shower, sinks, fire extinguisher, biological safety cabinet, first aid kit, emergency gas valve, emergency call button, spill response kit);
27. Dispose of uncontaminated broken glass in the broken glass container;
28. Dispose of razor blades, syringe needles, and other sharp objects in the “sharps” container. Call for removal when ¾ full. Do not attempt to retrieve objects;
29. Report spills and accidents immediately to your instructor. Clean small spills only if trained and proper PPE and spill response materials are available; and then, only with care (see instructions in this Manual). Seek help for large spills;
30. Report all injuries, accidents, or exposures immediately to the instructor, no matter how small they seem. The instructor or PI is to relate this information to the Biological Safety Officer (BSO).
31. Do not allow unauthorized persons access to the laboratory when the biological agents are in use or exposed. Report any suspicious activities to the BSU Police Department;
32. Do not work alone with biohazardous materials unless approved and all conditions for such work are authorized in advance;
33. Avoid working with infectious substances if you are ill with other conditions such as colds, influenza, gastrointestinal distress, or other symptoms that may reduce your resistance or spread infection to others working in the tight spaces of the laboratory. Try to maintain a six (6) foot physical distance from co-workers;
34. Be aware of the symptomology of the infectious agents or toxins you may be working with. Report any potential symptoms of exposure or infection to the PI, faculty, instructor, and the BSO if you are working with infectious agents;
35. Make the PI, faculty, or instructor and BSO aware of you have any relevant pre-existing conditions (e.g., pregnancy) that may increase concerns with exposure to infectious agents, or if immunocompromised in any way that may necessitate exposure restrictions to biohazards or other precautions.

Handwashing

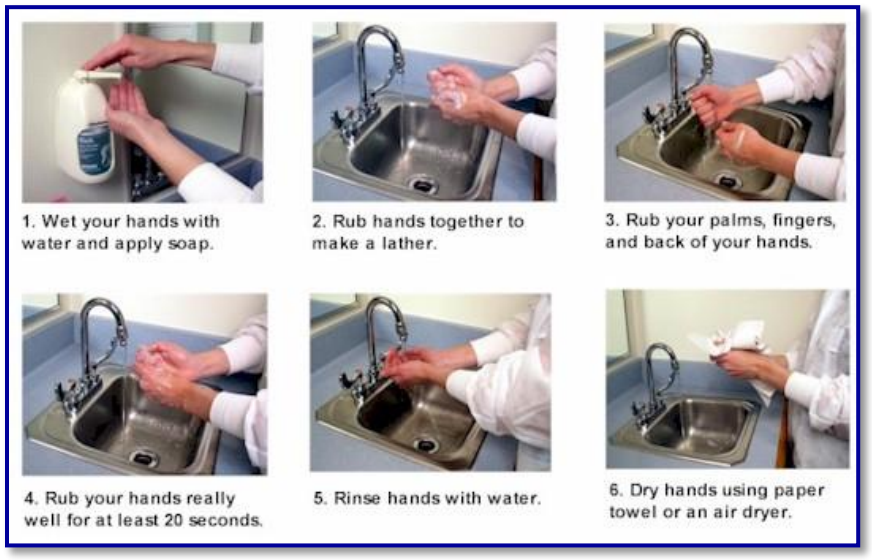
Whenever possible, suitable gloves should be worn when handling biohazardous materials. However, this does not replace the need for regular and proper hand-washing by laboratory personnel. Hands must be washed after handling biohazardous materials and animals, and before leaving the laboratory.

In most situations, thorough washing of hands with ordinary soap and water is sufficient to decontaminate them, and is the preferred method. The use of antibacterial soaps may be recommended in high-risk situations. Hands should be thoroughly lathered with soap, using friction, for at least 20 seconds, rinsed in clean water and dried.

Foot- or elbow-operated faucets are recommended and often required. Where not available, a paper towel should be used to turn off the faucet handles to avoid re-contaminating washed hands.

To satisfy inspection requirements, all hand washing facilities in BSL-2 or higher laboratories

should be located near the laboratory exit, or a location between the “dirty” and “clean” areas, of the laboratory space. All hand washing sinks must be dedicated fixtures (bench sinks may not serve this purpose), be provided with soap and disposable towels, as well as a convenient waste container.



Housekeeping

Good housekeeping in laboratories is essential to reduce risks and protect the integrity of biological experiments. Routine housekeeping must be relied upon to provide work areas free of significant sources of contamination. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected.

Laboratory personnel are responsible for cleaning laboratory benches, equipment and areas that require specialized technical knowledge. To facilitate decontamination, the laboratory should be kept neat and free of clutter - surfaces should be clean and free of infrequently used chemicals, glassware and equipment. Access to hand washing sinks, eyewash stations, emergency showers and exits, and fire extinguishers or spill kits must not be blocked or impeded.

Biological laboratories working with infectious agents are subject to RODAC or ATP testing performed by the BSO to verify the effectiveness of cleaning, sanitizing, and disinfecting practices.

Working Alone

According to the National Safety Council, the term *alone* means that a person is beyond the visual or auditory range of any other individual for more than a few minutes at a time.

All faculty, staff, students, and visitors working in an area (e.g., laboratory, animal holding room) where hazardous conditions exist should have knowledge of the following:

- Emergency Contacts;
- Emergency Response Procedures;

- Evacuation Routes;
- First Aid Procedures;
- Health and Safety Training Requirements;
- Personal Protective Equipment Requirements;
- Procedures to Report Unhealthy and Unsafe Conditions;
- Safety Policies and Procedures; and,
- Spill Response Equipment and Procedures

All personnel working alone in a laboratory where hazardous conditions exist must:

- Obtain written permission (e.g., e-mail, letter) from the Principal Investigator or Laboratory Supervisor to work alone in the laboratory;
- Ensure that a means to contact emergency response personnel is available when working alone in the laboratory; and
- Require that individuals working alone contact their supervisor before beginning work and upon completion.

Laundering

Laundering of Personal Clothing

Laboratory coats/gowns and contaminated clothing or clothing suspected to be contaminated with chemicals or biohazards are never be taken home or to a public laundry facility. Personal laundering is not acceptable for clothing contaminated with chemicals, blood, blood products, or other bodily fluids Clothing contaminated with biohazardous material must be autoclaved prior to any laundering at home. Documentation of effective autoclaving must be maintained.

BSU Laundry Facilities

Laundry facilities may be provided in departments at BSU. If so, follow departmental procedures for cleaning *mild to moderately* contaminated clothing. Generally, these facilities are for intra-department use only. Laboratory managers may launder mildly contaminated clothing using departmental laundry facilities where available. Contaminated clothing shall be washed, at a minimum in accordance with the manufacturer's directions. However, departments are encouraged to launder contaminated clothing in hot water (160º F or greater). Where departmental facilities are not available, contaminated clothing must be laundered by a professional laundry service. All personal protective clothing must be cleaned, laundered and disposed of by the employer at no cost to employees.

Professional Laundering Services

A professional service company may be used if the department does not have the capability to wash *mild to moderately* contaminated clothing. It is each laboratory's responsibility to determine if the cleaning company is capable and willing to launder the contaminated clothes. Where departmental facilities are not available, contaminated clothing must be laundered by a

professional laundry service. Laboratory managers shall ensure that all laundry sent off-site is containerized in leak-proof bags or boxes marked with the biohazard symbol and shall advise the vendor that the laundry is contaminated with blood and/or potentially infectious bodily fluids for textiles that are mildly contaminated.

Student PPE

Students are responsible for their own protective clothing. It may be advisable for students to store their lab coats or similar apparel in sealed plastic bags between uses if secure lockers or other accommodations for storage or hanging of personal items are not available.

Overtly Contaminated Clothing

Clothing that is overtly contaminated with chemicals must be disposed as hazardous waste. Clothing contaminated with radiological material must be disposed as radiological waste. Clothing that is contaminated with blood, blood products, or other bodily fluids must be removed and containerized in leak-proof bags or boxes at the location where it was used. Containers or bags must be marked with the biohazard symbol. Apparel contaminated with human blood or other potentially infectious materials should be handled as little as possible and needs to be collected in special hampers (labeled or color coded) or in biohazard bags. Appropriate PPE must be worn by employees who handle contaminated laundry.

Protective Clothing Beyond the Laboratory

The improper use or lack of protective clothing and equipment in a laboratory can lead to chemical burns, biological exposures, or other potential dangers. To help reduce the risk of exposure, personnel in BSU laboratories are required to wear gloves, safety glasses, lab coats and other personal protective clothing. However, in public areas, such as hallways and lounges, wearing personal protective clothing and equipment is not recommended unless required for the transport of chemicals or biohazards between laboratory areas. This is because contaminated clothing may present a hazard, and the perception of contaminated protective clothing and equipment in a public area may project a careless image to both colleagues and visitors.

Wearing gloves outside the laboratory should be minimized, except to move hazardous materials between laboratories. Chemicals should be transported from place to place on a cart, in a clean secondary container, or in a bottle carrier with secure handles. When this is not an option, personnel should use a clean, ungloved hand to touch common surfaces and a gloved hand to carry the items: the one-glove rule. Alternatively, the material should be packaged so the outer container may be transported without the need for personal protective equipment.

Protective gloves should never come into contact with door handles, elevator buttons, telephones, lavatory faucets, vending machines, bottled water dispensers, ice-making machines, or other surfaces outside the laboratory. Also, please be aware that strict federal and state regulations address the transport of hazardous (e.g., biological, chemical, radiological) materials on public roads.

For the sake of safety, appearances, and courtesy, personnel are asked not to wear contaminated, stained, or potentially contaminated lab coats and other research clothing and equipment in any public area, especially dining areas, lounges, auditoriums, conference rooms, or other non-hazardous areas.

Training

Good microbiological and laboratory practices are essential for a safe work environment. It is required at BSU that training and education on these practices and procedures starts at the undergraduate level. Many of the basic procedures and prohibitions necessary for biosafety working with RG-1 agents / BSL-1 facilities will have been provided in the BSU chemical laboratory training programs. All personnel or students working with RG-2 or 3 agents, or at BSL-2 or 3, must receive enhanced biosafety training and adequate laboratory-specific training from the Principal Investigator (PI) or laboratory supervisor. See **Appendix O** for a site-specific training checklist that can facilitate and document this training. Training should include at a minimum:

- Good laboratory and animal practices as applicable;
- Laboratory-specific information on risks, hazards and procedures; and
- Laboratory or environment specific BSL-2 or 3 procedures as applicable.

In addition, it is important that all personnel working in a laboratory handling infectious biological materials take the appropriate biological safety-related trainings required or offered by EHS:

- **Biosafety Principles Training:** This course covers general training requirements for working in environments at Biosafety Level 1 or higher. There are modules for different disciplines or work environments (see the list below).
- **Bloodborne Pathogens Initial:** This is OSHA required training that is needed by anyone who will be handling human-derived materials, including blood and cell lines.
- **Bloodborne Pathogens Refresher:** This is refresher training that is required by OSHA each year after taking the Bloodborne Pathogens Initial course.
- **Autoclave Safety Training:** This training is now required for those individuals who operate an autoclave as part of their job duties.
- **Security Awareness Training:** This training is required for anyone who works in or who has access to a laboratory.
- **Others:** The EHS Office can sometimes offer or arrange specialized training as requested. These include, but are not limited to, Sanitation and Disinfection Practices, Infectious Substance and Biological Materials Shipping, and Biosafety Cabinet training. If you have a need for a specialized class, please contact our office.

Health and Medical Surveillance

Medical surveillance of personnel in general is essential to identify health factors that may increase one's risk for laboratory-acquired infections. Under specific circumstances, work with high-risk agents or diagnostic specimens that may contain high-risk agents may require

consideration of vaccinations for some personnel or restricted access for others. In the case of exposures to potentially infectious materials, medical surveillance will include health monitoring as prescribed by EHS and the Health Center in order to facilitate recovery (Refer to **Appendix F** for exposure response procedures).

The Principal Investigator is responsible for ensuring that all lab and support personnel and visitors are fully informed of:

- Risks associated with handling the biological materials in use, including routes of transmission and signs and symptoms;
- Restricted access policies for those at elevated risk of infection for any infectious agent in use;
- Conditions that can lead to one becoming immunocompromised or immunosuppressed, and the option to notify one's supervisor or EHS in that instance to assure one's health.

Lab personnel and visitors should observe the following:

- Entry or work in any lab where biological materials are in use (regardless of the biosafety level) may pose an elevated risk of infection for individuals who are immunocompromised.
- Consultation with an occupational health provider before working in a lab is strongly advised if you believe that you may be immunocompromised. Please remember that events such as pregnancy, recent illnesses caused by an infectious agent (i.e., the flu), chemotherapy, etc. can result in an immunocompromised state of health.

Immunoprophylaxis

Specific projects may arise using infectious materials and techniques that warrant consideration of vaccines. In these instances, the Principal Investigator should notify the Biosafety Officer and the BSU health Center to further assess this need. The University follows the recommendations of the Centers for Disease Control and Prevention (CDC) and the Public Health Service Advisory Committee for Immunization Practices (ACIP) for vaccination of at-risk personnel. When considering the need for immunization, a risk assessment will be conducted by the health care provider in conjunction with information regarding the experimental agent provided by the EH&S Biological Safety Office.

Health Surveillance for Personnel Working with Infectious Agents

A health surveillance program is available for laboratory personnel who use agents that require it. Laboratory personnel should receive immunizations (see preceding section entitled "Immunoprophylaxis"), such as hepatitis B vaccination, and medical tests, such as tuberculosis skin testing, when appropriate. The Animal Contact and Bloodborne Pathogen Programs (see appropriate chapters) provide for health assessments, risk assessments, medical tests, and immunizations for certain at-risk personnel.

In the event that restricted access entry or vaccination requirements are implemented for a study underway, this information will be clearly posted on the lab door to communicate elevated risk.

Recordkeeping

The principal investigator must maintain the following records and be prepared to present these at all times if requested by IBC representatives, subject academic department personnel, faculty, staff, or students who are working with, or potentially exposed to, the infectious agents or toxins, and authorized governmental representatives:

1. **Inventory Log:** Laboratories should have a process for controlling inventory of infectious agents. A written or computerized inventory log must be kept for each biological agent or toxin of RG-2 or higher risk classification stored in that room in freezers, refrigerators, dehydrated storage, or otherwise. The inventory should be complete enough so that the PI would know if materials are missing, what those materials are, the quantity of materials, and the potential hazards of the materials. The log should be reconciled with the physical inventory on a periodic basis. All microorganisms stored in the lab should be documented and labeled. Any stocks or cultures that are not needed should be decontaminated and disposed of properly. If any *Select Agents* or *Toxins* are discovered, contact EHS immediately for assistance
2. A **Risk Assessment** for each biological agent or toxin stored or exposed in that room;
3. **Pathogen Safety Data Sheets**, or similar information and data on each biological agent or toxin stored or used in that room;
4. A copy of this **BSU Biological Safety Manual;**
5. The **Laboratory-Specific Biosafety Manual**;
6. The protocol or standard operating procedures for the biological agents (unless included in the Biosafety Manual;
7. Safety, security, and emergency response plans (unless included in the Biosafety Manual);
8. Training records for the PI, researcher, lab manager, and any graduate or undergraduate students with access to the laboratory when RG-2 agents are exposed or any RG-3 materials are stored, used, or exposed in the laboratory space.

The above records, logs, or plans may be available as hardcopy or electronic form. If electronic, they must be readily available to all persons at any time they may be present in the subject laboratory space. This will necessitate training all persons regarding the electronic files, and the necessary skills to quickly access the required records or plans.

Personal Protective Equipment (PPE)

Personal protective equipment is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may also protect the materials from contamination. Personal protective devices and safety equipment as well as training in the proper use of those devices and equipment, must be provided to all employees under the appropriate circumstances. The employees have the responsibility of properly using the equipment. The required PPE for entry and biohazard activities in the laboratory should be designated on the BSU Laboratory Hazard Sign on the entry door(s).

Personal protective equipment will obviously vary depending upon the biological safety

level to which a worker or student may be exposed. Please refer to **Table 7** below for a brief summary of PPE requirements for each of the four biological safety levels:

Table 7. Biological Safety – Personal Protective Equipment (PPE) Requirements			
BSL-1	**BSL -2**	**BSL -3**	**BSL -4**
• **Protective laboratory coats, gowns, or uniforms** recommended preventing contamination of personal clothing. • **Protective eyewear** worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. • Personnel who wear contact lenses in labs should also wear eye protection. • **Gloves** must be worn to protect hands from exposure to hazardous materials.	• **Protective laboratory coats, gowns, smocks, or uniforms** must be worn while working with hazardous materials. • **Eye and face protection** (goggles, mask, face shield, or other splatter guard) must be used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms are handled outside the Biological Safety Cabinet (BSC) or physical containment device. • Personnel who wear contact lenses in labs should also wear eye protection. • **Gloves** must be worn to protect hands from exposure to hazardous materials. • Eye, face and respiratory protection should be used in rooms containing infected animals.	• **Protective laboratory clothing with a solid-front**, such as tie-back or wrap-around gowns, scrub suits, or coveralls must be worn. • **Eye and face protection** (goggles, mask, face shield, or other splash guard) must be used for anticipated splashes or sprays of infectious or other hazardous materials. [All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. • Personnel who wear contact lenses in labs should also wear eye protection. • **Gloves** must be worn to protect hands from exposure to hazardous materials. • Eye, face and respiratory protection should be used in rooms containing infected animals.	• Not permitted at BSU. • Please refer to the CDC/NIH document, *Biosafety in Microbiological and Biomedical Laboratories* for PPE requirements.

**Safety is improved when PPE is used in combination with physical containment devices or equipment, such as Biological Safety Cabinets (BSCs).*

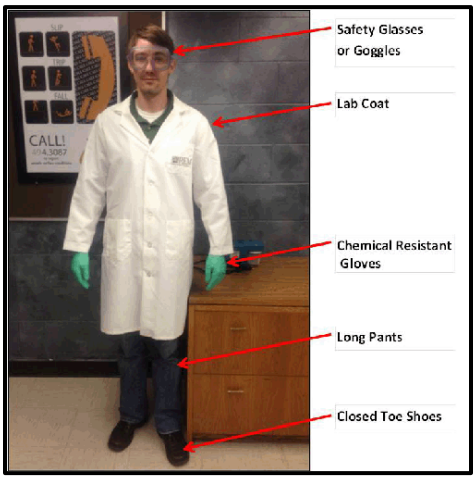
General PPE

As with all BSU laboratories, the following PPE precautions and prohibitions apply to work or study in regulated biosafety laboratories:

- Clothes should cover the body to the extent practicable, but must include long pants or other leg coverings which are not so tight as to promote contact with spilled materials or prevent

rapid removal in the event of spills or flame contact;

- Closed-toe shoes are required. Sandals and flip-flops, high heels, or shoes made of woven materials are not allowed. Oil-resistant slip-proof soles are preferred;
- Long hair must be tied back or otherwise restrained;
- Loose jewelry must be removed before entering the laboratory;
- Loose fitting clothing (baggy sleeves, etc.) is normally unacceptable as it may allow or transfer contamination;
- Non-synthetic clothing (cotton or wool) is preferable and may be required if working with flammable materials or ignition sources.



Personal possessions such as outer garments (coats, gloves, hats, etc.), backpacks, phones, and other items not essential to laboratory activities are not allowed in the laboratory unless a designated and protected location is available. In most cases, secured lockers will need to be provided and utilized to prevent the possibility of cross-contamination or escape of infectious agents.

Eye and Face Protection

Safety glasses must be worn in the lab whenever procedures are underway involving a low probability of splash, work with low hazard chemicals, or an impact hazard.

Whenever possible, lab operations should be performed in containment devices such as a biological safety cabinet or fume hood, or behind a bench-top shield in order to minimize the potential for skin or mucous membrane contact with a hazardous splash. If procedures do not permit containment of the hazard with a containment device, then appropriate PPE must be worn as outlined:

- Splash goggles are the only form of eye protection approved for splash hazards. If a chemical (including bleach) or biological splash hazard exists, splash goggles must be worn.

- Full face protection (i.e., face shield) must be used for procedures that have anticipated splashes or sprays of infectious or other hazardous materials to the face or if there is a high potential for aerosol generation. Face shields are not a replacement for eye protection. Information on the availability of low cost prescription safety eyewear may be obtained by calling EHS at 285-2815.

Protective Laboratory Outerwear

This category includes laboratory coats, smocks, scrub suits, and gowns. Long-sleeved garments should be used to minimize the contamination of skin or street clothes. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect clothing from contamination. If the garment is not disposable, it

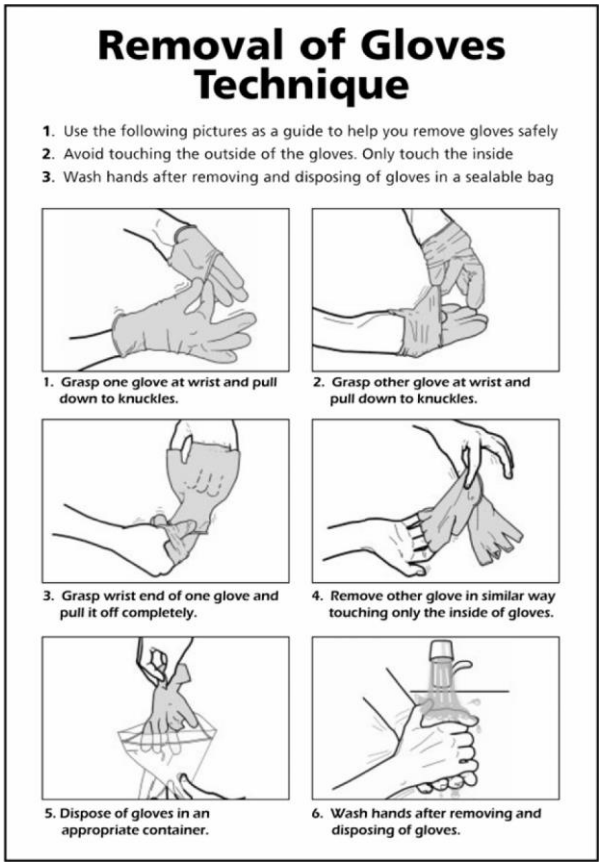
must be capable of withstanding sterilization, in the event it becomes contaminated.

A laboratory coat is recommended for all work at BSL-1 and it, or other suitable protective clothing, is required when handling potentially infectious materials at BSL-2 or higher. Preferably, like a lab coat with few buttons, the outer garment will be relatively loose fitting and capable of quick removal in the event of a spill or other exposure in order to protect the wearer. Additional criteria for selecting clothing are: comfort, appearance, closure types and location, antistatic properties and durability. Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas. Disposables should be available for visitors, maintenance and service workers in the event it is required. All protective clothing should be either discarded in the laboratory or commercially laundered. Personnel must not take laboratory clothing home.

Gloves

Gloves must be selected based on the hazards involved and the activity to be conducted. Gloves must be worn when working with biohazards, toxic substances, hazardous chemicals and other physically hazardous agents. Temperature resistant gloves must be worn when handling hot material or dry ice. Delicate work requiring a high degree of precision dictates the use of thin walled gloves.

When latex gloves have been chosen, alternatives should be made available. Powdered gloves are allowed for use in BSU laboratories Gloves should be changed as soon as possible after they have become contaminated; when their integrity has been compromised or when necessary. Hands should be properly washed with soap and water after removing gloves. Disposable gloves must not be washed or reused.



Gloves should be removed and hands washed when work with potentially infectious materials is complete or when leaving the laboratory. As a gloved hand creates an ideal environment for microorganisms (moist, temperate, organic substrate) they should be replaced at least every 2 hours even if not damaged, followed by hand washing. If you must transport potentially infectious materials (i.e., cultures, waste, etc.) to another part of the building use the one glove rule: use one gloved hand for handling the materials and use the other **ungloved** hand for touching common surfaces such as door knobs and elevator buttons. For assistance in glove selection, contact EHS at 285-2807.

If the lab procedure involves use of chemicals, the gloves should be selected based on

resistance to the particular chemical(s). Numerous charts are available from glove manufacturers showing the relative resistance of glove materials to common laboratory chemicals. The BSU *Chemical Hygiene Plan* is another resource.

Respiratory Protection

For certain protocols and projects, additional PPE such as respiratory protection may be required. Respirator selection is based on the hazard and the Protection Factor (PF) required. Personnel who require respiratory protection must contact the EHS for assistance in selection of proper equipment and training in its usage. All personnel wearing respirators need to be included in BSU's *Respiratory Protection Program* which includes a medical evaluation, initial training and initial and annual fit-testing and retraining. Voluntary use of filtering face mask (NIOSH approved) respirators may be accommodated in cooperation with EHS requirements and that Office should be contacted in that regard.

Biosecurity

Recent events have brought to the forefront the necessity of having a comprehensive laboratory security program. However, before outlining the biosecurity requirements that have been implemented by the University it is important to understand the distinction between “biosafety” and “biosecurity.”

“Biosafety” is the application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or other biohazards. “Biosecurity” refers to measures designed to protect microbiological agents from loss, theft, misuse or intentional release, and to protect research-related information from loss, theft or misuse. This can be accomplished by limiting access to facilities, biological materials and research-related information. Sufficient security for the biological materials in use may already be in place for laboratories that do not handle select agents, exempt levels of toxins on the select agents list or exempt strains of select agents. These security measures include access controls and training requirements outlined for BSL-1 and BSL-2 laboratories previously. If you wish to handle select agents, exempt levels of select agent toxins, exempt strains of select agents, other agents of public health or agricultural concern, or agents of high commercial value please contact the IBC, University Police, or EHS for additional biosecurity requirements.

Elements of the biosecurity program at BSU include:

1. **Physical security:** Access control and monitoring are intended to prevent the removal of materials for unauthorized purposes. Access should be limited to authorized personnel based on the necessity of entering sensitive areas. At a minimum, laboratory doors must be locked when no one is present in the lab, all storage units housed in shared space (i.e., hallway, storage room, etc.) must be locked, and all persons entering the laboratory should be asked for identification and questioned as to their purpose for being there.

2. **Inventory and accountability:** As required under the preceding Recordkeeping section, **i**t is the responsibility of each laboratory to establish material accountability procedures. These should be designed to track the inventory, storage, use, transfer and destruction of biological materials. The purpose is to know what agents are housed in a lab, where they are located and if they are all accounted for. See **Appendix H** for an example of an inventory log.

3. **Transport of biological agents:** Material transport policies are in place that outline requirements for transporting locally on campus and outside of campus. See the following section of this manual for additional information: *Introduction to the Transport of Biological Materials*.

4. **Reporting and communication:** In addition to following departmental reporting requirements should a security breach occur, the laboratory must also notify UPD and the Biological Safety Officer. Investigation into the breach will occur as appropriate.

5. **Training:** Laboratory security awareness training is required for anyone who has access to a laboratory. This training is available through our Biosafety Principles, Bloodborne Pathogens Initial, as well as the refresher trainings.

Laboratory Biosafety Equipment

As aerosols are important sources of infection, care should be taken to reduce the extent of their formation and dispersion. Hazardous aerosols can be generated by many laboratory operations, e.g. blending, mixing, grinding, shaking, stirring, sonicating, and centrifuging of infectious materials. Even when safe equipment is used, it is best to carry out these operations in an approved biological safety cabinet whenever possible. The use of safety equipment is no assurance of protection unless the user is trained and uses proper techniques. Equipment should be tested regularly to ensure its continued safe performance. **Table 8** provides a list of safety equipment designed to eliminate or reduce certain hazards and briefly outlines the safety features. Further details of much of this equipment are given in subsequent pages.

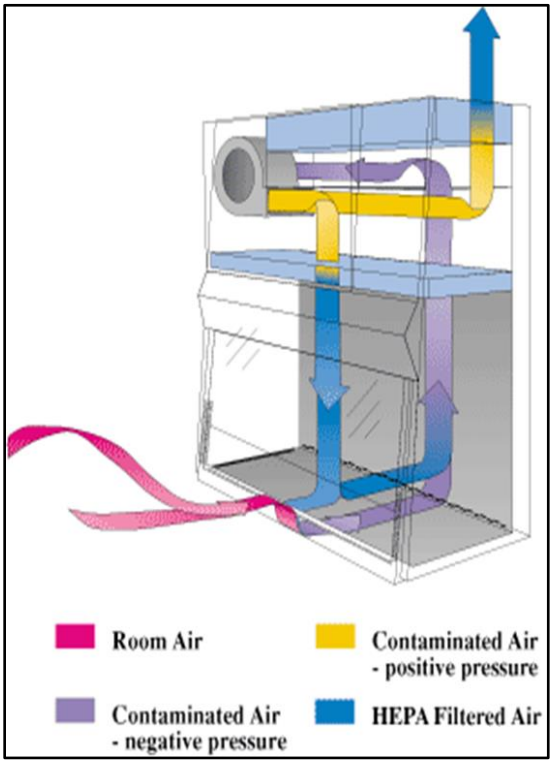
Table 8: Laboratory Biosafety Equipment

Equipment	Hazard Controlled	Safety Features
Biological Safety Cabinet		
--Class I	Aerosol and spatter	Minimum inward airflow (face velocity) at work access opening. Adequate filtration of exhaust air. Does not provide product protection
--Class II	Aerosol and spatter	Minimum inward airflow (face velocity) at work access opening. Adequate filtration of exhaust air. Provides product protection.
--Class III	Aerosol and spatter	Maximum containment. Provides product protection if laminar flow air is included.
Pipetting aids	Hazards from pipetting by mouth, e.g. ingestion of pathogens, inhalation of aerosols produced by mouth suction on the pipette, blowing out of liquid or dripping from pipet, contamination of suction end of pipette.	Ease of use. Controls contamination of suction end of pipette, protecting pipetting aid, user, and vacuum line. Can be sterilized. Controls leakage from pipette tip.
Loop microincinerators, disposable loops	Spatter from transfer loops	Shielded in open-ended glass or ceramic tube. Heated by gas or electricity. Disposable, no heating necessary.
Leakproof vessels for collection and transport of infectious materials	Aerosols, spillage, and leakage	Leakproof construction with lid of cover. Durable. Autoclavable.
Sharps disposal containers	Puncture wounds	Robust, puncture-proof
Autoclaves, manual or automatic	Infectious material (made safe for disposal or reuse)	Approved design Effective heat sterilization
Screw-capped bottles	Aerosols and spillage	Effective containment
Vacuum line protection	Contamination of laboratory vacuum system with aerosols and overflow fluids	Cartridge-type filter prevents passage of aerosols (particle size 0.45 µm). Overflow flask contains appropriate disinfectant. Rubber bulb may be used to close off vacuum automatically when storage flask is full. Entire unit is autoclavable.

Biological Safety Cabinets (BSCs)

Biological safety cabinets (BSCs) are designed to protect the operator, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating materials containing infectious agents. Aerosol particles are created by any activity that imparts energy into a liquid, such as shaking, pouring, stirring or dropping liquid onto a surface or into another liquid. Other laboratory activities, such as streaking agar plates, inoculating cell culture flasks with a pipette, using a multichannel pipette to dispense liquid suspensions of infectious agent into microculture plates, homogenizing and vortexing infectious materials, and centrifugation of infectious liquids, or working with animals, can generate infectious aerosols. Aerosol particles of less than 5 µm in diameter and small droplets of 5-100 µm in diameter are not visible to the naked eye. These particles may be inhaled or may cross contaminate work surface materials. BSCs, when properly used, have been shown to be highly effective in reducing laboratory-acquired infections and cross-contaminations of cultures due to aerosol exposures. BSCs also protect the environment.

BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of biological safety cabinets, designated as Class I, II and III are available. Biological safety cabinets use high efficiency particulate air (H EPA) filters in their exhaust and/or supply systems. The HEPA filter traps 99.97% of particles of 0.3 µm in diameter and 99.99% of particles of greater or smaller size. Biological safety cabinets must not be confused with other laminar flow devices or “clean benches.” Horizontal flow cabinets direct air towards the operator and should never be used for handling infectious or toxic materials.



For information on biological safety cabinets, beyond what is in this Manual, please refer to the CDC/NIH publication: *Primary Containment for Biohazards Selection, Installation and Use of Biological Safety Cabinets.*

Class I Biological Safety Cabinet

This is a ventilated cabinet for personnel protection with an un-recirculated inward airflow away from the operator. The air from the cabinet is exhausted through a HEPA filter: (a) into the laboratory and then to the outside of the building exhaust; (b) to the outside through the building exhaust; or (c) directly to the outside. The HEPA filter may be located in the exhaust plenum of the BSC or in the building exhaust. Some Class I BSCs are equipped with an integral exhaust fan, whereas others rely on the exhaust fan in the building exhaust system.
The Class I BSC was the first recognized BSC and, because of its simple design, is still in wide

use throughout the world. It has the advantage of providing personnel and environmental protection and can also be used for work with radionuclides and volatile toxic chemicals. Because unsterilized room air is drawn over the work surface through the front opening, it does not provide product protection.

Class II Biological Safety Cabinet

This is a ventilated cabinet for personnel, product and environmental protection which provides inward airflow and H EPA-filtered supply and exhaust air. The Class II cabinet has four designs depending on how much air is recirculated and/or exhausted and if the BSC is hard-ducted to the ventilation system or not. Class II cabinets may be of use with low to moderate risk biological agents, minute quantities of toxic chemicals, and trace quantities of radionuclides; however, care must be exercised in selecting the correct Class II cabinet design for these purposes.

Class II Type A1 Biological Safety Cabinet

An internal fan draws room air (supply air) into the cabinet through the front opening and into the front intake grill. The supply air then passes through a supply HEPA filter before flowing downwards over the work surface. As the air flows downwards it "splits" about 6-18 cm from the work surface, one half of the downwards flowing air passing through the front exhaust grill, and the other half passing through the rear exhaust grill. Any aerosol particles generated at the work surface are immediately captured in this downward airflow and passed through the front or rear exhaust grills, thereby providing the highest level of product protection. The air is then discharged through the rear plenum into the space between the supply and exhaust filters located at the top of the cabinet. About 70% of the air recirculates through the supply HEPA filter back into the work zone; the remaining 30% passes through the exhaust filter into the room or to the outside.

Air from the Class IIA1 BSC exhaust can be recirculated to the room or discharged to the outside of the building through a thimble connection to a dedicated duct or through the building exhaust system. A connection to a ducted exhaust system also allows some BSCs to be used for work with volatile radionuclides and volatile toxic chemicals (Table 3).

Class II Type A2 Vented to the Outside, B 1 and B2 Biological Safety Cabinets

Class IIA2 vented to the outside, IIB1 and IIB2 BSCs are variations of the Class IIA1. Each variation allows the BSC to be used for specialized purposes (see Table 3). These BSCs differ from one another in several aspects: the air intake velocity through the front opening; the amount of air recirculated over the work surface and exhausted from the cabinet's exhaust system, which determines whether air from the cabinet is exhausted to the room, or to the outside, through a dedicated exhaust system or through the building exhaust and the pressure arrangements (whether cabinets have biologically contaminated ducts and plenums are surrounded by negative-pressure ducts and plenums).

Class III Biological Safety Cabinet

This type provides the highest level of personnel protection and in used for Risk Group 4 agents. All penetrations are sealed "gas tight." Supply air is H EPA-filtered and exhaust air passes through

two HEPA filters. Airflow is maintained by a dedicated exhaust system exterior to the cabinet, which keeps the cabinet interior under negative pressure. Access to the work surface is by means of heavy duty rubber gloves, which are attached to ports in the cabinet. The Class III BSC should have an attached pass- through box that can be sterilized and is equipped with a H EPA-filtered exhaust. The Class III cabinet may be connected to a double-door autoclave used to decontaminate all materials entering to exiting the cabinet. Several glove boxes can be joined together to extend the work surface. Class III BSCs are suitable for work in Biosafety Level 3 and 4 laboratories. This is summarized in **Table 9** below:

Table 9: Selection of a Biological Safety Cabinet (BSC), (By Type of Protection Needed)

Type Of Protection	BSC Selection
Personnel protection, microorganisms in Risk Groups 1-3	Class I, Class II, Class III
Personnel protection, microorganisms in Risk Group 4, Glove-box laboratory	Class III
Personnel protection, microorganisms in Risk Group 4, Suit laboratory	Class I, Class II
Product protection	Class II, Class III only if laminar flow included
Volatile radionuclide/chemical protection, minute amounts	Class IIB1, Class IIA2 vented to the outside
Volatile radionuclide/chemical protection	Class I, Class IIB2, Class III

Selection of a Biological Safety Cabinet

A BSC should be selected primarily in accordance with the type of protection needed: product protection; personnel protection against Risk Group 1-4 microorganisms; personnel protection against exposure to radionuclides and volatile toxic chemicals; or a combination of these. Table 3 shows which BSCs are recommended for each type of protection.

Volatile or toxic chemicals should not be used in BSCs that recirculate exhaust air to the room, i.e. Class I BSCs that are not ducted to building exhaust systems, or Class IIA1 or Class IIA2 cabinets. Class IIB1 BSCs are acceptable for work with minute amounts of volatile chemicals and radionuclides. A Class IIB2 BSC, also called a total exhaust cabinet, is necessary when significant amounts of radionuclides and volatile chemicals are expected to be used.

Using Biological Safety Cabinets in the Laboratory

Location

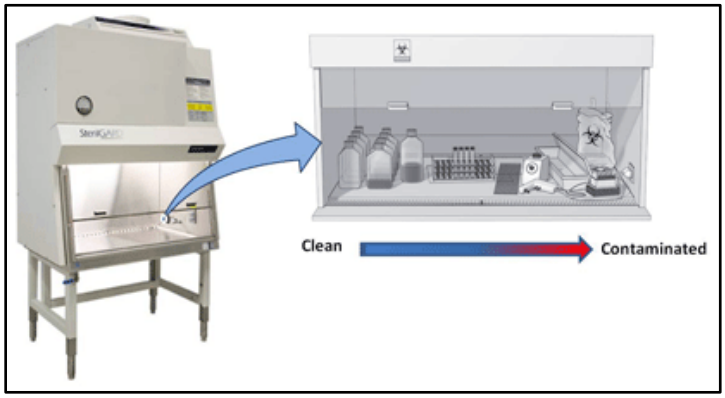
The velocity of air flowing through the front opening into a BSC is about 0.45 m/s. At this velocity the integrity of the directional air inflow can be easily disrupted by air currents generated by people walking close to the BSC, open windows, air supply registers, and opening and shutting doors. Ideally, BSCs should be situated in a location away from traffic and potentially disturbing air currents. Whenever possible a 30-cm clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance. A clearance of 30-35 cm above the cabinet may be required to provide for accurate air velocity measurement across the exhaust filter and for exhaust filter changes.

Operators

If BSCs are not used properly, their protective benefits are reduced. Operators need to be careful not to disrupt the air inflow when moving their arms into and out of cabinets. Arms should be moved in and out slowly, perpendicular to the front opening. Operators should not begin work until one minute after placing hands and arms inside. This will allow the cabinet to adjust and to "air sweep" the surface of the hands and arms. The number of movements across the front opening should be minimized by placing all necessary items inside the cabinet before beginning procedures.

Material Placement

The front intake grill of Class II BSCs must not be blocked with paper, equipment or other items. Materials to be placed inside the cabinet should be surface-decontaminated with 70% alcohol. Work may be performed on disinfectant-soaked absorbent towels to capture splatters and splashes. All materials should be placed as far back in the cabinet, towards the rear edge of the work surface, as practical without blocking the rear grill. Aerosol-generating equipment (e.g. mixers, centrifuges, etc.) should be placed towards the rear of the cabinet. Bulky items, such as biohazard bags, discard pipette trays and suction collection flasks should be placed to one side of the inside of the cabinet. Active work should flow from clean to contaminated areas across the work surface.



The autoclavable biohazard collection bag and pipette collection tray should not be placed outside the cabinet. The frequent in-and-out movement needed to use these containers is disruptive to the integrity of the cabinet's air barrier, and can compromise both personnel and product protection.

Operation and Maintenance

Most BSCs are designed to permit operation 24 hr/day, and investigators find that continuous operation helps to control the levels of dust and particulate materials in the laboratory. Class IIA1 and IIA2 BSCs exhausting to the room or connected by thimble connections to dedicated exhaust ducts can be turned off when not in use. Other types such as IIB1 and IIB2 BSCs, which have

hard-duct installations, must have airflow through them at all times to help maintain room air balance. Cabinets should be turned on at least 5 min before beginning work and after completion of work to allow the cabinet to “purge” (i.e. to allow time for contaminated air to be removed from the cabinet environment).

NSF/ANSI Standard 49 requires that BSCs be serviced and certified on an annual basis or whenever a new cabinet is installed, relocated, or the cabinet is in need of repairs. All repairs made on BSCs should be made by a qualified technician. Any malfunction in the operation of the BSC should be reported and repaired before the BSC is used again.

Ultraviolet Lights

Ultraviolet lights are not required in BSCs. The CDC and NIH do not recommend the use of UV lights in BSCs though manufacturers espouse their effectiveness. If they are used, they must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the light. Ultraviolet light intensity should be checked when the cabinet is recertified to ensure that light emission is appropriate. Ultraviolet lights must be turned off while the cabinet in in use by persons or whenever the room is occupied, to protect eyes and skin from inadvertent exposure.

Open Flames

Open flames are not allowed inside the BSC. The Centers for Disease Control and Prevention (CDC) reports that “open-flames are not required in the near microbe-free environment of a biological safety cabinet” and create “turbulence which disrupts the pattern of air supplied to the work surface” jeopardizing the sterility of the work area. This is also the recommendation of the World Health Organization (WHO) as well as the major Biosafety cabinet manufacturers.

Flames compromise the protection of the worker and the work by: disrupting the airflow patterns and causing excessive heat buildup damaging the HEPA filter and its components. Recirculation of cabinet air can create flammable atmospheres that directly result in a fire or explosion. The use of flames in the cabinet inactivates the manufacturer’s warranties on the cabinet: cabinet manufacturers will assume no liability in the event of fire, explosion or worker exposure due to the use of a flammable gas in the cabinet. Additionally, the UL approval will automatically be void. The heat generated may also damage the HEPA filters or cause a fire. If a flame is needed, consider alternative sterilizers as pictured below.

Sterile, disposable inoculating loops, needles and cell spreaders are available as an alternative to using open flames in the BSC for sterilizing equipment. Electric “furnaces” are also available. If it is deemed absolutely necessary for the work being done, use a pilotless burner or touch-

plate microburner to provide a flame on demand.

Spills

When a spill of biohazardous material occurs within a BSC, clean-up should begin immediately, while the cabinet continues to operate. An effective disinfectant should be used and applied in a manner that minimizes the generation of aerosols. All materials that come into contact with the spilled agent should be disinfected or autoclaved. See the following section of this manual for additional information on spill cleanup procedures: *Biohazard Spill Cleanup Procedures*.

Certification

The functional operation and integrity of each BSC should be certified to NSF Standard 49 at the time of installation and annually thereafter by qualified technicians. Certification includes tests for cabinet integrity, HEPA filter leaks, downflow velocity profile, face velocity, negative pressure/ventilation rate, airflow smoke pattern, and alarms and interlocks. Optional tests for electrical leaks, lighting intensity, ultraviolet light intensity, noise level and vibration may also be conducted. Special training, skills and equipment are required to perform these tests. Annual certification is required for BSCs that are used for work with human pathogens, recombinant DNA or human derived materials (e.g., cell lines, blood, etc.). To request service or certification contact the Biology Department Office or EHS.

Cleaning and Disinfection

All items within BSCs, including equipment, should be surface-decontaminated and removed from the cabinet when work is completed, since residual culture media may provide an opportunity for microbial growth.

The interior surfaces of BSCs should be decontaminated before and after each use. The work surfaces and interior walls should be wiped with a disinfectant that will kill any microorganisms that might be found inside the cabinet. At the end of the work day, the final surface decontamination should include a wipe- down of the work surface, the sides, back and interior of the glass. A solution of bleach or 70% alcohol should be used where effective for target organisms. A second wiping with sterile water is needed when a corrosive disinfectant, such as bleach, is used.

It is recommended that the cabinet is left running. If not, it should be run for 5 min in order to purge the atmosphere inside before it is switched off.

Decontamination

BSCs must be decontaminated before filter changes and before being moved. The most common decontamination method is by fumigation with formaldehyde gas. BSC decontamination should performed by a qualified professional.

Personal Protective Equipment

Personal protective clothing should be worn whenever using a BSC. Laboratory coats are normally acceptable for work being performed at biosafety levels 1 and 2. A solid front, back-closing laboratory gown provides better protection and should be used at biosafety level 3.

Gloves should be pulled over the wrists of the gown rather than worn inside. Elasticized sleeves can be worn to protect the investigator's wrists. Masks and safety glasses may be required for some procedures.

Alarms

BSCs can be equipped with one of two kinds of alarm. Sash alarms are found only on cabinets with sliding sashes. The alarm signifies that the sash has been moved to an improper position. Airflow alarms indicate a disruption in the cabinet's normal airflow pattern. This represents an immediate danger to the operator or product. When an airflow alarm sounds, work should cease immediately and the laboratory supervisor should be notified. Manufacturer's instruction manuals should provide further details.

Other Safety Equipment

Pipetting aids

A pipetting aid must always be used for pipetting procedures. Mouth pipetting must be strictly forbidden. The most common hazards associated with pipetting procedures are the result of mouth suction. Oral aspiration and ingestion of hazards associated with pipetting procedures are the result of mouth suction. Oral aspiration and ingestion of hazardous materials have been responsible for many laboratory- associated infections.

Aerosols can be generated when a liquid is dropped from a pipette onto a work surface, when cultures are mixed by alternate sucking and blowing, and when the last drop is blown out of a pipette. The inhalation of aerosols unavoidably generated during pipetting operations can be prevented by working in a biological safety cabinet.

Pipetting aides should be selected with care. Their design and use should not create an additional infectious hazard and they should be easy to sterilize and clean. Plugged (aerosol resistant) pipette tips should be used when manipulating microorganisms and cell cultures.

Pipettes with cracked or chipped suction ends should not be used as they damage the seating seals of pipetting aids.

Homogenizers, shakers, blenders, and sonicators

Domestic (kitchen) homogenizers are not sealed and release aerosols. Only equipment designed for laboratory use should be used. Their construction minimizes or prevents such release. Homogenizers used for Risk Group 3 microorganisms should always be loaded and reopened in biological safety cabinets. Sonicators may release aerosols. They should be operated in biological safety cabinets or covered with shields during use. The shields and outsides of sonicators should be decontaminated after use.

Disposable transfer loops, needles and cell spreaders

The advantage of disposable transfer loops, needles and cell spreaders is that they do not have to be sterilized and can therefore be used in biological safety cabinets where Bunsen burners and microincinerators would disturb the airflow. These loops should be placed in disinfectant

after use and discarded as contaminated waste.

Recommended Work Practices

Autoclaves

The following procedure is recommended for the decontamination of biohazardous waste:

- Items should be autoclaved in approved autoclave bags and in a rigid, autoclavable secondary container.
- Follow the guidelines set by the posted autoclave parameter signs when setting the cycle time.
- Add one cup of water to each bag of solid waste and keep the bags open. Steam cannot penetrate closed bags.
- To prevent spills and accidents, be sure that the exhaust setting is appropriate for the type of material you are autoclaving. Fast exhaust should be used for solid items and solid waste and slow exhaust for liquids and liquid waste.
- After the cycle is complete, let the bag cool before removing it from the autoclave.
- Securely close the orange autoclave bag.
- Place treated autoclave bags into opaque black bags and close them securely before disposing.

The following PPE should be worn when operating an autoclave:

- Heat resistant autoclave gloves- for loading and unloading the autoclave;
- Fluid resistant gloves- to eliminate contact with contaminated wastes;
- Lab coat- to protect your personal clothing; and
- Splash goggles- if a splash hazard is present.

Flow Cytometers

Cells should be sorted under the same containment conditions (e.g., BSL-2 for human cells) in which they are handled for other manipulations. When sorting potentially infectious unfixed cells, it is important to keep in mind that potentially infectious aerosols are generated. When the cell sorter fails to operate properly (e.g., a clogged sort nozzle) there can be an increased production of aerosols. High speed sorters also produce an increased amount of aerosols. Because of this risk it is recommended that the aerosol containment of the cell be verified. The following precautions should also be taken:

- Universal precautions should be followed (see the BSU Exposure Control Plan for details);
- Appropriate PPE should be worn (i.e., lab coat, gloves, N-95 respirator, splash goggles, face shield if desired);
- If possible, the cell sorter should be located in a separate room;

- The sorter should be operated according to the manufacturer's recommendations; and
- Decontaminate the sorter after each run using an appropriate disinfectant. The disinfectant should be run through the machine for at least 10 minutes.

Additional biosafety features can be installed to the sorter as appropriate.

Pipettes and Pipetting Aids

Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used. Confine pipetting of biohazardous or toxic fluids to a biosafety cabinet if possible. Use the following precautions:

- Always use cotton-plugged pipettes when pipetting biohazardous or toxic fluids.
- Never prepare any kind of biohazardous mixtures by suction and expulsion through a pipette.
- Biohazardous materials should not be forcibly discharged from pipettes. Use "to deliver" pipettes rather than those requiring "blowout."
- Do not discharge biohazardous material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.
- Place contaminated, reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them.
- Autoclave the pan and pipettes as a unit before processing them for reuse.
- Discard contaminated Pasteur pipettes in an appropriate size sharps container.
- When work is performed inside a biosafety cabinet, all pans or sharps containers for contaminated glassware should be placed inside the cabinet as well while in use.

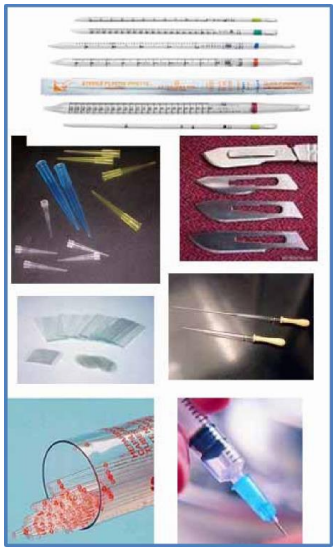
Sharps

Generally, the use of sharps should be restricted to procedures for which there is no alternative. Situations where the use of sharps may be appropriate include parenteral injection, phlebotomy, and aspiration of fluids. Plastic alternatives should be substituted for glassware whenever possible to prevent the unnecessary potential for sharps related exposure incidents. **Appendix R** includes additional procedures and precautions in the handling and disposal of sharps.

If it has been determined that the use of sharps is unavoidable, the following practices should be adhered to:

1. All personnel should be trained in safe sharps handling procedures.

2. Use disposable sharps devices (i.e., scalpels, biopsy punches, needles) if at all possible.
3. Procedures should be organized in a manner that limits personnel exposure to the sharp device. For example:
    - Do not expose/unsheath sharp devices until the procedure actually requires the use of these items
    - Do not leave exposed sharp items unattended
    - If feasible, place an BSU-approved sharps container within arm's reach of the point of use for the sharp item to allow for immediate disposal (For reusable sharps, use a hard- walled container that encloses the sharp end of the device)



4. Do not bend or break sharps.
5. Do not recap sharps if possible. If recapping is required, use a one-handed scoop technique. Note: The need for recapping can be eliminated through the use of safer sharps devices.
6. Do not handle sharps with two hands.
7. Dispose of waste sharps in a properly labeled BSU-approved sharps container.
8. Permanently close and dispose of sharps containers when they are 3/4 full. Do NOT overfill or shake containers because these actions can result in accidental sharps exposure.
9. Reusable sharps should be placed in a hard walled container for storage until processing for reuse.
10. Broken glassware should be handled with a mechanical device, such as tongs, forceps, or a broom and dustpan rather than directly by hand.
11. ALL syringes (with and without needles), scalpels, and razors, whether contaminated or not, are to be disposed in an infectious waste sharps container.

Safer Sharps Program

Laboratories that use human derived materials or work with bloodborne pathogens are subject to the requirements of the OSHA *Bloodborne Infectious Diseases Standard*. This standard requires that available safer sharps devices be used and that those devices be reviewed annually in consideration of newly marketed ones. For additional information on safer sharps refer to the BSU *Bloodborne Pathogens Exposure Control Plan* or contact the EHS Office at 5-2807.

Cryostats

Frozen sections of unfixed human tissue or animal tissue infected with an etiologic agent pose a risk because accidents can occur. Freezing tissue does not necessarily inactivate infectious agents. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material. Gloves should be worn during preparation of frozen sections. When working with biohazardous material in a cryostat, the following is recommended:

- Consider the contents of the cryostat to be contaminated and decontaminate it frequently with a disinfectant suitable for the agent(s) in use.
- Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.
- Decontaminate the cryostat with a tuberculocidal type disinfectant regularly and immediately after tissue known to contain bloodborne pathogens, M. tuberculosis or other infectious agents is cut.
- Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
- Consider solutions for staining potentially infected frozen sections to be contaminated.

Centrifuge Equipment

Hazards associated with centrifuging include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions.

Aerosols are created by practices such as filling centrifuge tubes, removing supernatant, and re-suspending sedimented pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation. To minimize the generation of aerosols when centrifuging biohazardous material, the following procedures should be followed:

- Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Fill and open centrifuge tubes, rotors and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
- Add disinfectant to the space between the tube and the bucket to disinfect material in the event of breakage during centrifugation.
- Always balance buckets, tubes and rotors properly before centrifugation.
- Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters.
- Work in a BSC when re-suspending sedimented material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
- Small low - speed centrifuges may be placed in a BSC during use to reduce the aerosol escape.

Safety Blenders

Safety blenders, although expensive, are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation, and to withstand sterilization by autoclaving. Blenders should be loaded, operated and unloaded in a biosafety cabinet when used in conjunction with potentially infectious materials. The use of glass blender jars is not recommended because of the breakage potential. A towel moistened with disinfectant should be

placed over the top of the blender during use. Blender jars should be allowed to rest for at least one minute to allow the aerosol to settle before opening them. The device should be decontaminated promptly after use.

Lyophilizers and Ampoules

Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material should be loaded in a BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized and vapor traps should be used wherever possible.

Opening ampoules containing liquid or lyophilized infectious culture material should be performed in a BSC to control the aerosol produced. Gloves must be worn. To open, nick the neck of the ampoule with a file, wrap it in disinfectant soaked towel, hold the ampoule upright and snap it open at the nick. Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the container. Discard the towel and ampoule top and bottom as biohazardous waste.
Ampoules used to store biohazardous material in liquid nitrogen have exploded causing eye injuries and exposure to the infectious agent. The use of polypropylene tubes eliminates this hazard. These tubes are available dust free or pre-sterilized and are fitted with polyethylene caps with silicone washers. Heat sealable polypropylene tubes are also available.

Loop Sterilizers and Bunsen Burners

Sterilization of inoculating loops or needles in an open flame generates small particle aerosols which may contain viable microorganisms. The use of a shielded electric incinerator or hot bead sterilizers minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work.
Continuous flame gas burners should not be used in BSCs. These burners can produce turbulence which disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter.

Guidelines for Working with Tissue Culture/Cell Lines

When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line can be classified at the same RG level as that recommended for the agent.

The Centers for Disease Control and Prevention (CDC) and OSHA require that all cell lines of human origin be handled at BSL-2. All personnel working with or handling these materials need to be included in BSU's Bloodborne Pathogen Program (Refer to the BSU Exposure Control Plan for additional information).

Cell lines which are non-primate or are of normal primate origin, which do not harbor a primate

virus, which are not contaminated with bacteria, mycoplasma or fungi, and which are well established may be considered Class I cell lines and handled at a Biosafety Level 1. Appropriate tests should confirm this assessment.

Primate cell lines derived from lymphoid or tumor tissue, all cell lines exposed to or transformed by a primate oncogenic virus, all clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy), all primate tissue, all cell lines new to the laboratory (until shown to be free of all adventitious agents) and all virus and mycoplasma-containing primate cell lines are classified as RG-2 and should be handled at a Biosafety Level 2. Studies involving suspensions of HIV prepared from T-cell lines must be handled at BSL-3.
Recent product recalls for bovine serum have raised the awareness of potential Bovine Spongiform Encephalopathy (BSE) or TSE (Transmissible Spongiform Encephalopathy) contamination of those sera. For more information on testing and purity of bovine serum used in your laboratory, contact your supplier.

Guidelines for Preventing the Transmission of Tuberculosis

Since 1985, the incidence of tuberculosis in the United States has been increasing steadily, reversing a 30 year downward trend. Recently, drug resistant strains of Mycobacterium tuberculosis have become a serious concern. Outbreaks of tuberculosis, including drug resistant strains, have occurred in healthcare environments. Several hundred employees have become infected after workplace exposure to tuberculosis, requiring medical treatment. A number of healthcare workers have died.

In October 1994, CDC first published its *Guidelines for Preventing the Transmission of Mycobacterium tuberculosis in Health-Care Facilities*. These guidelines were reviewed and updated by the CDC in 2005. The guidelines contain specific information on ventilation requirements, respiratory protection, medical surveillance and training for those personnel who are considered at risk for exposure to tuberculosis. For more information, contact EHS at 355-0153. Investigators intending to work with Mycobacterium sp. in the laboratory must contact EHS well in advance. Propagation and/or manipulation of Mycobacterium tuberculosis and M. bovis cultures in the laboratory or animal room must be performed at BSL-3.

Guidelines for Clinical Laboratories

Clinical laboratories receive clinical specimens with requests for a variety of diagnostic services. The infectious nature of this material is largely unknown. In most circumstances, the initial processing of clinical specimens and identification of microbial isolates can be done safely at BSL-2.

A primary barrier, such as a biological safety cabinet, should be used:

- When it is anticipated that splashing, spraying or splattering of clinical materials may occur;
- For initial processing of clinical specimens where it is suggested that an agent transmissible by infectious aerosols may be present (e.g., M. tuberculosis); or

- To protect the integrity of the specimen.

All laboratory personnel who handle human source materials are included in the Bloodborne Pathogens Program as outlined in BSU's Exposure Control Plan. "Universal Precautions" need to be followed when handling human blood, blood products, body fluids or tissues.

The segregation of clinical laboratory functions and restricting access to specific areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented. A copy of the Exposure Control Plan must be available in all laboratories. Additional recommendations specific for clinical laboratories may be obtained from the *National Committee for Clinical Laboratory Standards (NCCLS)*.

Guidelines for Prion Use

Research-related activities involving prions or tissues containing prions have been on the rise at BSU in both the animal health and human health arenas. Because the infectious nature of prions is not well characterized and destruction of these particles goes beyond the techniques typically required for biohazard inactivation, work with these agents requires special considerations for biocontainment to minimize both occupational and environmental exposure risk.
At this time, work with prion-risk materials at BSU is limited to research and diagnostic laboratory applications. A guidance document has been prepared that applies to these procedures only (See **Appendix L**). Guidelines for use of prion-risk materials in conjunction with live animals will be developed if needed. Therefore, if future project plans call for use of live animals and prion-risk materials, please notify the BSU Biosafety Officer at the proposal-writing stage to perform a risk assessment and identify containment requirements.

Guidelines Regarding Select Agents

The Centers for Disease Control and Prevention (CDC) regulates the possession, use, and transfer of select agents and toxins that have the potential to pose a severe threat to public health and safety. The CDC Select Agent Program oversees these activities and registers all laboratories and other entities in the United States of America that possess, use, or transfer a select agent or toxin.

The U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) published final rules for the possession, use, and transfer of select agents and toxins (42 C.F.R. Part 73, 7 C.F.R. Part 331, and 9 C.F.R. Part 121) in the Federal Register on March 18, 2005. All provisions of these final rules supersede those contained in the interim final rules and became effective on April 18, 2005.

The purpose of the CDC's Select Agents regulation (42CFR72) is to provide a means of accountability for the use of select agents- biological agents that could pose a severe threat to public health and safety. On June 10, 2002, President George W. Bush signed into law the "Public Health Security and Bioterrorism Preparedness and Response Act of 2002." This Act expands

current regulations governing listed biological agents or toxins to require that all persons who possess, use, and/or transfer these materials register with the Department of Health and Human Services and the U.S. Department of Agriculture. All such persons are subject to safety and security requirements and inspections.

As a result of the bioterrorism events of 2001 and 2002, federal legislation (USA Patriot Act) has been passed that restricts specific groups of people from handling or accessing Select Agents. Therefore, anyone who plans to work with these materials may be asked to complete an affidavit to verify that he/she is not a restricted person in addition to registering with the CDC via EHS.

Registration of Select Agent & Toxin Possession is MANDATORY

Under previous select agent regulations, an individual was permitted to use select agent materials without registering with the federal authorities. Registration was only required if an individual planned to send or receive materials on the select agent list. Under the revised regulations which take effect on February 7, 2003, all individuals who possess select agents must register with the CDC and/or APHIS through the designated institutional responsible official (RO). At BSU, the EHS Director and Biosafety Officer serve in this capacity. The registration process is rigorous and includes many provisions such as:

- Description of research space including HVAC details, safety equipment and security features
- Research summary outlining use of agent
- Agent-specific safety and biocontainment procedures
- Safety and technical training of lab personnel
- Security & emergency response plans
- • Security risk assessment, including U.S. Attorney General background check of personnel with access to agent

Once the registration document is prepared and submitted to the appropriate federal authorities, the turnaround time for approval is expected to be at least 2 months. **For new registrations, the agent cannot be transferred to BSU facilities until approval is granted by the CDC and/or APHIS.**

Considerations for Colleges & Departments

It is critical for departments to identify any potential for use or possession of select agents by research personnel in order to protect both the university and the researcher from unknowingly violating a regulatory requirement that bears both civil and criminal penalties. University policies are likely to be developed in order to address this potential. In the meantime, the following actions can be taken to prevent this from happening:

- Screen all research materials received in order to assure that no items on the select agent list have been inadvertently sent to campus. This is especially true for items received from foreign countries because the select agents regulations apply to the United States. International colleagues may not be aware of these new restrictions.

- Query all visiting research personnel, or newly recruited faculty before they come to campus to assure that they are not planning to bring any materials that are restricted under the select agent regulations. Again, international colleagues may not be aware of these new restrictions.

- Consult the EHS if any researcher plans to pursue grant money for research involving select agents. At this time, there are several bioterrorism-related funding opportunities for researchers. In order to plan for this potential work, research personnel need to be aware of the scope of regulatory requirements and limitations associated with this type of work.

Guidelines for Handling Exempt Strains of Select Agents

The United States Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA) have established regulations for the possession, use and transfer of select agents and toxins (see 42 CFR Part 73, 7 CFR Part 331 and 9 CFR Part 121). These regulations have also established a procedure by which an attenuated strain of a select agent that does not pose a severe threat to public health and safety, animal health, or animal products may be excluded from the requirements of the regulations when used for specific purposes. Please note that if an excluded attenuated strain is manipulated in such a way that virulence is restored or enhanced, or if factors associated with virulence are reintroduced, it will then be subject to the regulations. Because of the nature of these exempt strains and the potential for them to be manipulated for use as a biological weapon, Environmental Health and Safety/ has implemented the containment and security requirements outlined in **Appendix M** for handling exempt strains of select agents.

The containment and security requirements apply to the following exempt strains of select agents:

- *Bacillus anthracis* strains devoid of both plasmids pX01 and pX02
- *Bacillus anthracis* strains devoid of the plasmid pX02 (e.g., Bacillus anthracis Sterne, pX01 $^{+}$pX02^{-})
- *Brucella abortus* strain RB51 (vaccine strain)
- *Brucella abortus* strain 19
- *Coxiella burnetii* Phase II, Nine Mile Strain, plaque purified clone 4
- *Francisella tularensis* subspecies *novicida* (also referred to as *Francisella novicida*) strain, Utah 112 (ATCC 15482)
- *Francisella tularensis* subspecies *holartica* LVS (live vaccine strain; includes NDBR 101 lots, TSIGSD lots, and ATCC 29684)
- *Francisella tularensis* ATCC 6223 (also known as strain B38)
- Rift Valley fever virus, MP-1 2 vaccine strain
- Venezuelan equine encephalitis virus, TC-83 strain
- Venezuelan equine encephalitis virus vaccine candidate strain V3526
- Highly pathogenic avian influenza virus, recombinant vaccine reference strains of the H5N1 and H5N3 subtypes
- Japanese encephalitis virus, SA-1 4-1 4-2 strain

Guidelines for the Use of Exempt Levels of Select Agent Toxins

Several toxins that appear on the NIH/CDC Select Agent list may be used in reduced quantities without completing the rigorous CDC registration. A list of such toxins can be accessed through a reference in **Appendix E**. Regardless, registration with EHS is required for exempt levels, and Standard Operating Procedures (SOPs) regarding storage, disposal, and handling must be implemented before toxins are used in the laboratory.

Decontamination

Decontamination is defined as the reduction of microorganisms to an acceptable level. Methods applied to reach this goal can vary and most often include disinfection or sterilization. Generally speaking, disinfection is used when the acceptable level of microorganisms is defined as being below the level necessary to cause disease. This means that viable microorganisms are still present. In contrast, sterilization is defined as the complete killing of all organisms present. Depending on the circumstances and tasks, decontamination of a surface (e.g., lab bench) is accomplished with a disinfectant, while decontamination of biomedical waste is done by sterilization in an autoclave.

Many different terms are used for disinfection and sterilization. The following are among the more common in biosafety:

- *Antimicrobial* – An agent that kills microorganisms or suppresses their growth and multiplication.
- *Antiseptic* – A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.
- *Biocide* – A general term for any agent that kills organisms.
- *Chemical germicide* – A chemical or a mixture of chemicals used to kill microorganisms.
- *Disinfectant* – A chemical or mixture of chemicals used to render items free of pathogenic or infectious microorganisms kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.
- *Microbicide* – A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of “biocide”, “chemical germicide” or “antimicrobial.”
- *Sanitizer* – A chemical or mixture of chemicals that reduces the microorganism load to a level considered safe for public health purposes – i.e., log 3 to log 5 reduction.
- *Sporocide* – A chemical or mixture of chemicals used to kill microorganisms and spores.
- *Sterilant* – An agent that renders items free of **all** microorganisms.

When choosing a method of decontamination, it is important to consider the following aspects:

- Type of biohazardous agents, concentration and potential for exposure;
- Physical and chemical hazards to products, materials, environment and personnel.

Cleaning Laboratory Materials

Cleaning is the removal of dirt, organic matter and stains. Cleaning includes brushing, vacuuming, dry dusting, washing or damp mopping with water containing a soap or detergent. Dirt, soil and organic matter and shield microorganisms an can interfere with the killing action of decontaminants (antiseptics, chemical germicides and disinfectants).

Pre-cleaning is essential to achieve proper disinfection or sterilization. Many germicidal products claim activity only on pre-cleaned items. Pre-cleaning must be carried out with care to avoid exposure to infections agents.
Cleaning materials chemically compatible with the germicides to be applied later must be used. It is quite common to use the same chemical germicide for pre-cleaning and disinfection.

Decontamination Methods

Physical and chemical means of decontamination fall into four main categories:

- Heat
- Liquid chemicals
- Vapors and gases, and
- Radiation.

Disinfection is normally accomplished by applying liquid chemicals or wet heat during boiling or pasteurization. To sterilize, vapors and gases (e.g., ethylene oxide), radiation, and wet heat (steam sterilization in an autoclave) are used. Some liquid chemicals are also applied for sterilization, if used in the right concentration and incubation time.

Heat (Autoclave)

In order to kill microbial agents, heat can be applied in dry or wet form. The advantage of wet heat is a better heat transfer to and into the cell resulting in shorter exposure time and lower temperature. Steam sterilization uses pressurized steam at 121-132°C (250-270°F) for 30 or 40 minutes. This type of heat kills all microbial cells including spores, which are normally heat resistant. In order to accomplish the same effect with dry heat in an oven, the temperature needs to be increased to 160-170°C (320-338°F) for periods of 2 to 4 hours.

Decontamination of Biohazardous Waste by Autoclaving

Autoclaving is accepted as a safe and effective procedure for sterilization. There are currently over one hundred fifty operating autoclaves on the BSU campus. To ensure that any biohazardous waste created by the BSU community is properly decontaminated, the EHS tests each autoclave on an annual basis. Biological and chemical tests are used to monitor the autoclave cycle inside the chamber. Ampoules with heat resistant spores (*Bacillus stearothermophilus*) and steam sterilization integrator strips are used to indicate that adequate sterilization conditions are reached.

Procedures for BSU Autoclaves:

- All autoclaves used for decontamination of biohazardous waste need to be registered with EHS and tested on at least an annual basis.
- Strong oxidizing material (chemicals) must not be autoclaved with organic material: Oxidizer + Organic Material + Heat = Possible Explosion
- All biohazardous waste must be placed in orange biohazard bags with a heat sensitive "Autoclaved" indicator.
- Prior to autoclaving, a biohazard bag containing waste must be kept closed to prevent airborne contamination and nuisance odors. However, when autoclaving, the bag must be open to allow the steam to penetrate. Upon removal of the bag from the autoclave, it should be closed and disposed of in an opaque (black) waste bag.
- It is recommended to add water to each bag before autoclaving.
- Autoclave biohazardous materials using the recommended parameters posted on the autoclave.

Liquid Disinfection

The appropriate liquid disinfectant should be chosen after carefully assessing the biohazardous agent and the type of material to be decontaminated. Liquid disinfectants are preferably used for solid surfaces and equipment. They vary greatly in their efficiency, depending on the chemical constituents and the agents involved. Variables to remember when disinfecting:

- Nature of surface being disinfected - Porous or smooth; the more porous and rough the surface, the longer a disinfectant will need to be effective.
- Number of microorganisms present - Higher concentrations require a longer application time and/or higher concentration of disinfectant.
- Resistance of microorganisms - Microbial agents can be classified according to increasing resistance to disinfectants and heat (see Table 4).
- Presence of organic material - The proteins in organic materials such as blood, bodily fluids, and tissue can prevent or slow the activity of certain disinfectants.
- Duration of exposure and temperature - Increased exposure time increases the effectiveness of disinfectants. Low temperatures may slow down the activity requiring more exposure time.

TABLE 10: Increasing Resistance to Chemical Disinfectants

		Examples
LEAST RESISTANT	**LIPID OR MEDIUM-SIZE VIRUSES**	Herpes simplex Virus Cytomegalovirus Hepatitis B virus HIV
	VEGETATIVE BACTERIA	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Salmonella choleraesuis*
	FUNGI	Trichophuton sp. Cryptococcus sp. Candida sp.
	NONLIPID OR SMALL VIRUSES	Poliovirus Coxsackievirus Rhinovirus
	MYCOBACTERIA	*Mycobacterium tuberculosis;* *M. bovis*
MOST *RESISTANT*	**BACTERIAL SPORES**	*Bacillus subtilis* *Clostridium sporogenes*

There are many different liquid disinfectants available under a variety of trade names. In general, these can be categorized as halogens, acids or alkalines, heavy metal salts, quaternary ammonium compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the most effective disinfectants are often corrosive and toxic.

Alcohols

The most commonly used alcohols, ethanol and isopropanol, are most effective at concentrations of 70% in water. Both higher and lower concentrations are less effective. Alcohols are active against vegetative bacteria, fungi, and lipid viruses but not against spores. They are only moderately effective against nonlipid viruses. Alcohols are difficult to use as contact disinfectants because they evaporate rapidly and do not penetrate organic matter well. When using alcohols, it is best to clean an object, then submerge it in alcohol for the appropriate time. Alcohols are often used in concert with other disinfectants such as formaldehyde (but see toxicity warning below) or chlorine (2000 ppm chlorine in alcohol). Alcohol is NOT a registered tuberculocidal or HIV listed disinfectant.

Chlorine compounds

The most commonly used and generally effective disinfectant is sodium hypochlorite (common household bleach). It is a strong oxidizing agent and therefore can be corrosive to metal. A 1:50 dilution, supplying 1000 ppm available chlorine, of the common household product (e.g. chlorox) is very effective as a general laboratory disinfectant and a 1:10 dilution supplying 5000 ppm available chlorine is effective against spills involving blood or other organic material. Please note that the presence of high concentrations of protein can inactivate the action of chlorine products. Dilute hypochlorite solution must be prepared daily to be maximally effective. There are more concentrated sodium hypochlorite solutions available for industrial use, so please read the product information carefully to determine the proper dilution.

Formaldehyde

Formaldehyde is a gas that is available either dissolved in water and methanol as a 37% formaldehyde solution, known as formalin, or as a solid, paraformaldehyde, that may be melted to release the gas. Formaldehyde gas is very active against a variety of microorganisms and is used for space decontamination and to decontaminate biological safety cabinets. Formaldehyde dissolved in water is active at 1-8% solutions and can be used to decontaminate hard surfaces. However, because formaldehyde is very irritating at low concentrations (0.1 to 5 ppm) and a known carcinogen, its use as a hard surface disinfectant is limited to situations in which it is particularly needed. Due to its toxic effects, there are no EPA-registered disinfectants that contain formaldehyde.

Glutaraldehyde

Glutaraldehyde is usually supplied as a 20% solution and requires activation by the addition of an alkaline agent prior to use. The activated product may be kept for about two weeks and should be discarded when turbid. Glutaraldehyde is active against almost all microorganisms, but is toxic, irritating, and mutagenic and should be used only when necessary. Please follow the manufacturer's guidance when using glutaraldehyde-based products because there are many different formulations that have been designed for specific uses.

Hydrogen peroxide

Hydrogen peroxide is usually available as a 30% solution. It may be diluted 1:5 for use as a disinfectant. It is active against a wide array of microorganisms. However, it is an oxidizing agent and should not be used on aluminum, copper, zinc, or brass. Hydrogen peroxide is unstable at high temperatures and in light.

Iodine and Iodophors

Iodine and iodophors, compounds in which the iodine is combined with a solubilizing or carrier agent, are general, all-purpose disinfectants with an action similar to that of chlorine products. The appropriate concentration for iodine-containing products is 75 ppm available iodine for disinfecting work surfaces. Concentrations may be much higher for other purposes. Like chlorine compounds, the effectiveness of iodine compounds may be diminished in the presence of protein/organic material. Iodophor compounds that are used for antisepsis (germicide applied to tissue or skin) are not appropriate for use as hard surface disinfectants and vice versa. Please read the product material for appropriate dilutions and applications.

Phenol and phenolic compounds

Phenolic compounds are active at 0.2 to 3% concentrations against all forms of vegetative microorganisms but not against spores. They have only limited effectiveness against nonlipid viruses. There are many common disinfectants based on phenol and they should be used

according to the manufacturer's recommendations. Phenol is a hazardous chemical and requires hazard assessment as well as proper PPE selection and use.

Quaternary ammonium compounds

Compounds in this class are active at concentrations of 0.1 - 2%. They are active against vegetative bacteria, lipid viruses, but not against bacterial spores, non-lipid viruses, or tubercle bacilli. These compounds should be used only when a low-level disinfectant is required.

Table 11: Summary and Comparison of Liquid Disinfectants (Table 1 of 2)

Commonly used disinfectants, recommended when appropriate.

Class	Recommended Use	How They Work	Advantages	Disadvantages	Comments/Hazards	Examples
70% Isopropyl alcohol solution	• Cleaning some instruments • Cleaning skin	• Changes protein structure of microorganism • Presence of water assists with killing action	• Fairly inexpensive	• <50% solution not very effective • Not active when organic matter present • Not active against certain types of viruses • Evaporates quickly - contact time not sufficient for killing	•Flammable •Eye Irritant •Toxic	
Chlorine compounds	• Spills of human body fluids • Bactericidal - Good • Fungicidal - Good • Sporicidal - Good at >1000 ppm Sodium Hypochlorite	• Free available chlorine combines with contents within microorganism - reaction byproducts cause its death • Need 500 to 5000 ppm • Produce chemical combination with cell substances • Depend upon release of hypochlorous acid	• Kill hardy viruses (e.g., hepatitis) • Kill a wide range of organisms • Inexpensive • Penetrates well • Relatively quick microbial kill • May be used on food prep surfaces	• Corrode metals such as stainless, aluminum • Organics may reduce activity • Increase in alkalinity decreases bactericidal property • Unpleasant taste and odor • Tuberculocidal - with extended contact time	• Follow spill procedure and dilution instructions • Make fresh solutions before use • Eye, skin, and respiratory irritant •Corrosive •Toxic	• Bleach solutions (sodium hypochlorite) • Clorox • Cryosan • Purex
Gluteraldehyde	• Bactericidal - Good • Fungicidal - Good • Tuberculocidal - Excellent • Virucidal - Good • Sporicidal - Good	• Coagulates cellular proteins	• Non-staining, relatively non-corrosive • Useable as a sterilant on plastics, rubber, lenses, stainless steel, and other items that can't be autoclaved	• Not stable in solution • Has to be in alkaline solution • Inactivated by organic material	•Eye, skin and respiratory irritant. •Sensitizer •Toxic	• Calgocide 14 • Cidex • Vespore

Table 12: Summary and Comparison of Liquid Disinfectants (Table 2 of 2)

Class	Recommended Use	How They Work	Advantages	Disadvantages	Comments/Hazards	Examples
Iodophors (iodine with carrier)	• Disinfecting some semi-critical medical equipment • Bactericidal - Very Good • Fungicidal - Excellent • Virucidal - Excellent	• Free iodine enters microorganism and binds with its cellular components • Carrier helps penetrate soil/fat • Need 30 to 50 ppm • Probably by disorder of protein synthesis due to hindrance and/or blocking of hydrogen bonding	• Kill broad range of organisms • Highly reactive • Low tissue toxicity • Kill immediately rather than by prolonged period of stasis • Not affected by hard water • May be used on food prep surfaces	• May stain plastics or corrode metal • May stain skin/laundry • Stains most materials • Odor • Some organic and inorganic substances neutralize effect • Tuberculocidal - with extended contact time • Sporicidal - some	• Dilution critical - follow directions! • Use only EPA-registered hard surface iodophor disinfectants • Don't confuse skin antiseptic iodophors for disinfectants •Skin and eye irritant •Corrosive •Toxic	• Bactergent • Hy-Sine • Ioprep • Providone-iodine; betadine • Wescodyne
Phenolic Compounds	• Bactericidal - Excellent • Fungicidal - Excellent • Tuberculocidal - Excellent • Virucidal - Excellent	• Gross protoplasmic poison • Disrupts cell walls • Precipitates cell proteins • Low concentrations inactivate essential enzyme systems	• Nonspecific concerning bactericidal and fungicidal action • When boiling water would cause rusting, the presence of phenolic substances produces an anti-rusting effect	• Unpleasant odor • Some areas have disposal restrictions • Effectiveness reduced by alkaline pH, natural soap, or organic material • Sporicidal - NO	•Skin and eye irritant •Sensitizer •Corrosive •Toxic	• Hil-Phene • Lph • Metar • Vesphene
Quaternary ammonium compounds (QUATS)	• Ordinary housekeeping (e.g., floors, furniture, walls) • Bactericidal - Excellent • Fungicidal - Good • Virucidal - Good (not as effective as phenols)	• Affect proteins and cell membrane of microorganism • Release nitrogen and phosphorous from cells	• Contain a detergent to help loosen soil • Rapid action • Colorless, odorless • Non-toxic, less corrosive • Highly stable • May be used on food prep surfaces	• Do not eliminate spores, TB bacteria, some viruses • Effectiveness influenced by hard water • Layer of soap interferes with action	• Select from EPA list of hospital disinfectants •Skin and eye irritant •Toxic	• Coverage 258 • End-Bac • Hi Tor

Table 13: Summary of Practical Disinfectants

	Quatenary Ammonium Compounds	Phenolic Compounds	Chlorine Compounds	Iodophor	Ethyl Alcohol	Isopropyl Alcohol	Formaldehyde	Glutaraldehyde
Inactivates								
Vegetative Bacteria	+	+	+	+	+	+	+	+
Lipoviruses	+	+[a]	+	+	+[a]	+	+	+
Nonlipid Viruses	-	-	+	+	-	-	+	+
Bacterial Spores	-	-	+	+	-	-	+	+
Treatment Requirements								
Use Dilution	0.1-2.0%	1.0-5.0%	500ppm[b]	25-1600ppm[b]	70-85%	70-85%	0.2-0.8%	2%
Contact Time-minutes								
Lipovirus	10	10	10	10	10	10	10	10
Broad Spectrum	NE	NE	30	30	NE	NE	30	30
Important Characteristics								
Effective Shelf Life > 1 week[c]	+	+	-	+	+	+	+	+
Corrosive	-	+	+	+	-	-	-	-
Flammable	-	-	-	-	+	+	-	-
Explosion Potential	-	-	-	-	-	-	-	-
Inactivated by organic matter	+	-	+	+	-	-	-	-
Skin Irritant	+	+	+	+	-	-	+	+
Eye Irritant	+	+	+	+	+	+	+	+
Respiratory Irritant	-	-	+	-	-	-	+	+
Toxic[d]	+	+	+	+	+	+	+	+
Applicability								
Waste Liquids	-	-	+	-	-	-	-	-
Dirty Glassware	+	+	+	+	+	+	+	+
Equipment, Surface Decontamination	+	+	+	+	+	+	+	+
Proprietary Products[e]	A-33, CDQ, End-Bac, Hl-Tor, Mikro-Quat	Hil-Phene, Metar, Mikro-Bac, O-Syl	Chloramine T, Clorox, Purex	Hy-Sine, Ioprep, Mikroklene, Wescodyne			Sterac	Cidex

Source: Adapted from Laboratory Safety Monograph, A Supplement to the NIH Guidelines for Recombinant DNA Research. National Institutes of Health, Office of Research Safety, National Cancer Institute, and the Special Committee of Safety and Health Experts, Bethesda, Maryland. January 1989: 104-105

\+ Yes
\- No
NE Not Effective

[a] Variable results depending on virus
[b] Available Halogen
[c] Protected from light and air
[d] By skin or mouth or both
[e] Space limitations preclude listing all products available. Individual listings (or omissions) do not imply endorsement (or rejection) of any product by the National Institutes of Health.

Vapors and Gases

A variety of vapors and gases possess germicidal properties. The most commonly used are formaldehyde and ethylene oxide. Applied in closed systems under controlled conditions (e.g., humidity) these gases achieve sterility.

Formaldehyde gas is primarily used in the decontamination of spaces or biological containment equipment like biological safety cabinets. Formaldehyde is a toxic substance and a suspected human carcinogen. Considerable caution must be exercised in handling, storing, and using formaldehyde. Ethylene oxide is used in gas sterilizers under controlled conditions. Ethylene oxide is also a human carcinogen and monitoring is necessary during its use.

Radiation

Gamma and X-ray are two principal types of ionizing radiation used in sterilization. Their application is mainly centered on the sterilization of prepackaged medical devices. Ultraviolet (UV) radiation is a practical method for inactivating viruses, mycoplasma, bacteria and fungi. UV radiation is successfully used in the destruction of airborne microorganisms. The sterilizing capabilities of UV light, such as that found in biosafety cabinets, are limited on surfaces because of its lack of penetrating power.

Federal Regulation of Disinfectants

The Food and Drug Adminstration (FDA) regulates those products that are marketed as sterilants or sanitizing agents on medical devices. They have published a list of products currently on the market that are labeled as sterilants.

The Environmental Protection Agency (EPA) regulates pesticides, including chemical disinfectants, under the Federal Insecticide, Fungicide, and Rodenticide Act. They are required to be registered with the EPA. It is important to follow the directions on the manufacturer's label, including those for concentration and contact time, when using disinfectants to ensure compliance with the EPA requirements. The following is a link to the U.S. EPA website for Selected EPA-registered disinfectants: https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants

Because disinfectants are antimicrobial, they may, by their nature, also have a toxic effect to the user. Therefore, Safety Data Sheets (SDSs) and other manufacturer's product information should be available and thoroughly reviewed before using these products.

Please contact the Biological Safety Office for information about any of these lists or for a list of manufacturers. Be aware that most disinfectants assume pre-cleaning to remove gross organic/protein prior to use. Whenever a disinfectant or sterilant is used, proper safety precautions must be followed. Appropriate clothing (gloves, safety goggles, aprons) must be worn. In addition, these compounds must be used in well-ventilated areas.

Biohazardous Waste Management

At BSU, the term **biohazardous waste** is used to describe different types of waste that might include infectious agents. Currently, the following waste categories are all considered to be biohazardous waste:

1. **Medical waste:** Defined as any solid waste which is generated in the diagnosis, treatment (e.g., provision of medical services), or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biologicals, as well as all categories defined by Indiana regulations. Medical waste includes:

    a. Cultures and stocks of infectious agents and associated biologicals, including laboratory waste, biological production waste, discarded live and attenuated vaccines, culture dishes, and related devices.
    b. Liquid human and animal waste, including blood and blood products and body fluids, but not including urine or materials stained with blood or body fluids.
    c. Pathological waste: defined as human organs, tissues, body parts other than teeth, products of conception, and fluids removed by trauma or during surgery or autopsy or other medical procedure, and not fixed in formaldehyde.
    d. Sharps: Defined as needles, syringes, scalpels, and intravenous tubing with

needles attached regardless of whether they are contaminated or not.

e. Contaminated wastes from animals that have been exposed to agents infectious to humans, these being primarily research animals.

2. **Regulated waste** as defined by the *Occupational Safety and Health Act on Bloodborne Infectious Diseases* (IOSHA) including:

   a. Liquid or semi-liquid blood or other potentially infectious materials;
   b. Contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state if compressed;
   c. Items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling;
   d. Contaminated sharps which includes any contaminated object that can penetrate the skin;
   e. Pathological and microbiological wastes containing blood or other potentially infectious materials.

4. **Laboratory waste and regulated waste** as defined in the *Guidelines For Research Involving Recombinant DNA Molecules* (NIH) and the *CDC/NIH Biosafety in Microbiological and Biomedical Laboratories*. The CDC/NIH Biosafety Guidelines cover contaminated waste that is potentially infectious or hazardous for humans and animals. The same is true for the NIH Guidelines on recombinant DNA which also cover contaminated waste potentially infectious or hazardous for plants.

5. **Inactivation of Waste Containing Biologically-derived Toxins:** Although biologically-derived toxins are often considered hazardous chemicals, they will be addressed in this manual because of their frequent use in biomedical research. Equipment and apparatus contaminated with toxins and waste generated from procedures involving toxins must undergo decontamination to inactivate the toxins. Waste contaminated with toxins may be autoclaved or chemically deactivated via sodium hypochlorite or a mixture of sodium hypochlorite and sodium hydroxide prior to disposal.

   Table 14 lists effective methods for inactivating or denaturing toxins. In general, high molecular weight, proteinacious bacterial toxins are inactivated by steam sterilization (autoclaving 1 hour at 121°C), whereas low molecular weight toxins (e.g., mycotoxins, marine and reptile venoms) are more effectively inactivated by treatment with sodium hypochlorite (NaOCl) or a mixture of sodium hypochlorite and sodium hydroxide

Table 14. Inactivation of Biologically-derived Toxins

Toxin	Autoclave at 121º C for 1 hr.	2.5% NaOCl for 1 hr.	2.5% NaOCl 0.25N NaOH for 1 hr.	2.5% NaOCl 0.25N NaOH for 4 hr.
Abrin	Yes	NO	NO	NO
Botulinum neurotoxins	Yes	Yes	Yes	Yes
***Clostridium perfringenes* epsilon toxin**	Yes	Yes	Yes	Yes
Diacetoxyscirpenol (DAS), T-2	NO	NO	NO	Yes
Palytoxin	NO	Yes	Yes	Yes
Ricin	Yes	Yes	Yes	Yes
Saxitoxin	NO	Yes	Yes	Yes
Shigatoxin and Shiga-like toxins	Yes	Yes	Yes	Yes
Staphylococcal enterotoxins	Yes	Yes	Yes	Yes
Tetrodotoxin	NO	NO	Yes	Yes

General Labeling, Packaging and Disposal Procedures

The responsibility for decontamination and proper disposal of biohazardous waste lies with the producing facility (e.g., laboratory and department). The EHS Office assists only in the disposal of sharps and biohazardous wastes, including animal carcasses.

All biohazardous waste needs to be packaged, contained and located in a way that protects and prevents the waste from release at any time at the producing facility prior to ultimate disposal. If storage is necessary, putrefaction and the release of infectious agents into the air must be prevented.

If not stated otherwise (see below), most biohazardous waste from BSL facilities will be autoclaved prior to disposal in biohazard bags. Unless to be placed in a secondary outer biohazard bag, BSU requires the use of biohazard bags that include the biohazard symbol and a built-in heat indicator with the word ("AUTOCLAVED"). Bags that meet these requirements are available through numerous medical supply houses and vendors. All waste disposed of in these bags is to be autoclaved in an approved autoclave until the waste is decontaminated. The built-in heat indicator will turn dark. All autoclaves used for the decontamination of biohazardous waste will be tested by the EHS at least on an annual basis. After successful autoclaving (decontamination), unless to be placed in a secondary outer biohazard bag for disposal as biohazardous waste, all autoclave bags need to be bagged in opaque (black) plastic non-biohazard bags that are leakproof. These opaque bags can be put in a secure dumpster, roll-off container, or be picked up by custodial services. Biohazardous waste that has been successfully decontaminated by autoclaving is no longer considered hazardous, although the generator is free to handle it as such regardless.

Since autoclaves are an integral part of BSU's biohazardous waste treatment procedure, proper operation and maintenance is very important. All users of autoclaves need to be trained in the proper operating procedures either through the laboratory supervisor or Principal Investigator or whoever was put in charge by the department. Maintenance and repair of autoclaves used for the decontamination of biohazardous waste are the responsibility of the individual departments. If the department chooses to not use autoclaves for their biohazardous waste treatment, all wastes must be placed into properly labeled red biohazard bags, preferably inside labeled biohazard boxes or containers for storage, pickup and disposal by EHS for interim storage or directly on a schedule with the BSU biohazardous waste vendor.

Biohazard Box and 5 Gallon Biohazard Pail

Waste Specific Procedures for BSL-1 and 2

Cultures, Stocks and Related Materials

Cultures and stocks of infectious agents and associated biologicals (as defined above), shall be placed in biohazard bags and decontaminated by autoclaving. Double or triple bagging may be required to avoid rupture or puncture of the bags.

Bulk Liquid Waste, Blood and Blood Products

All liquid waste from humans or animals such as blood, blood products and certain body fluids, not known to contain infectious agents, can be disposed of directly by flushing down a sanitary sewer. However, due to coagulation, flushing of large quantities of blood is impractical. Contact the EHS for additional information on disposal of large volumes of blood. All other liquid biohazardous waste needs to be autoclaved prior to disposal or treated with a disinfectant.

Sharps

All sharps must be placed in a rigid, puncture resistant, closable and leak-proof container, which is labeled with the word **"Sharps"** and the biohazard symbol. Approved sharps containers, of assorted sizes, are normally available through the EHS Office. Food containers (e.g., empty coffee cans) and other containers are **not permissible** as sharps containers. All sharps must be handled with extreme caution and that is a key requirement for all BSL laboratories. The clipping, breaking, and recapping of needles is not to be performed. Sharps containers should not be filled more than ¾ full. Contact the EHS for pick-up as soon as possible. **Never place any type of sharps in the BSU waste dumpsters or roll-off containers.**

Contaminated Solid Waste

Contaminated solid waste includes cloth, plastic and paper items that have been exposed to agents that may be infectious or hazardous to humans, animals, or plants. These contaminated items shall be placed in biohazard bags and decontaminated by autoclaving. Double or triple bagging may be required to avoid rupture or puncture of the bags. **Contaminated Pasteur pipettes are considered sharps** and need to be disposed of in a sharps container.

Waste Specific Procedures for Biosafety Level 3 (BSL-3)

Any biohazardous waste including RG-2 and 3 agents that are handled at BSL-3 is to be autoclaved at the point of origin (laboratory, or facility). Transportation of non-autoclaved BSL-3 waste outside of the building is generally not permitted. Exceptions might include animal carcasses that need to be incinerated.

Pathological Waste

The EHS office, or IU-Ball Memorial Hospital (for BSU medical education), provides removal, transportation and disposal services for University units that generate pathological waste. Pathological waste consists of human organs, tissues, body parts other than teeth, products of conception, and fluids removed by trauma or during surgery or autopsy or other medical procedure, and not fixed in formaldehyde. At BSU, animal carcasses are also considered pathological waste. Although not all pathological waste is infectious, it is prudent to handle such waste as if it were because of the possibility of unknown infection in the source. All human pathological waste is also covered by *Universal Precautions* according to the OSHA *Bloodborne Pathogen Standard*. For more information on this subject, refer to BSU's *Exposure Control Plan*. Typically, carcasses or tissues are collected in plastic bags, labeled, stored in area freezers, cold rooms or refrigerators and removed for off-site treatment and disposal by a vendor contracted by EHS. Several department have routine pickups by the university's biohazardous waste vendor. For non-scheduled pickups, contact the EHS Office.

Animal Waste

Collect animal carcasses, tissues, or bedding in non-transparent, 4-6 mil plastic bags.

Small animal carcasses may be individually bagged and collected together in a larger leak-proof container. For small animals, do not exceed 35 pounds total weight per bag. Large animals shall be securely packaged in large plastic bags. Bind any limbs or sharp protrusions so they will not puncture the bag. Leaky or punctured bags will not be picked up.

Attach a BSU Materials Pickup Tag (see BSU's *Waste Disposal Guide*) to each individual container or bag to be removed. Tags are available through General Stores or EHS. Tags must be completely filled out or the waste will not be removed. Affix tags to the waste container(s) or bag(s). Attach the tags so they will not fall off during transportation and storage. Tags should not be permanently cemented or excessively taped as this prevents the tag from being removed for record keeping purposes.

If the waste contains known viable pathogens e.g., the animal had an infectious zoonotic disease or was inoculated with a known pathogen, enter the name of the biohazardous agent on the waste tag and attach a biohazard sticker to the container. Alternatively, put the opaque plastic bag inside a biohazard bag. If no known viable pathogens are present, mark the waste as noninfectious on the waste tag.

Store carcasses in a freezer or cold storage area. Do not mix pathological wastes contaminated with hazardous chemicals or radioisotopes with uncontaminated waste. Pathological wastes containing radioactive materials shall also be labeled with a radioactive waste tag for pick-up by the Radiation Safety Officer or EHS.

Department or Facility Specific Waste Procedures
If required, departments or facilities may establish biohazardous waste procedures that are more stringent than the above listed procedures. A written copy of these procedures should be made available to EHS prior to initiation.

Biohazard Spill Clean-Up Procedures

A spill of biological materials that takes place in the open laboratory may create a serious problem. Every effort should be taken to prevent spilling materials.

Laboratory personnel are not required to respond to a spill. An individual who is uncomfortable responding to a spill should contact the BSO or EHS. If a spill poses imminent danger to health and safety and cannot be isolated or contained, evacuate the area and contact University Police by dialing 911 and provide the following information:

1. Name and telephone number of the caller.
2. Location of the emergency (building name, room number, and building specific address, if known).
3. Nature of the emergency (e.g., agent or material involved, fire, injuries).
4. Special considerations (e.g., inhalation hazards present, potential for explosion, people trapped in rooms or buildings, number of people injured and type of injuries, electrical hazards, property damage, and access routes to the emergency).

A spill poses less of a problem if it happens inside a biological safety cabinet, provided splattering to the outside of the cabinet does not occur. Direct application of concentrated liquid disinfectant and a thorough wipe down of the internal surfaces of the cabinet will usually be effective for decontaminating the work zone, but gaseous sterilants may be required to disinfect the interior sections of the cabinet and HEPA filter(s).

If potentially hazardous biological material is spilled in the laboratory, avoid inhaling any airborne material by holding your breath and leaving the laboratory. Warn others in the area by posting signs and go directly to wash or change room area. If clothing is known (or suspected) to be contaminated, remove the clothing with care, folding the contaminated area inward. Discard the clothing into a bag or place the clothing directly in an autoclave. Wash all potentially contaminated body areas as well as the arms, face and hands. Shower if facilities are available. Re-entry into the laboratory should be delayed for a period of at least thirty minutes to allow reduction of the aerosol generated by the spill.

Protective clothing should be worn when entering the laboratory to clean the spill area. Rubber gloves, autoclavable or disposable footwear, an outer garment, and a respirator equipped with a HEPA filter should be worn. If the spill was on the floor, do not use a surgical gown that may trail on the floor when bending down. Take the " spill kit" into the laboratory room, place a discard container near the spill, and transfer large fragments of material into it; replace the cover. Using a freshly prepared hypochlorite containing 5000 ppm (10% by volume) available chlorine, iodophor solution containing 1600 ppm iodine, or other appropriate EPA registered disinfectant, carefully pour the disinfectant around and into the visible spill. Avoid splashing and work from the outside toward the center. Allow 30 minutes' contact time. Use paper or cloth towels to wipe up the disinfectant and spill, working toward the center of the spill. Discard all towels and other clean up materials into a discard container as they are used. Wipe the outside of the discard containers, especially the bottom, with a towel soaked in a disinfectant. Place the discard container and other materials in an autoclave and sterilize. Alternately, place all materials in the appropriate biomedical waste system for incarceration. Remove shoes or shoe covers, outer clothing, respirator, and gloves and sterilize by autoclaving. Wash hands, arms and face, or if possible, shower.

Since spills of biological materials will inevitably happen, it is important to be prepared prior to dealing with the problem. All personnel working in a laboratory should have received training in the response and cleanup procedures for minor spills, releases, or exposures. Laboratories working with biohazards should have a basic biological spill kit ready to use at all times. For most instances the basic kit can be assembled with materials already used in the laboratory. All labs operating at BSL-2 or higher must have an assembled spill kit available in the lab. In BSL-1 labs, although it is preferable to have the contents of the spill kit in one location, as long as the materials are easily accessible to everyone in the lab, or at a known nearby location, prior assembly might not be necessary.

The following is a list of items that should go into a basic biological spill kit. It should be enhanced to meet the needs of your unique situation. For example, if formalin or glutaraldehyde is used in the laboratory, a formaldehyde or glutaraldehyde absorbent should be included in the kit.

Basic Biological Spill Kit Contents:

- Disinfectant (e.g., bleach 1:10 dilution, prepared fresh)
- Spray bottle
- Absorbent material (e.g., paper towels, absorbent pads or powder)
- Leakproof waste container
- Personal protective equipment (e.g., protective gown, gloves, eye and face protection)
- Mechanical tools (e.g., tongs, dustpan and broom)
- Antimicrobial towelettes
- Red bag / sharps container
- Tongs
- Warning sign and/or barricade tape
- Spill cleanup procedures
- Personal protective equipment (e.g., gloves, eye and face protection)

The following procedures are provided as a guideline to biohazardous spill clean-up and will need to be modified for specific situations. As with any emergency situation, stay calm, call 911 if necessary, and proceed with common sense. Call EHS at 285-2807 if cleanup assistance is required, especially if the spill outgrows the resources in the laboratory. Only persons who are trained, equipped with suitable PPE, and provided with the necessary response and cleanup materials should attempt to clean up a spill or release of biohazardous materials.

Spill kit maintenance:

Your biological spill kit should be restocked after each use. It should also be checked for completeness on an annual basis. The following maintenance activities should be done:

- Check expiration on disinfectant and replace as needed (e.g., bleach should be replaced annually and the container discarded after opening);
- Replace gloves;
- Replace antimicrobial towelettes;
- Check integrity of protective gown; and,
- Check straps on splash goggles for deterioration.

Spills inside the Laboratory

Clear spill area of all personnel. Wait for any aerosols to settle before entering spill area. Remove any contaminated clothing and place in biohazard bag for disposal of further processing by laundry (department). Have a complete *Biological Spill Kit* ready to go before you start the clean-up.

Spills with NO broken glass/sharps:

1. Remove spill supplies from container and line the container with a biohazard bag.
2. Put on two layers of gloves. Put on splash goggles.
3. Prepare the disinfectant solution, following the manufacturer's recommendations for

concentration.

4. Cover the spill area with absorbent material (i.e., absorbent pads or paper towels).
5. Using the broom and dustpan, remove absorbent powder and deposit it in the biohazard bag, or if using paper towels, place them in the biohazard bag for disposal.
6. Spray the contaminated area with disinfectant and wait the appropriate contact time (10 minutes for bleach solution). Remove disinfectant with paper towels and place the paper towels in the biohazard bag for disposal.
7. Repeat step 6 to allow for sufficient disinfection of contaminated surfaces.
8. Remove outer pair of gloves only and dispose of them in the biohazard bag.
9. Remove goggles with inner gloves still on, and clean the goggles with an antimicrobial towelette. Also wipe down contact surfaces of disinfectant container.
10. Remove inner gloves and dispose of them in biohazard bag.
11. Place the biohazard bag in a biohazardous waste container for treatment and disposal.
12. Wash your hands with soap and water as soon as possible.
13. Restock the kit for next use.

Spills involving broken glass/sharps:

1. Remove spill supplies from container and line the container with a biohazard bag. Retrieve a sharps container for disposal of glass/sharps.
2. Put on two layers of gloves. Put on splash goggles.
3. Prepare the disinfectant solution, following the manufacturer's recommendations for concentration.
4. Using tongs or forceps, place broken glass/sharps in sharps container.
5. Cover the spill area with absorbent powder.
6. Using the broom and dustpan, remove absorbent powder and deposit it in the biohazard bag.
7. Spray the contaminated area with disinfectant and wait the appropriate contact time. Remove disinfectant with paper towels and place the paper towels in the biohazard bag for disposal.
8. Repeat step 7 to allow for sufficient disinfection of contaminated surfaces.
9. Remove outer pair of gloves only and dispose of them in the biohazard bag.
10. Remove goggles with inner gloves still on, and clean the goggles with an antimicrobial towelette. Also wipe down contact surfaces of disinfectant container.
11. Remove inner gloves and dispose of them in biohazard bag.
12. Place the biohazard bag in a biohazardous waste container for treatment and disposal.
13. Wash your hands with soap and water as soon as possible.
14. Restock the kit for next use.

Spills inside the Biological Safety Cabinet

A spill that is confined to the interior of the biological safety cabinet should present little or no hazard to personnel in the area. However, chemical disinfection procedures should be initiated at once **while the cabinet ventilation system continues to operate** to prevent escape of contaminants from the cabinet.

Have a complete biological spill kit ready to go before you start the clean-up.

- Wear labcoat, safety goggles and gloves during clean-up.
- Allow cabinet to run during clean-up.
- Soak up spilled material with paper towels (work surface and drain basin) and apply disinfectant using the manufacturer's recommended concentration and contact time.
- Wipe up spillage and disinfectant with disposable paper towels.
- Wipe the walls, work surface and any equipment in the cabinet with a disinfectant soaked paper towel.
- Discard contaminated disposable materials in biohazard bag(s) and autoclave before discarding as waste.
- Place contaminated reusable items in biohazard bags, or heat resistant pans or containers with lids before autoclaving and further clean-up.
- Spray or wipe walls, work surfaces, and equipment with a disinfectant. A disinfectant with a detergent has the advantage of detergent activity that will help clean the surfaces by removing both dirt and microorganisms. A suitable disinfectant is a 3% solution of an iodophor such as Wescondyne or a 1:100 dilution of household bleach (e.g. Chlorox) with 0.7% nonionic detergent. The operator should wear gloves during this procedure. Use sufficient disinfectant solution to ensure that the drain pans and catch basins below the work surface contain the disinfectant. Lift the front exhaust grill and tray and wipe all surfaces. Wipe the catch basin and drain the disinfectant into a container.
- The disinfectant, gloves, wiping cloth and sponges should be discarded into an autoclave pan and autoclaved.
- Remove protective clothing used during cleanup and place in a biohazard bag for further autoclaving or disposal using a red biohazard bag.
- Run cabinet at least 10 minutes after clean-up and before resuming work.
- Inform all users of the BSC as well as the laboratory supervisor about the spill and clean-up as soon as possible.

The above procedure will not disinfect the filters, blower, air ducts, or other interior parts of the cabinet. If the entire interior of the cabinet needs to be sterilized, contact the EHS Office.

Spills inside a Centrifuge

Have a complete biological spill kit ready to go before you start the clean-up.

- Clear area of all personnel. Wait 30 minutes for aerosols to settle before attempting to clean up the spill.
- Wear a lab coat or protective gown, safety goggles and gloves during clean-up.
- Remove rotor and place it in the biosafety cabinet.
- Open rotor, remove tubes using tongs or forceps.
- Disinfect the rotor with an appropriate chemical disinfectant and contact time.
- Dry the rotor thoroughly after disinfection.
- Cover the bottom of the centrifuge with disinfectant-soaked towels. Concentrated

disinfectant should be used. Allow at least a 20-minute contact time.

- Wipe the inside of the centrifuge and the lid with an appropriate disinfectant. Dry the inside of the centrifuge thoroughly.
- Remove contaminated debris after disinfection, place in appropriate biohazard waste container(s) and/or autoclave before disposal.

Spills during Transport

If a spill occurs in a public area:

- Don't attempt cleanup without the proper supplies.
- Contact EHS (285-2807) for assistance.

If a spill occurs in a vehicle:

- Leave the vehicle with closed windows and locked doors.
- Contact EHS (285-2807) for assistance.

Biohazard Exposure

In the event of a personal exposure, an individual's primary concern must be to minimize the degree of exposure and the possible effects. The emergency procedures employed depend on the type of biohazardous material to which the individual was exposed and the extent of exposure. Each laboratory where biological materials are used or stored should have a *Laboratory-specific Biosafety Plan* that outlines specific procedures to be followed in the event of an exposure to biohazards present in their laboratory. Immediate emergency response procedures for inhalation or skin exposure incidents are provided below.

In general, laboratory personnel who have experienced an exposure should immediately:

1. Decontaminate themselves;
2. Call 911 for medical assistance or report to the closest medical facility, when needed;
3. Notify the PI or lab supervisor and BSO; and,
4. Submit a *First Report of Accident Form* to the Workers' Compensation department within Human Resources and Payroll.

Medical care as a result of work-related exposure may be provided at no cost to the employee and is dependent on the type of exposure. Students who have been exposed should report to Student Health Services. In the event that Student Health Services is closed, seek medical attention at the closest medical facility.

Facilities closest to the Ball State University campus are listed at the front of this Manual under **Emergency Contact Information**.

Inhalation Exposure

Follow the steps below when there is a potential for inhalation exposure:

1) Stop breathing in order to avoid inhaling airborne material, and quickly leave the room.
2) Signal to others to leave, close the door, and post a warning sign. No one should enter the laboratory for at least 30 minutes.
3) Go to a support space or adjacent laboratory. Avoid the hallway and publicly-accessed areas.
4) Remove contaminated PPE and clothing, turning exposed areas inward, and place in a biohazard bag.
5) Wash all exposed skin and hands with antiseptic soap and warm water for 15 minutes. Wash gently so as not to break the skin.
6) Call 911 for medical assistance or report to the closest medical facility, when needed.
7) Immediately notify PI/LS and EHS. Notify EHS who must clear the laboratory for reentry. If EHS is not available or it is after normal business hours, contact University Police.
8) If a BSU employee, complete and submit a *First Report of Accident Form* to the Workers' Compensation department within Human Resources.

Skin or Mucous Membrane Exposure

Skin or mucous membrane exposure can occur through splashes to the eye, face, exposed skin, or clothing; by touching mucous membranes with contaminated hands; or from a needle-stick, puncture with a contaminated sharp object, an animal scratch or bite, or through wounds, abrasions, and eczema. In the event of a skin or mucous membrane exposure:

1) Remove contaminated PPE and clothing, turning exposed areas inward, and place in a biohazard bag.
2) For mucous membrane exposure, flush the affected area with the eyewash for at least 15 minutes.
3) For skin exposure, wash the affected skin with antiseptic soap and warm water for at least 15 minutes. Wash gently so as not to break the skin.
4) Call 911 for medical assistance or report to the closest medical facility, when needed. Immediately notify PI/LS and EHS. Notify EHS who must clear the laboratory for reentry. If EHS is not available or it is after normal business hours, contact University Police.
5) Immediately notify PI/LS and EHS. Notify EHS who must clear the laboratory for reentry. If EHS is not available or it is after normal business hours, contact University Police.
6) Complete and submit a *First Report of Accident Form* to the Workers' Compensation Department within Human Resources.

Transport of Biological Substances on Campus

Biological materials can be safely transported both within and between buildings on campus when they are appropriately packaged, labeled, and transported in a manner that minimizes the potential for environmental release.

It is recognized that the transport of biological materials may be necessary between laboratory spaces sharing equipment (BSCs, autoclaves, etc.), though this should be minimized to the extent practicable. The following precautions are required for movement of RG-2 or higher biohazardous

materials:

Transport Between Laboratories

1. Use primary containers that are designed to contain the material to be stored. Do not use food containers or other containers not originally designed for laboratory storage purposes;
2. Place primary sample containers into an appropriate secondary container for transport. If sample material is liquid or may release liquids, use a leak-proof secondary container with a secure lid (i.e. cooler with a latchable lid). Additionally, place enough absorbent material (i.e. paper towels) in the secondary container to absorb all free liquids in the event that primary containers rupture or break during transport;
3. Affix biohazard labels on the exterior of the primary or secondary container;
4. Either utilize a lab cart for the transportation, or use a "buddy system", for the movement of the biohazardous material between laboratories with one person preceding the material carrier for the trip and opening and closing doors en route to the destination.

Transport Between Buildings

The following procedure for preparing and transporting biological materials between university buildings should be used:

1. Use primary containers that are designed to contain the material to be stored. Do not use food containers or other containers not originally designed for laboratory storage purposes.
2. Place primary sample containers into an appropriate secondary container for transport. If sample material is liquid or may release liquids, use a leakproof secondary container with a secure lid (i.e. cooler with a latchable lid). Additionally, place enough absorbent material (i.e. paper towels) in the secondary container to absorb all free liquids in the event that primary containers rupture or break during transport.
3. Package primary containers in the secondary container in a manner that will reduce shock, rupture, and/or breakage. Bubble wrap or similar shock-absorbing materials may also be used to minimize the potential for primary container rupture.
4. Label all secondary containers with a brief description of the contents and an emergency contact name and phone number. Containers used for transporting blood specimens (regardless of source) or specimens known or suspected to contain a pathogen should be additionally labeled with the biohazard symbol.

5. Either utilize a lab cart for the transportation, or use a “buddy system”, for the movement of the biohazardous material between buildings with one person preceding the material carrier for the trip and opening and closing doors en route to the destination.

6. A University-owned vehicle must be used for transport. Store and secure the transport container in a location in the vehicle whereby if an accident were to occur, the container or its contents will not be an exposure risk to the driver or to the environment. For example, in transporting materials by car or van, store the container in the back seat or cargo bay. Secure the container with bungee cords or belts to keep the container upright and stable.
7. If a university vehicle is not available, the EHS Office should be contacted (285-2807) to assist in the transportation of the biohazardous material.

Shipping of Biological Materials to an Off Campus Destination

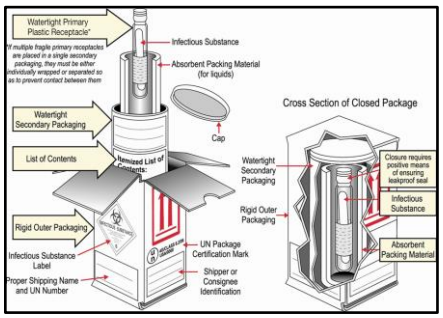
Transportation of biological materials is an activity that affects all research and diagnostic service entities. In some instances, these materials may be regulated for transportation and will require specific packaging, labeling and documentation. Additionally, the shipper must have documented training relative to his or her tasks associated with the shipment. This is the case for shipment of diagnostic specimens (from humans or animals), cultures of infectious substances (infectious to humans and/or animals), genetically modified organisms and any biological materials shipped on dry ice. In light of recent current events, there is an increased level of surveillance on the part of federal and international authorities for all hazardous materials/dangerous goods shipments that may include diagnostic specimens and infectious substances. As a shipper, it is essential to ensure that materials are properly classified and that all applicable regulatory provisions for shipment are met.

EHS can often assist with training and consultation for campus personnel who plan to ship biological materials including: diagnostic specimens, infectious substances, genetically modified organisms, and biological materials on dry ice. These regulations require the proper identification, packaging, labeling, and completion of shipping papers for the material. International shipments require additional preparation and precautions. Persons involved in nearly all aspects of shipping hazardous materials or dangerous goods require training under either DOT or FAA regulations including those activities mentioned above which are necessary for the shipping and receiving of these materials.

The following regulations apply to the packaging and shipment of biological materials:

- U.S. Department of Transportation, 49 CFR Parts 171-180 and amendments
- U.S. Public Health Service, 42 CFR Part 72, *Interstate Shipment of Etiolgic Agents*
- U.S. Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 19 10.1030, *Bloodborne Pathogens*
- International Air Transport Association (IATA), *Dangerous Goods Regulations*

- U.S. Postal Service, 39 CFR Part 111, *Mailability of Etiologic Agents, Mailability of Sharps and Other Medical Devices*, and Publication 52, *Acceptance of Hazardous, Restricted or Perishable Matter*
- International Civil Aviation Organization*, Technical Instructions for the Safe Transport of*
- *Dangerous Goods by Air*
- United Nations*, Recommendations of the Committee of Experts on the Transportation of Dangerous Goods*



All North American airlines and FedEx, the largest shipper of infectious materials, use the IATA regulation (also referred to as the Dangerous Goods Regulation or DGR) as their standard. Meeting the conditions of this standard will ensure meeting the provisions of the other US regulations.

General information is provided below, but **please contact the EH&S Biological Safety Office for specific information**. Note that for any biological materials for which a state or federal permit or license is required, registration with the EH&S Biological Safety Office is also required.

Any BSU faculty/staff, regardless of job or task, must be trained to package, ship or receive packages of materials/samples classified by regulation as "dangerous goods". This training is occasionally offered by EHS, or on-online training is available through the U.S. DOT or private vendors. Regulations require retraining every other year.

Impact of non-compliance:

- Increased risk of material release during the shipping process.
- May result in refusal or return of packages during the shipping process. This could be critical if materials are temperature sensitive.
- May result in fines from the Federal Aviation Administration (FAA) or Department of Transportation (DOT).
- Criminal charges for deliberate violation of the regulations.

Preparing to Ship Biological Materials:

Before you package and ship materials to an off campus destination then, there are several items that should be checked or completed. These paperwork requirements can take several weeks to complete, therefore you should prepare well in advance for them.

Material Transfer Agreements

BSU Technologies requires that a Material Transfer Agreement be completed for materials entering or leaving campus. Before you send your shipment it is important that you contact BSU Technologies to ensure that the appropriate agreements are completed and processed.

Export Controls and Trade Sanctions

Export controls and trade sanctions are regulatory areas that may apply to you, depending on

your activity. Exports are any items (commodities, software, technology, select biological agents) sent from the United States to a foreign destination.

Export control laws may apply when one or more of the following concerns pertain to your research project:
It has actual or potential military applications, including dual use items (i.e., commercial items with potential military application)

- The destination country, organization, or individual is restricted by federal law
- The declared or suspected end use or the end user of the export compromises national security
- Economic protection issues are associated with the destination country

If you have questions about whether there are export controls issues associated with your activity, contact the Office of Research Integrity (285-5213) or view the BSU Export Controls Web Site: http://cms.bsu.edu/about/administrativeoffices/researchintegrity/export-control-compliance-program

Permits

The CDC, USDA, U.S. Fish and Wildlife Service and Department of Commerce require permits for shipping certain etiological agents and other materials.

FAQ: *Can I take my materials on the airplane with me (either in carried-on or checked baggage)?* The answer to this question is, it depends. It depends on the materials that you wish to take and if you have the proper paperwork in place. You **CANNOT** carry on or check biological materials if any of the following apply:

- The materials are classified as "dangerous goods;"
- Carriage of the materials is against rules established by the Transportation Security Administration (TSA);
- You do not have a completed material transfer agreement in place for the materials;
- Transport of the materials does not comply with export control and trade sanctions regulations; or
- Transport of the materials does not comply with Department of Transportation regulations.

When in doubt, PLEASE ASK!

For more information on biological materials shipping requirements, please contact the EHS Office at 285-2807.

Use of Animals in Research

The use of animals in research, teaching, and outreach activities is subject to state and federal laws and guidelines. University policy specifies that:

- All animals under University care will be treated humanely;
- Prior to their inception, all animal projects receive approval by the Institutional Animal Care and Use Committee (IACUC);

- BSU will comply with state and federal regulations regarding animal use and care.

Project directors are responsible for the humane treatment of animals under their supervision, and for adherence to applicable University, state, and federal regulations. Faculty members planning to use live vertebrate animals for any University-related activity must submit an animal use form (AUF) to the IACUC for review, or request an exemption from the Committee Chairperson and receive approval, prior to the start of the project, regardless of the source of funding for the project.

Additional information is available on the ORI website at:
http://cms.bsu.edu/about/administrativeoffices/researchintegrity/animalcareanduse

Use of Human Subjects and Materials in Research

Federal and University regulations and policies require that all research involving human subjects or materials be reviewed and approved before initiation by the University's Institutional Review Board (IRB) to protect the rights and welfare of human subjects.

Ball State University's IRB is the Human Subject Participation Program. Prescribed by the National Research Act of 1974 (PL 93-348) and endorsed by the Academic Council, the IRB reviews applications for research involving human subjects. Reviews are performed in accordance with the U.S. Department of Health and Human Services (HHS) regulations for the Protection of Human Research Subjects (45 CFR 46, as amended) as codified and extended by the University's formal Assurance to HHS: M-1239.

It is the responsibility of the Project Investigator to assure that all research involving human subjects is reviewed and approved by the IRB prior to initiation. All personnel with a reasonable anticipated risk of exposure to bloodborne pathogens through the contact with human blood or other human materials must be included in BSU's Bloodborne Pathogen Program.

Additional information is available on the ORI website at:
http://cms.bsu.edu/about/administrativeoffices/researchintegrity/humansubjects

Appendices

Appendix A	Definitions
Appendix B	Acronyms
Appendix C	Biosafety Resources
Appendix D	Toxin Table
Appendix E	Select Agents and Toxins
Appendix F	Risk Assessment Form
Appendix G	Laboratory-specific Biosafety Manual Contents Example
Appendix H	Examples of RG-2, RG-3, and RG-4 Agents
Appendix I	Equipment Release Form
Appendix J	Exposure Response Procedures
Appendix K	Example Inventory Log
Appendix L	Recommended Biosafety Practices - Prions
Appendix M	Requirements for Exempt Strains of Select Agents
Appendix N	Dual Use Chemicals
Appendix O	Site-specific Training Checklist
Appendix P	Registration Form
Appendix Q	Institutional Biosafety Committee (Section IV-B-2)

Appendix A. Definitions

Administrative controls: Work procedures, such as written safety policies, rules, supervision, and training, with the goal of reducing the duration, frequency, and severity of exposure to hazardous materials or situations.

Aerosol: Solid particles or liquid droplets, ranging in diameter from 0.01 to a few microns, suspended in a gaseous medium (e.g., air).

Animals: Any member of the animal kingdom except a human including an animal product (e.g., a mount, rug, or other display item composed of the hide, hair, skull, teeth, bones, or claws).

Arthropods: Any living insect including crustaceans, spiders, scorpions, etc. capable of being a host or vector of human disease.

Biohazards are microorganisms, microbial toxins, or other biological agents that can infect and/or cause disease in humans, animals, or plants. Biohazards include infectious agents of humans, animals and plants, toxins of biological origin, human-derived materials, recombinant DNA and any materials potentially containing infectious agents or biological hazards. In addition, biohazards include human blood, body fluid, tissues, and cell lines of human origin. Biohazards are often referred to as *infectious agents* or *etiological agents*.

Biohazardous material: All infectious agents, vectors known to carry and transmit infectious agents, infected or potentially-infected animals, infectious material, recombinant DNA, and biologically-derived toxins that present either a risk or a potential risk to the health of humans, animals, or plants either directly through infection or indirectly through damage to the environment.

Biological inventory: List of all biological materials present, used, or stored in the laboratory.

Biological material: As used in this manual, a general term referring to all prokaryotic and eukaryotic organisms (and their components), viruses, subviral agents, recombinant DNA, and biologically-derived toxins used in research and instructional laboratories.

Biological Product: A biological prepared and manufactured in accordance with regulations that govern the manufacture of vaccines, reagents, etc.

Biological safety: (see Biosafety).

Biologically-derived toxin: All molecules produced by animals, plants, microorganisms or other agents that have an LD50 value of <50 mg/kg when administered orally to rats.

Biological Safety Officer: The BSO is the designated scientific-administrative officer who assures compliance and biosafety of research involving biohazards and/or recombinant DNA conducted at BSU and affiliated institutions. The BSO should be experienced in the control and safe handling of laboratory biosafety hazards and the regulations which govern and provide guidance to biosafety issues.

Biosafety: The discipline addressing the safe handling and containment of infectious microorganisms and hazardous biological materials. The practice of safe handling of pathogenic microorganisms and their toxins in the biological laboratory is accomplished through the application of risk assessment and containment principles. Biosafety encompasses the knowledge, techniques, equipment, and facilities necessary to prevent or minimize an exposure to, or release

of, a biohazard including research or applications involving recombinant DNA and synthetic nucleic acids.

Biosafety cabinet: A devise enclosed (except for necessary exhaust purposes) on three sides and top and bottom, designed to draw air inward by means of mechanical ventilation, operated with insertion of only the hands and arms of the user, and in which virulent pathogens are used.

Biosafety Committee: At BSU, this may refer to either the *University Biosafety Committee* or the BSU *Institutional Biosafety Committee* depending on which Committee has been constituted to oversee work with biohazardous agents or molecules at the time.

Biosafety level (BSL): Classification system established by the CDC and NIH in BMBL for work involving biological materials; four levels (BSL1-4) provide combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate to minimize the risk of exposure to infectious agents. The NIH Guidelines for Research Involving Recombinant DNA Molecules also makes use of this classification system in its requirements for safety practices regarding laboratory activities involving organisms that contain recombinant DNA.

Biosafety Level 1 Containment: Suitable for work involving well-characterized agents not known to consistently cause disease in immunocompromised adult humans, and present minimal potential hazard to laboratory personnel and the environment.

Biosafety Level 2 Containment: Builds upon BSL-1 and is suitable for work involving agents that pose moderate hazards to personnel and the environment.

Biosafety Level 3 Containment: 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 the inhalation route of exposure.

Biosafety Level 4 Containment: Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission.

Bloodborne Pathogens: Pathogenic microorganisms that ae present in human blood that can cause disease in humans. These pathogens include, but are not limited to hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV).

Blood: Means human blood, human blood components, and products made from human blood.

Centers for Disease Control and Prevention (CDC): The Centers for Disease Control and Prevention of the United States Department of Health and Human Services.

Contact time: Length of time that a chemical disinfectant must be in contact with a surface or instrument in order to decontaminate that instrument or surface.

Containment: Used to describe safety methods for managing infectious agents in the laboratory environment where they are being handled and maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

Decontamination: Process by which contaminated surfaces, equipment, instruments, or waste are rendered nonhazardous. Physical and chemical means of decontamination include the use of heat, liquid decontaminants, and gasses.

Disinfection: Use of antimicrobial substances to destroy or suppress the growth of microorganisms such as bacteria, viruses, or fungi.

Employee: A person who works for the university full-time or part-time and is paid through the university's payroll system or receives compensation in any form.

Engineering controls: Controls that eliminate or reduce exposure to laboratory hazards through the use or substitution of engineered machinery or equipment. Examples include self-capping syringe needles, ventilations systems such as a chemical fume hood, sound-dampening materials to reduce noise levels, safety interlocks, and radiation shielding.

Diagnostic Specimen: Any human or animal material including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluids, etc., which is reasonably believed to contain an etiologic agent and is being shipped for purposes of diagnosis.

Host: Organism in which the rDNA replicates; or, (2) Organisms, usually humans or animals, which are exposed to and harbor a disease.

Etiologic Agent: A viable microorganism or its toxin that causes, or may cause, human disease

Exempt recombinant DNA: Recombinant DNA molecules exempt from requirements set forth by the NIH *Guidelines for Research Involving Recombinant DNA Molecules*.

Exposure incident: A blood/body fluid exposure incident occurs when blood or other potentially infectious material enters the body via one of the following routes: inhalation, a percutaneous injury (e.g., a needle-stick or cut with a sharp object), contact with mucous membranes (eyes, nose, mouth) contact with non-intact skin (especially when the exposed skin is chapped, abraded, or afflicted with dermatitis, or the contact is prolonged or involving an extensive area).

Faculty: An employee who is appointed as a member of the instructional, research, or administrative faculty, including visiting faculty and post-doctoral fellows.

Hazardous chemical (as defined in 29 CFR 1910.1450): *A chemical for which there is* statistically significant evidence based on at least one study conducted in accordance with established scientific principles that acute or chronic health effects may occur in exposed employees. The term "health hazard" includes chemicals which are carcinogens, toxic or highly toxic agents, reproductive toxins, irritants, corrosives, sensitizers, hepatotoxins, nephrotoxins, neurotoxins, agents which act on the hematopoietic systems, and agents which damage the lungs, skin, eyes, or mucous membranes.

Hazardous waste: A waste with properties that make it dangerous or potentially harmful to human health or the environment and exhibits at least one of four characteristics: ignitability, corrosivity, reactivity, or toxicity.

High Efficiency Particulate Air (HEPA) filtration: Filtration of air through filters that have an efficiency of 99.97% for particles with a diameter greater or equal to of 0.3 microns.

Immunocompromised: A state in which the immune system's ability to fight infectious disease is compromised or entirely absent. Most cases of immunodeficiency are either congenital or acquired. An immunocompromised person is very vulnerable to opportunistic infections.

Infectious agents: All human, animal, and plant pathogens (bacteria, parasites, fungi, viruses, prions).

Infectious material: Infectious agents and all biological material that contains or has the potential to contain infectious agents. Examples of infectious material include all human or NHP materials (e.g., blood and other body fluids, organs, tissues, cultured cells), infected animals and material from infected animals, and environmental samples likely to contain infectious agents.

Instructional laboratory: Facility located on Ball State University property that meets the requirements for a laboratory set forth in 29 CFR 1919.1450 and where academic laboratory courses are conducted.

Infectious Substance: Any material that is known or reasonably expected to contain a biohazard.

Institutional Biosafety Committee: The BSU Committee that will be constituted from the *University Safety Committee* at such time that the university becomes formally subject to the National Institutes of Health Guidelines for Recombinant DNA or Synthetic Nucleic Acid research or activities in order to fully comply with those guidelines.

Interstate Shipping: Transporting across state lines within the continental United States.

Intrastate Shipping: Transporting within the State of Indiana.

Laboratory exposure: Occupational exposure to an infectious material or other hazardous material that takes place in the laboratory.

Laboratory personnel: Faculty (professional, administrative, post-doctoral, and research), staff (classified, wage, and student wage), affiliates (visiting faculty, volunteers, visiting research associates), and students (graduate students, undergraduate students, laboratory assistants, etc.) working in laboratories and laboratory support areas. This term does not refer to students enrolled in instructional laboratory courses.

Laboratory support room: Space auxiliary to a laboratory that is used by laboratory personnel to prepare reagents or store materials for their laboratory.

Lethal dose 50 (LD50): Quantity of material than when ingested, injected, or applied to the skin as a single dose will cause death to 50% of test animals who are exposed to it. The test conditions should be specified; the value is expressed in g/kg or mg/kg of body weight.

Mutagen: Agent giving rise to an increased occurrence of mutation in populations of cells and/or organisms.

Negative Airflow: Directional airflow from areas exterior to a laboratory into the laboratory.

Nonbiohazardous material: Material that is not normally infectious, including nonpathogenic microorganisms, viruses, and subviral agents; plants and nonprimate animals (except those listed as biohazardous material), biological material not likely to contain infectious agents, recombinant DNA molecules exempt from NIH Guidelines, environmental samples not likely to contain infectious agents, and biologically-derived nontoxic molecules.

Nonexempt recombinant DNA: Recombinant DNA molecules subject to requirements set forth by the NIH Guidelines for Research Involving Recombinant DNA Molecules.

Other Potentially Infectious Materials: (1) The following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any human body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids; (2) Any unfixed tissue or organ (other than intact skin) from a human (living or dead); and (3) HIV-containing cell or tissue cultures, organ cultures, and HIV- or HBV containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV.

Parenteral: Taken into the body or administered in a manner other than through the digestive tract, as by intravenous or intramuscular injection.

Pathogenic: Capable of causing disease.

Personal Protective Equipment (PPE): Clothing and other work accessories designed to create a barrier against workplace hazards. Examples include safety goggles, blast shields, hard hats, hearing protectors, gloves, respirators, aprons, and work boots.

Primary Containment: Use of appropriate safety equipment, microbiological techniques, and PPE to protect personnel and the immediate laboratory environment from an exposure.

Prion: (proteinaceous infectious particle) — Infectious protein structure that propagates through conversion of normal host proteins of the same type. Though the exact mechanisms of their actions and reproduction are unknown, it is now commonly accepted that prions are responsible for a number of previously known but little-understood diseases generally classified under transmissible spongiform encephalopathy diseases. Prions are highly resistant to common decontamination techniques.

Recombinant: DNA produced in a laboratory by joining segments of DNA from different sources. Recombinant can also describe proteins, cells, or organisms made by genetic engineering.

Recombinant and Synthetic Nucleic Acid (rDNA/sNA) Molecules: In the context of the *NIH Guidelines*, recombinant and synthetic nucleic acids are defined as:

a. Molecules that are (i) constructed by joining nucleic acid molecules and (ii) that can replicate in a living cell, i.e., recombinant nucleic acids;
b. nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, i.e., synthetic nucleic acids, or
c. molecules that result from the replication of those described in (a) or (b) above.

rDNA: DNA prepared by breaking up and splicing together DNA from several different species of organisms.

rDNA Insert: - That (those) strand(s) of foreign DNA being inserted into the host/vector.

Reproductive hazard: A material (chemical, agent, or toxin) that has the potential to affect reproductive capabilities or to cause damage to the unborn embryo/fetus.

Research laboratory: Facility located on Ball State University property that meets the requirements

for a laboratory set forth in 29 CFR 1910.1450 and where scientific research is conducted.

Responsible Official: The individual designated by an institution to act on its behalf pertaining to Select Agent and Toxin regulations. This individual must have the authority and control to ensure compliance with the regulations.

Risk Group 1 Organisms: (BSL-1) - Organisms not known to cause disease in healthy adults.

Risk Group 2 Organisms: (BSL-2) - Organisms associated with human disease, infectious through auto- inoculation ingestion, mucous membrane exposure.

Risk Group 3 Organisms: (BSL-3) - Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences.

Secondary containment: Use of facility design and operational practices, such as restricted access, ventilation, directional airflow and air treatment systems to protect the protection of the environment external to the laboratory from exposure.

Select Agent: Defined by CDC and USDA as biological agents or toxins deemed as a threat to the public, animal or plant health, or to animal or plant products.

Sharps: Any object that can penetrate the skin, e.g., needle, scalpel, knife, etc.

sNA: Synthetic nucleic acid

Staff: A part-time or full-time employee who is not a member of the faculty or a student.

Select Agent: Biological agent or toxin that could pose a severe threat to public health and safety; to animal or plant health; or to animal or plant products and are therefore covered under the Select Agent Rule (7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73).

Sterilization: Use of mechanical or chemical means to inactivate all forms of microbial life.

Student: A person who is officially enrolled in a course or program of study offered by the university.

Subviral agent: Infectious particle, such as a prion, that is capable of infecting and causing disease in a living organism.

Toxin: The toxic material or product of plants, animals, microorganisms (including, but not limited to, bacteria, viruses, fungi, rickettsia, or protozoa), or infectious substances, or a recombinant or synthesized molecule, whatever their origin and method of production, and includes:

- Any poisonous substance or biological product that may be engineered as a result of biotechnology produced by a living organism; or
- Any poisonous isomer or biological product, homolog or derivative of such a substance.

University Biosafety Committee: The BSU Committee created and charged with oversight of biosafety in university research and educational activities, and the precursor to any *Institutional Biosafety Committee* to which it may transform if and when necessary..

Vector: Any animals (vertebrate or invertebrate) including arthropods or any noninfectious self-replicating system (e.g., plasmids or other molecular vector) or animal products (e.g., a mount, rug, or other display item composed of the hide, hair, skull, teeth, bones, or claws of an animal) that are

known to transfer or are capable of transferring an infectious biological agent to a human.

Visitor: A person that is not an employee, faculty, staff, or student but is participating in laboratory activities in facilities owned or under the control of Ball State University.

Appendix B. Acronyms

AC	Animal Care
ABSA	American Biological Safety Association
APHIS	Animal and Plant Health Inspection Service
BBP	Bloodborne Pathogens
BSU	Ball State University
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BSC	Biological Safety Cabinet
BSL-1	Biosafety Level 1
BSL-2	Biosafety Level 2
BSL-3	Biosafety Level 3
BSL-4	Biosafety Level 4
BSO	Biological Safety Officer
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
DACT	Department of Animal Care and Technologies
DEA	Drug Enforcement Administration
EHS	Environmental Health and Safety Office
IACUC	Institutional Animal Care and Use Committee
IBC	Institutional Biosafety Committee
IDEM	Indiana Department of Environmental Management
IO	Institutional Official
IRB	Institutional Review Board
ISDH	Indiana State Department of Health
NHP	Non-Human Primate
NIH	National Institutes of Health
OBA	Office of Biotechnology Activities (NIH)
OPIM	Other Potentially Infectious Materials
OLAW	Office of Laboratory Animal Welfare (PHS)
ORI	Office of Research Integrity
OSHA	Occupational Safety and Health Administration
PHS	Public Health Service
PI	Principal Investigator
PPE	Personal Protective Equipment
PSDS	Pathogen Safety Data Sheet
rDNA/sNA	Recombinant DNA and/or synthetic Nucleic Acid
RG-1	Risk Group 1
RG-2	Risk Group 2
RG-3	Risk Group 3
RG-4	Risk Group 4
RO	Responsible Official
SDS	Safety Data Sheet
UBC	University Biosafety Committee
USDA	United States Department of Agriculture

Appendix C - Biosafety Resources

BSU Safety-Related Manuals:

For copies contact EHS at 765-285-2807, or visit the EHS website.

Biological Safety Plan
Waste Management Plan
Lab Waste (RCRA) Management Plan
Spill Preparedness and Response Plan
Chemical Hygiene Plan
Exposure Control Plan for Bloodborne Pathogens

Websites:

Environmental Health and Safety (EHS) Office:
http://cms.bsu.edu/about/administrativeoffices/riskmanagement/ehs

Office of Research Integrity (ORI):
http://cms.bsu.edu/about/administrativeoffices/researchintegrity

Biology Department:
http://cms.bsu.edu/academics/collegesanddepartments/biology

Radiation Safety:
http://cms.bsu.edu/about/administrativeoffices/researchintegrity/radiationsafety

National Institutes of Health (NIH) Office of Science Policy (Biosafety):
http://osp.od.nih.gov/office-biotechnology-activities/biosafety

Centers for Disease Control (CDC) Biosafety
https://www.cdc.gov/biosafety/

Federal Select Agent Program (CDC/USDA):
https://www.selectagents.gov/selectagentsandtoxinslist.html

CDC Permit to Import or Transport Etiologic Agents:
http://www.cdc.gov/od/eaipp/

Links to Relevant Agency Guidance

Biosafety in Microbiological and Biomedical Laboratories, 5th edition (BMBL):
https://www.cdc.gov/biosafety/publications/bmbl5/

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (*NIH Guidelines*):
http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

Appendix D: Toxin Table

Toxins with a mammalian LD_{50} of < 100 ug/kg must be registered with the Biosafety Committee. Therefore, use of the following toxins requires registration. If a toxin is not on the list, it still may require registration, depending upon the LD_{50}. For more information, please contact the EHS Office.

Toxicity

	LD_{50} (µg/kg)*
Abrin	0.7
Aerolysin	7.0
Botulinin toxin A	0.00 12
Botulinin toxin B	0.00 12
Botulinin toxin C1	0.0011
Botulinin toxin C2	0.00 12
Botulinin toxin D	0.0004
Botulinin toxin E	0.0011
Botulinin toxin F	0.0025
J3 -bungarotoxin	14.0
Caeruleotoxin	53
Cereolysin	40-80
Cholera toxin	250
Clostridium difficile enterotoxin A	0.5
Clostridium difficile cytotoxin B	220
Clostridium perfringens lecithinase	3
Clostridium perfringens kappa toxin	1500
Clostridium perfringens perfringolysin O	13-16
Clostridium perfringens enterotoxin	81
Clostridium perfringens beta toxin	400
Clostridium perfringens delta toxin	5
Clostridium perfringens epsilon toxin	0.1
Conotoxin	12-30
Crotoxin	82
Diphtheria toxin	0.1
Listeriolysin	3-12
Leucocidin	50
Modeccin	1-10
Nematocyst toxins	3 3-70
Notexin	25
Pertussis toxin	15

Toxicity

	LD_{50} (ïg/kg)*
Pneumolysin	1.5
Pseudomonas aeruginosa toxin A	3
Ricin	2.7
Saxitoxin	8
Shiga toxin	0.250
Shigella dysenteriae neurotoxin	1.3
Streptolysin O	8
Staphylococcus enterotoxin B	25
Staphylococcus enterotoxin F	2-10
Streptolysin S	25
Taipoxin	2
Tetanus toxin	0.001
Tetrodotoxin	8
Viscumin	2.4-80
Volkensin	1.4
Yersinia pestis murine toxin	10

*Please note that the LD_{50} values are from a number of sources (see below). For specifics on route of application (i.v., i.p., s.c.), animal used, and variations on the listed toxins, please go to the references listed below.

Reference:

1. Gill, D. Michael; 1982; Bacterial toxins: a table of lethal amounts; *Microbiological Reviews*; 46: 86-94
2. Stirpe, F.; Luigi Barbieri; Maria Giulia Battelli, Marco Soria and Douglas A. Lappi; 1992; Ribosome-inactivating proteins from plants: present status and future prospects; *Biotechnology*; 10: 405-412
3. Registry of toxic effects of chemical substances (RTECS): comprehensive guide to the RTECS. 1997. Doris V. Sweet, ed., U.S. Dept of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health; Cincinnati, Ohio

Appendix E: Select Agents and Toxins

The following biological agents and toxins have been determined to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant products. An attenuated strain of a select agent or an inactive form of a select toxin may be excluded from the requirements of the Select Agent Regulations. The list of excluded agents and toxins can be found at: http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20Exclusions.html.

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
Ebola virus*
*Francisella tularensis**
Lassa fever virus
Lujo virus
Marburg virus*
Monkeypox virus
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

USDA SELECT AGENTS AND TOXINS

African horse sickness virus
African swine fever virus
Avian influenza virus
Classical swine fever virus
Foot-and-mouth disease virus*
Goat pox virus
Lumpy skin disease virus
Mycoplasma capricolum
Mycoplasma mycoides
Newscastle disease virus[1]
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 rayssiae
Synchytrium endobioticum
Xanthomonas oryzae

*Denotes Tier 1 Agent

[1] A virulent Newcastle disease virus (avian paramyxovirus serotype 1) 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. 10/01/2012

Appendix F - Risk Assessment Form

Biological Safety Risk Assessment for Proposed Procedures

Date: _____ Principal Investigator: _____

Description of Materials & Procedures: _____

Location(s): _____

This template consists of 3 sections with headings on the following pates. Please contact the BSU Biosafety Officer for any guidance.

It may be helpful to begin the Risk Assessment process by utilizing the forms/data from the following links to initially determine the recommended Biosafety Level (BSL) and/or elementary precautions for the agent of study:

American Biological Safety Association International (ABSA) Risk Group Database:

https://my.absa.org/tiki-index.php?page=Riskgroups

Centers for Disease Control Biological Risk Assessment Worksheet:

file:///Z:/Biological%20Safety/BSU%20BSP/CDC%20biologicalriskassessmentworksheet.pdf

SECTION 1

Material Source Information Use the space below to identify:

- Types of materials to be used including quantities and biological activation status
- Source, and any known infectious disease considerations associated with either the source species or the geographic location of the source species
- Procedural steps for the analysis, from material preparation through waste disposal

SECTION 2 **Infectious Disease Considerations** Complete this section for each agent identified as an infectious disease consideration in the previous section. Make additional copies of this section if needed.		
Agent		
Pathogenicity of the organism & Routes of transmission	Infectious Dose	
	Routes of Transmission	
	Host Range	
	Disease Severity	
	Previous History of Lab-Associated Infection	
Medical Surveillance	Pre-exposure recommendations (vaccines availability, indications, etc.)	
	Post-exposure recommendations (therapy or post-exposure prophylaxis availability, indications, etc.)	
	Personnel considerations (identify any health status conditions that would make a person more susceptible to infection or for who exposure to this agent is contraindicated.)	
Agent Stability & Specific Features	Means of chemical or physical inactivation	
	Any specific qualities of the agent that will hinder inactivation or medical treatment (i.e. antibiotic-resistance, genetic modification, etc.	

Biosafety Level & Containment Practices Assignment *(Consult with the Biosafety Officer as needed))*

Use this space to summarize:

- Regulatory recommendation or restriction factors (USDA, ABSA, NIH, CDC, etc.)
- Factors associated with the process that impact biosafety level assignment
- Biosafety level assignment along with any additional procedural considerations

Date of implementation: Date due for review:

Note that any biological exposure incident associated with the outlined procedure may be indicative of a need for procedural change. In this instance, a review of the procedure and the risk assessment document must be conducted within 30 days of a biological exposure incident.

Appendix G: Laboratory-specific Biosafety Manual Contents

Click here to enter text. **Laboratory Biosafety Manual**

Date: Click here to enter text.

[Credit: Form Developed by *Montana State University*]

PI's Last Name Laboratory

Building(s) and room number(s)

Note:

This template is provided to assist Principal Investigators (PIs) and faculty in the development of a *laboratory-specific* biosafety manual with instructions to safely handle and manipulate a particular agent or agents under particular laboratory conditions. The PI is responsible for including basic background information for each agent, writing an exposure risk, detailing surface decontamination, and writing standard operating procedures for experiments where safety is a concern. Also, please provide lab-specific information where you see gray text fields. Training dates should be maintained in the provided table. Additions/changes to this template that will render the final manual more useful for the laboratory's safety needs are encouraged. If any laboratory determines the need to deviate from standard BSL2 work practices discussed in this manual, then these alterations, along with a written explanation must be submitted to the Biosafety Officer for approval. While this Plan is specific to BSL-2 activities it is adaptable to BSL-1 studies through that approach.

In addition to this Laboratory-Specific Biosafety Manual, the BSU Biosafety Committee requires the lab to follow the BSU *Biological Safety Manual* and follow BSL procedures as outlined in the National Institutes of Health (NIH) *Biosafety in Microbiological and Biomedical Laboratories, 5th Edition* (http://www.cdc.gov/biosafety/publications/bmbl5/). For research involving recombinant DNA, the lab must also follow the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.htm).

A loose-leaf binder that can easily accommodate changes or new materials is the recommended means for maintaining and organizing this Laboratory-specific Biosafety Manual.

All lab personnel must read the contents of this manual and sign & date below. By signing this page, lab personnel agree to abide by the safety precautions and procedures discussed herein.

I have read, understand, and agree to adhere to the biosafety procedures contained within:

Principal Investigator:

Typed Name	Title	Signature	Date
First, Last	Principal Investigator		

Laboratory Staff:

Typed Name	Job/Student Title	Signature	Date
First, Last	Enter info		
First, Last	Enter info		
First, Last	Enter info		
First, Last	Enter info		
First, Last	Enter info		
First, Last	Enter info		
First, Last	Enter info		

First, Last	Enter info		
First, Last	Enter info		
First, Last	Enter info		
First, Last	Enter info		
First, Last	Enter info		
First, Last	Enter info		
First, Last	Enter info		
First, Last	Enter info		
First, Last	Enter info		

Table of Contents

Responsibilities

Principal Investigator Responsibilities

Dr. Click here to enter text. has the primary responsibility for ensuring that their laboratory is safe. Dr. Click here to enter text. is responsible for the safe use of biological materials used in the lab.

In addition, Dr. Click here to enter text. is responsible for the following:

- Limit personnel, student, and visitor exposure to hazards to the lowest practical level.
- Be familiar with the required medical surveillance for each type of biological agent used in the laboratory.
- Develop a written lab specific safety procedures and train personnel on them.
- Maintain documentation of training.
- Provide Personal Protective Equipment (PPE) and instruction on proper use.
- Ensure waste is properly disposed.
- Report spills, exposures or incidents to Kirk Lubick, Biosafety Officer, at 994-6998.

Laboratory Staff/Student Responsibilities

- Knowledge of the biological agents and procedures used in the laboratory.
- Follow approved lab procedures and safety guidelines.
- Know emergency procedures and equipment.
- Complete all required training before conducting any lab activity.
- Report any unsafe conditions to the PI and/or the BSU Biosafety Officer.
- Utilize appropriate lab equipment and containment facilities.

Lab-Specific Emergency Information

Emergency Contacts

Principal Investigator:	**First, Last**
Lab Location:	Lab Location
Office Phone:	Office Phone
Cell Phone:	Enter number
Secondary Contact	**First, Last**
Office Phone:	Office Phone
Cell Phone:	Enter number
Biosafety Officer	**Tom Russell, EHS Office**
Office Phone:	285-2807
Cell Phone:	765-499-3060
Registration Document #(s):	**Enter number(s)**
IACUC Protocol #(s) (if applicable):	**Enter number(s)**

If there is an emergency, call 911 to reach BSU police. If there is a fire or explosion that you cannot control, evacuate the area immediately. Pull the fire alarm and then call 911 from a safe location.

If any emergency or significant spill/exposure occurs in the laboratory, immediately notify the Biosafety Officer and your lab supervisor/PI.

Fire Alarms/Extinguishers

Locations of fire pull station alarms:

Click here to enter text.

Location of fire extinguishers:

Click here to enter text.

Know the location of each of these, and identify the location of the extinguisher closest to your lab bench. If the fire alarm sounds, leave the building immediately and move away to a safe distance.

Eyewashes

Location of eyewashes:

Click here to enter text.

In case of exposure, proceed to nearest eyewash station. Hold eyelids open with thumb and forefinger and rinse for at least 15 minutes. Wash from outside edges towards the inside to prevent washing back into the eye.

Rinse should be aimed at the inner corner of the eye (near the nose) not directly at the eyeball. "Roll" eyes around and up and down to ensure full rinsing.

Contact lenses (if worn) should be removed as soon as possible. Have another member of the lab call for emergency response immediately. The area around the eye wash station must remain clear at all times.

Biological Spill Kit

Location of spill kit:

Click here to enter text.

Standard Operating Procedures

Standard Microbiological Practices

Standard Microbiological Practices refer to the safe laboratory work practices for working with biological agents.

Hygiene and Housekeeping

Keep work areas clean and uncluttered to reduce the chance of cross-contamination and inadvertent exposure to biohazards. To avoid ingestion of contaminated material, use a mechanical pipetting device, keep food out of refrigerators and microwaves in work areas, eat, drink, or apply cosmetics only in designated "clean" areas outside the laboratory.

a. Wash hands after removing gloves, before leaving the lab, and when handling materials known or suspected to be contaminated.
b. Clean work surfaces and decontaminate with Click here to enter text. at the end of each day.

c. Remove gloves before leaving the lab, touching the face, keyboards, or control panels, and before using the elevator.

Personal Protective Equipment

a. Wear gloves if skin on the hand is broken, if rash is present, and when handling biological waste.
b. Remove rings or other jewelry that could puncture gloves.
c. Wear the appropriate glove for the hazard. Usually a type of latex or nitrile glove is recommended for working with biological material.
d. Avoid reusing gloves unless they can be decontaminated.
e. Wear eyewear approved for UV light or other rays that could damage eyes.

Biological Spills in the Laboratory

Call the Biosafety Officer when a significant spill occurs. A lab incident report form must be filled out for significant spills.

A significant spill is defined as:

- Spills greater than 5 ml outside primary containment
- Spills that result in an exposure
- Spills that present an inhalation hazard
- Spills that cannot be easily cleaned
- Spills that endanger people or the environment

Location of spill kit:

Click here to enter text.

Small Spill Decontamination and Clean Up (less than 1 liter):

a. Stop work and secure all items you are working with.
b. Replace any contaminated personal protective equipment (PPE).
c. Make sure you are wearing the appropriate PPE such as disposable gloves, lab coat and eye protection (safety glasses or goggles).
d. Using the Lab Spill Kit take the absorbent pads and place over the spill area.
e. Make a fresh solution of 10 % bleach and pour over the absorbent pads. The absorbent pads absorb the spill, help contain the 10 % bleach solution and help prevent splattering.
f. Let the bleach solution inactivate and decontaminate the biological material for a minimum of 15 minutes.
g. Report the spill to Dr. Click here to enter text. and the Biosafety Officer at x52807. This is very important as the University maintains an ongoing log/list of spills and injuries as applicable and reports these as required under the *NIH Guidelines for Research Involving Recombinant DNA Molecules*.
h. Soak up and clean up the excess bleach solution and decontaminated material with extra absorbent pads or paper towels.
i. Dispose of in biohazard bag.

Large Spill (greater than 1 liter):

a. Stop work immediately, secure all items and avoid inhaling airborne aerosols.
b. Notify others to leave the room immediately.
c. Label the area off-limits for at least 30 minutes. This allows the ventilation system to purge the air.
d. Remove contaminated PPE and or clothing, turn exposed clothing inward, and put in autoclave bag or biohazardous bag.

e. Wash all exposed skin with soap and water.
f. Report the spill to Dr. Click here to enter text. and the Biosafety Officer at x52807. This is very important as the University maintains an ongoing log/list of spills and injuries as applicable and reports these as required under the *NIH Guidelines for Research Involving Recombinant DNA Molecules.*
g. After at least 30 minutes, Biosafety personnel will enter the area to clean up the spill.

Biological Waste Disposal

Liquid Biohazardous Waste Disposal:

All liquid biological waste from the lab must be treated prior to disposal. Examples of biological waste include cell lines, recombinant DNA, recombinant proteins, and biological agents. The procedures below outline the steps to take to treat liquid biohazardous waste generated in Dr. Click here to enter text. lab:

a. Always wear appropriate PPE such as disposable gloves, lab coat and eye protection (safety glasses or goggles) when working with biohazardous waste.
b. When liquid biohazardous waste is anticipated to be generated, add 100 ml of undiluted bleach into a 1 l beaker.
c. As experiments are performed and completed pour the biological waste into the beaker with the bleach.
d. Once experiments are complete and if the beaker is less than 1 L add water to bring the volume to 1 L.
e. Once the beaker is full the bleach has been diluted to a 10 % solution.
f. Let 10 % bleach and biological waste solution stand for at least 1 hour.
g. Dispose of the solution with care to avoid splatter down the lab sink and rinse beaker.

Solid Biohazardous Waste Disposal:

The procedures below outline the steps to take to treat solid biohazardous waste generated in Dr. Click here to enter text. lab:

a. All solid lab waste that has come in contact with biological waste from the lab must be treated prior to disposal. Examples of biological waste include used personal protective equipment such as disposable gloves, paper towels, pipette tips, disposable Petri dishes, pipettes, and culture flasks.
b. Always wear appropriate PPE such as disposable gloves, lab coat, and eye protection (safety glasses or goggles) when working with biohazardous waste.
c. Place all potentially contaminated items in biohazardous waste bag.
d. Once the bag is ¾ full close bag and place autoclave tape on the bag.
e. Take the biohazard bag to the autoclave room per your buildings transportation guidelines as follows Click here to enter text. and place in the autoclave.
f. Complete autoclave log book entry and autoclave biohazardous waste for Click here to enter text. following manufactures' recommendations for autoclave operation.
g. Once the autoclave cycle is complete the load within has been sterilized if the autoclave tape has turned color and the autoclave display shows no errors.
h. Place the biohazardous bag into a black garbage bag prior to disposing of the waste in the dumpster.
i. If the autoclave tape did not turn color and/or the autoclave display indicates errors occurred during operation or an incomplete cycle the load has not been sterilized. Notify Click here to enter text. at Click here to enter text. as the load is still considered biohazardous.

Sharp Biohazardous Waste Disposal:

The procedures below outline the steps to carefully handle sharps in Dr. Click here to enter text. lab:

a. All used sharps must be immediately discarded into a sharps container.
b. Sharps containers must be kept upright and never reach into a sharps disposal container.
c. Sharps containers must be easily located in the immediate area where sharps are used.
d. When sharps waste has reached the pre-marked "fill-line" of the sharps container close and lock lid.
e. Contact Safety and Risk Management to have sharps containers picked-up and replaced.

Injury to an individual in the laboratory (i.e. needles stick, cut, biological/chemical exposure incident – splash, etc.):

a. Immediately stop work and flush affected area with soap and water for 15 minutes.
b. If the injury is a Medical Emergency call 911 or campus police x2807. If you need emergency medical treatment, call 911 for transport to the nearest hospital: IU Health Ball Memorial Hospital, located at 2401 W. University Ave.
c. Secure all infectious materials.
d. Notify Dr. Click here to enter text. and Biosafety Officer at (765) 285-2807 or Campus police 911 after working hours. This is a very important as the University maintains an ongoing log/list of spills and injuries and as applicable reports these as required under the *NIH Guidelines for Research Involving Recombinant DNA Molecules*.
e. Use the nearest First Aid Kit located in the laboratory.
f. If during working hours seek non-emergency medical attention at the BSU Health Center. After working hours seek medical attention at MedExpress: 1313 W. McGalliard Avenue: 765-287-8460, or US Healthworks: 3911 W. Clara Lane: 765-288-8800.
g. Take the pertinent Biological Data Safety Sheet or other information on the biohazard with you.
h. Dr. Click here to enter text. will complete the Workers Compensation Injury/Incident Report form documenting the route of exposure and the circumstances under which the incident occurred.

SOPs for Dr. Last name Laboratory when using BSL Agents

The purpose of this section is to develop SOPs that specifically outline instances during protocols where consideration for safety with a BSL2 agent is paramount. Detailed, step-by step protocols describing entire experiments with materials and methods are not necessary. Examples of SOPs where safety is emphasized are bulleted below:

- Propagation of viruses
- Experiments that require PPE in addition to a lab coat and gloves
- Experiments that require manipulation of a BSL2 agent outside a biosafety cabinet
- How to properly vortex or sonicate a viable BSL2 agent
- Safety concerning the handling of human or non-human primate cell lines or tissues
- Safety when injecting a research animal with a BSL2 agent
- How to safely centrifuge a sample containing BSL2 agents

Please enter SOPs under separate headings.

Click here to enter text.

Inactivation and Surface Decontamination

Describe the reagents and/or processes used to inactivate the agents(s) and the method to decontaminate surfaces.

Click here to enter text.

Training

All laboratory research personnel must take institutional provided training. Training must be documented (electronic or paper). Personnel should not initiate research until training is completed. Copies of completed training certificates should be included in this manual.

Lab Personnel	**Relevant Training Dates**						
Name	CITI-Blood-borne Pathogens	CITI-Biosafety Initial	CITI-Biosafety Refresher	CITI- rDNA	CITI-Animal Research	CITI-Human Subjects	Dangerous Goods Shipping
First, Last							
First, Last							
First, Last							
First, Last							
First, Last							
First, Last							
First, Last							
First, Last							
First, Last							
First, Last							
First, Last							
First, Last							
First, Last							
First, Last							

***Agent(s)*-specific Training.** Laboratory personnel are not allowed to work with agent(s) until they have been trained by the PI who supervises their work, or a designated technical expert. The worker should demonstrate good microbiological skills and an understanding of this SOP prior to being permitted to work with agent(s).

Personal Protective Equipment

The following PPE must be worn when working with agent(s):

Please check appropriate boxes by double clicking and selecting "checked."

☐ **Gloves**	☐ **Safety glasses**	☐ **N95 Respirator**	☐ **Shoe covers**
☐ **Latex**	☐ **Face shield**	☐ **Surgical mask**	☐ **Medical scrubs**
☐ **Nitrile**	☐ **Lab coat**	☐ **Hair net**	

Exposure Risk

Describe the means by which laboratory personnel could be exposed to the agent(s). Include practices that pose potential for exposure, such as those that could create aerosols. Include the symptomology associated with the biological agent or molecule.

Click here to enter text.

Biohazard Warning Signs and Posting

Each laboratory must clearly display a sign that provides safety information to visitors and service personnel. ORC/SRM will provide the signs.

a. All areas and laboratories which contain biohazardous agents must be posted with a biohazard sign.
b. If the biological agent or molecule is used or exposure possible in only particular locations in the lab space (i.e., fume hood, bench area, etc.), those should be designated and delineated with the BSU Designated Area signage.
c. The standard BSU Lab Hazard door sign should include the following information:
d. The sign must have information regarding biosafety level, materials used, entry requirements, PPE requirements, emergency contact name and phone number.

Biological Safety Cabinets (BSC's)

BSC's should be positioned in the laboratory away from normal traffic patterns to minimize airflow disruption.

Some work may be done on the open bench by persons wearing appropriate protective clothing or gear. Any work that may produce splatters or aerosols of infectious materials should be done inside a biological safety cabinet (BSC).

Before materials are introduced into the BSC, they should be wiped with disinfectant to remove any external contaminants.

Clean materials should be kept to one side of the work surface, dirty items on the other. Management of workflow within the BSC is crucial to preventing cross-contamination.

Rapid air movement outside the cabinet (caused by co-workers walking past, air supply vents directed across the face of the BSC, etc.) will interrupt the rather fragile air curtain, which may cause air-borne contaminants in the cabinet to be drawn into the lap of the worker.

The chair should be adjusted so that the lower portion of the sash is even with the worker's armpits.

Any paper or plastic materials introduced into the BSC should not be allowed to interfere with air flow through the front or rear grilles.

The downward airflow from the supply filter "splits" about one third of the way into the cabinet; in the front third, air moves to the front grille, with the remainder of the air flowing to the rear. This means that aerosol-generating activities should be performed towards the rear of the cabinet to provide further worker protection.

Agent(s) Specific Pathogen Safety Data Sheets

Pathogen Safety Data Sheets can be found at the Public Health Agency of Canada website (http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php)

Attached are the Pathogen Safety Data Sheets for each Biological Agent used in Dr. Click here to enter text. Laboratory.

Click here to enter text.

Departures from the BSU Biological Safety Manual

Following are any departures from the *BSU Biological Safety Manual*, justification for such departures, and the alternate protective measures employed for each Biological Agent used in Dr. Click here to enter text. Laboratory:

Click here to enter text.

Location of the Completed BSU Registration Document and Risk Assessment

Following are the locations of the Registration Document and the Risk Assessment, as required by the *BSU Biological Safety Manual*, for each Biological Agent used in Dr. Click here to enter text. Laboratory:

Click here to enter text.

Appendix H- Examples of RG-2, RG-3 and RG-4 Agents

Please remember that those agents not listed in Risk Groups 2, 3 and 4 are not automatically classified in RG 1. A risk assessment must be conducted based on the known and potential properties of the agents.

Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.

Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

--*Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)
--*Actinobacillus*
--*Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
--*Aeromonas hydrophila*
--*Amycolata autotrophica*
--*Archanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
--*Arizona hinshawii* - all serotypes
--*Bacillus anthracis*
--*Bartonella henselae, B. quintana, B. vinsonii*
--*Bordetella* including *B. pertussis*
--*Borrelia recurrentis, B. burgdorferi*
--*Burkholderia* (formerly *Pseudomonas* species) except those listed in Appendix B-III-A (RG3)) --*Campylobacter coli, C. fetus, C. jejuni*
--*Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
--*Clostridium botulinum*, *C. chauvoei, C. haemolyticum, C. histolyticum, C. novyi, C. septicum, C. tetani*
--*Coxiella burnetii* – specifically the Phase II, Nine Mile strain, plaque purified, clone 4
--*Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale*
--*Dermatophilus congolensis*
--*Edwardsiella tarda*
--*Erysipelothrix rhusiopathiae*
--*Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
--*Francisella tularensis* specifically **F. tualrensis* subspecies *novocida* [aka *F. novocida*], strain Utah 112; **F. tularensis* subspecies *holartica* LVS; **F tularensis* biovar *tularensis* strain ATCC 6223 (aka strain B38) [* For research involving high concentrations, BL3 practices should be considered (See *Appendix G-II-C-2.Special Practices (BL3)*).]
--*Haemophilus ducreyi, H. influenzae*
--*Helicobacter pylori*
--*Klebsiella* - all species except *K. oxytoca* (RG 1)
--*Legionella* including *L. pneumophila*
--*Leptospira interrogans* - all serotypes
--*Listeria*
--*Moraxella*
--*Mycobacterium* (except those listed in Appendix B-III-A (RG3)) including *M. avium* complex, *M. asiaticum, M. bovis* BCG vaccine strain, *M. chelonei, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi* --*Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
--*Neisseria gonorrhoeae*, *N. meningitidis*
--*Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis*
--*Rhodococcus equi*
--*Salmonella* including *S. arizonae, S. cholerBSUis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi,* A, B, C, *S. typhi*, *S. typhimurium*
--*Shigella* including *S. boydii, S. dysenteriae,* type 1, *S. flexneri, S. sonnei*

--*Sphaerophorus necrophorus*
--*Staphylococcus aureus*
--*Streptobacillus moniliformis*
--*Streptococcus* including *S. pneumoniae, S. pyogenes*
--*Treponema pallidum, T. carateum*
--*Vibrio cholerae, V. parahemolyticus, V. vulnificus*
--*Yersinia enterocolitica*
--*Yersinia pestis* specifically *pgm*$^{(-)}$ strains (lacking the 102 kb pigmentation locus and *lcr*$^{(-)}$ strains (lacking the LCR plasmid)

Risk Group 2 (RG2) - Fungal Agents

--*Blastomyces dermatitidis*
--*Cladosporium bantianum, C. (Xylohypha) trichoides*
--*Cryptococcus neoformans*
--*Dactylaria galopava (Ochroconis gallopavum)*
--*Epidermophyton*
--*Exophiala (Wangiella) dermatitidis*
--*Fonsecaea pedrosoi*
--*Microsporum*
--*Paracoccidioides braziliensis*
--*Penicillium marneffei*
--*Sporothrix schenckii*
--*Trichophyton*

Risk Group 2 (RG2) - Parasitic Agents

--*Ancylostoma* human hookworms including *A. duodenale, A. ceylanicum*
--*Ascaris* including *Ascaris lumbricoides suum* --
Babesia including *B. divergens, B. microti* --
Brugia filaria worms including *B. malayi, B. timori*
--*Coccidia*
--*Cryptosporidium* including *C. parvum*
--*Cysticercus cellulosae* (hydatid cyst, larva of *T. solium*)
--*Echinococcus* including *E. granulosis, E. multilocularis, E. vogeli*
--*Entamoeba histolytica*
--*Enterobius*
--*Fasciola* including *F. gigantica, F. hepatica*
--*Giardia* including *G. lamblia*
--*Heterophyes*
--*Hymenolepis* including *H. diminuta, H. nana*
--*Isospora*
--*Leishmania* including *L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruvania, L. tropica*
--*Loa loa* filaria worms
--*Microsporidium*
--*Naegleria fowleri*
--*Necator* human hookworms including *N. americanus*
--*Onchocerca* filaria worms including, *O. volvulus*
--*Plasmodium* including simian species, *P. cynomologi, P. falciparum, P. malariae, P. ovale, P. vivax* --*Sarcocystis* including *S. sui hominis*
--*Schistosoma* including *S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi* --*Strongyloides* including *S. stercoralis*
--*Taenia solium*
--*Toxocara* including *T. canis*
--*Toxoplasma* including *T. gondii*

--*Trichinella spiralis*
--*Trypanosoma* including *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi*
--*Wuchereria bancrofti* filaria worms

Risk Group 2 (RG2) - Viruses

Adenoviruses, human - all types

Alphaviruses (Togaviruses) - Group A Arboviruses
--Chikungunya vaccine strain 181/25
--Eastern equine encephalomyelitis virus
--Venezuelan equine encephalomyelitis vaccine strains TC-83 and V3526
--Western equine encephalomyelitis virus

Arenaviruses
--Junin virus candid #1 vaccine strain
--Lymphocytic choriomeningitis virus (non-neurotropic strains)
--Tacaribe virus complex
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Bunyaviruses
--Bunyamwera virus
--Rift Valley fever virus vaccine strain MP-12
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Caliciviruses

Coronaviruses - except SARS-Cov, (see Appendix B-III-D, Risk Group 3 (RG3) - Viruses and Prions)

Flaviviruses - Group B Arboviruses
--Dengue virus serotypes 1, 2, 3, and 4
--Japanese encephalitis virus strain SA 14-14-2
--Yellow fever virus vaccine strain 1 7D
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Hepatitis A, B, C, D, and E viruses

Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see Appendix B-IV-D, *Risk Group 4 (RG4) - Viral Agents*)
--Cytomegalovirus
--Epstein Barr virus
--*Herpes simplex* types 1 and 2
--*Herpes zoster*
--Human herpesvirus types 6 and 7

Orthomyxoviruses
--Influenza viruses types A, B, and C (except those listed in Appendix B-III-D, Risk Group 3 (RG3) - Viruses and Prions)
--Tick-borne orthomyxoviruses

Papilloma viruses
--All human papilloma viruses

Paramyxoviruses --
Newcastle disease virus

--Measles virus
--Mumps virus
--Parainfluenza viruses types 1, 2, 3, and 4
--Respiratory syncytial virus

Parvoviruses
--Human parvovirus (B19)

Picornaviruses
--Coxsackie viruses types A and B
--Echoviruses - all types
--Polioviruses - all types, wild and attenuated
--Rhinoviruses - all types

Poxviruses - all types except Monkeypox virus (see Appendix B-III-D, Risk Group 3 (RG3) - Viruses and Prions) and restricted poxviruses including Alastrim, Smallpox, and Whitepox (see Section V-L, *Footnotes and References of Sections I through IV*)

Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)

Rhabdoviruses
--Rabies virus - all strains
--Vesicular stomatitis virus non exotic strains: VSV-Indiana 1 serotype strains (*e.g.* Glasgow, Mudd-Summers, Orsay, San Juan) and VSV-New Jersey serotype strains (*e.g.* Ogden, Hazelhurst)

Rubivirus (Togaviruses)
--Rubella virus

Risk Group 3 (RG3) Agents

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.

Risk Group 3 (RG3) - Bacterial Agents Including Rickettsia

--*Bartonella*
--*Brucella* including *B. abortus, B. canis*, *B. suis*
--*Burkholderia (Pseudomonas) mallei, B. pseudomallei*
--*Coxiella burnetii* (except the Phase II, Nine Mile strain listed in Appendix B-II-A, Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia
--*Francisella tularensis* (except those strains listed in Appendix B-II-A, Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia
--*Mycobacterium bovis* (except BCG strain, see Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia*), *M. tuberculosis*
--*Pasteurella multocida* type B -"buffalo" and other virulent strains
--Rickettsia akari, R. australis, R. canada, R. conorii, R. prowazekii, R. rickettsii, R, siberica, R. tsutsugamushi, R. typhi (R. mooseri)
--*Yersinia pestis* (except those strains listed in Appendix B-II-A, Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

Risk Group 3 (RG3) - Fungal Agents

--*Coccidioides immitis* (sporulating cultures; contaminated soil)
--*Histoplasma capsulatum, H. capsulatum var.. duboisii*

Risk Group 3 (RG3) - Parasitic Agents

None

Risk Group 3 (RG3) - Viruses and Prions

Alphaviruses (Togaviruses) - Group A Arboviruses
--Chikungunya virus (except the vaccine strain 181/25 listed in Appendix B-II-D, Risk Group 2 (RG2) - Viruses
--Semliki Forest virus
--St. Louis encephalitis virus
--Venezuelan equine encephalomyelitis virus (except the vaccine strains TC-83 and V3526, see Appendix B-II-D, Risk Group 2 (RG2) - Viruses (RG2))
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Arenaviruses
--Flexal
--Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)

Bunyaviruses
--Hantaviruses including Hantaan virus
--Rift Valley fever virus

Coronaviruses
--SARS-associated coronavirus (SARS-CoV)

Flaviviruses - Group B Arboviruses
--Japanese encephalitis virus (except the vaccine strain 14-14-2 listed in Appendix B-II-D, Risk Group 2 (RG2) - Viruses
--West Nile virus (WNV)
--Yellow fever virus
--Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)

Orthomyxoviruses
--Influenza viruses 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968), and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1).

Poxviruses --
Monkeypox virus

Prions

--Transmissible spongioform encephalopathies (TME) agents (Creutzfeldt-Jacob disease and kuru agents)(see Section V-C, *Footnotes and References of Sections I through IV*, for containment instruction)

Retroviruses

--Human immunodeficiency virus (H IV) types 1 and 2 --
Human T cell lymphotropic virus (HTLV) types 1 and 2 --

Simian immunodeficiency virus (SIV)

Rhabdoviruses
--Vesicular stomatitis virus (except those strains listed in Appendix B-II-D, Risk Group 2 (RG2) - Viruses
Risk Group 2 Agents
RG-2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.

Bacterial Agents

--*Acinetobacter baumannii*
--*Actinobacillus*
--*Actinomyces pyogenes*
--*Aeromonas hydrophila*
--*Amycolata autotrophica*
--*Archanobacterium haemolyticum*
--*Arizona hinshawii* - all serotypes
--*Bacillus anthracis*
--*Bartonella henselae, B. quintana, B. vinsonii*
--*Bordetella* including *B. pertussis*
--*Borrelia recurrentis, B. burgdorferi*
--*Burkholderia* (except those listed as RG-3)
--*Campylobacter coli, C. fetus, C. jejuni*
--*Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
--*Clostridium botulinum*, *Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani*
--*Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale*
--*Dermatophilus congolensis*
--*Edwardsiella tarda*
--*Erysipelothrix rhusiopathiae*
--*Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
--*Haemophilus ducreyi, H. influenzae*
--*Helicobacter pylori*
--*Klebsiella* - all species except *K. oxytoca* (RG1)
--*Legionella* including *L. pneumophila*
--*Leptospira interrogans* - all serotypes
--*Listeria*
--*Moraxella*
--*Mycobacterium (*except those listed as RG-3) including *M. avium* complex, *M. asiaticum, M. bovis* BCG vaccine strain, *M. chelonei, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi*
--*Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens --*Neisseria gonorrhoeae*, *N. meningitidis*
--*Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis*
--*Rhodococcus equi*
--*Salmonella* including *S. arizonae, S. cholerBSUis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S.paratyphi,* A, B, C, *S. typhi*, *S. typhimurium*
--*Shigella* including *S. boydii, S. dysenteriae,* type 1, *S. flexneri, S. sonnei*
--*Sphaerophorus necrophorus*
--*Staphylococcus aureus*
--*Streptobacillus moniliformis*
--*Streptococcus* including *S. pneumoniae, S. pyogenes*
--*Treponema pallidum, T. carateum*
--*Vibrio cholerae, V. parahemolyticus, V. vulnificus*
--*Yersinia enterocolitica*

Fungal Agents --

--Blastomyces dermatitidis
--Cladosporium bantianum, C. (Xylohypha) trichoides
--Cryptococcus neoformans
--Dactylaria galopava (Ochroconis gallopavum)
--Epidermophyton
--Exophiala (Wangiella) dermatitidis
--Fonsecaea pedrosoi
--Microsporum
--Paracoccidioides braziliensis
--Penicillium marneffei
--Sporothrix schenckii
--Trichophyton

Parasitic Agents

--Ancylostoma human hookworms including *A. duodenale, A. ceylanicum*
--Ascaris including *Ascaris lumbricoides suum*
--Babesia including *B. divergens, B. microti*
--Brugia filaria worms including *B. malayi, B. timori*
--Coccidia
--Cryptosporidium including *C. parvum*
--Cysticercus cellulosae (hydatid cyst, larva of *T. solium*)
--Echinococcus including *E. granulosis, E. multilocularis, E. vogeli*
--Entamoeba histolytica
--Enterobius
--Fasciola including *F. gigantica, F. hepatica*
--Giardia including *G. lamblia*
--Heterophyes
--Hymenolepis including *H. diminuta, H. nana*
--Isospora
--Leishmania including *L. bra ziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruvania, L. tropica*
--Loa loa filaria worms
--Microsporidium
--Naegleria fowleri
--Necator human hookworms including *N. americanus*
--Onchocerca filaria worms including, *O. volvulus*
--Plasmodium including simian species, *P. cynomologi, P. falciparum, P. malariae, P. ovale, P. vivax*
--Sarcocystis including *S. sui hominis*
--Schistosoma including *S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi*
--Strongyloides including *S. stercoralis*
--Taenia solium
--Toxocara including *T. canis*
--Toxoplasma including *T. gondii*
--Trichinella spiralis
--Trypanosoma including *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi*
--Wuchereria bancrofti filaria worms

Viruses

Adenoviruses, human - all types
Alphaviruses (Togaviruses) - Group A Arboviruses --Eastern equine encephalomyelitis virus --Venezuelan equine encephalomyelitis vaccine strain TC-83
--Western equine encephalomyelitis virus

Arenaviruses
--Lymphocytic choriomeningitis virus (non-neurotropic strains)
--Tacaribe virus complex
Bunyaviruses
--Bunyamwera virus
--Rift Valley fever virus vaccine strain MP-1 2
Caliciviruses
Coronaviruses
Flaviviruses (Togaviruses) - Group B Arboviruses --
Dengue virus serotypes 1, 2, 3, and 4 --Yellow
fever virus vaccine strain 1 7D
Hepatitis A, B, C, D, and E viruses
Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see RG-4)
--Cytomegalovirus
--Epstein Barr virus
--*Herpes simplex* types 1 and 2
--*Herpes zoster*
--Human herpesvirus types 6 and 7
Orthomyxoviruses
--Influenza viruses types A, B, and C
--Other tick-borne orthomyxoviruses
Papovaviruses
--All human papilloma viruses
Paramyxoviruses
--Newcastle disease virus
--Measles virus
--Mumps virus
--Parainfluenza viruses types 1, 2, 3, and 4
--Respiratory syncytial virus
Parvoviruses
--Human parvovirus (B19)
Picornaviruses
--Coxsackie viruses types A and B
--Echoviruses - all types
--Polioviruses - all types, wild and attenuated
--Rhinoviruses - all types
Poxviruses - all types except Monkeypox virus *(see RG-3)*and restricted poxviruses including Alastrim, Smallpox, and Whitepox
Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)
Rhabdoviruses
--Rabies virus - all strains
--Vesicular stomatitis virus - laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow
Togaviruses (see Alphaviruses and Flaviviruses)
--Rubivirus (rubella)

Risk Group 4 (RG4) Agents

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

Risk Group 4 (RG4) - Bacterial Agents

None

Risk Group 4 (RG4) - Fungal Agents

None

Risk Group 4 (RG4) - Parasitic Agents

None

Risk Group 4 (RG4) - Viral Agents

--Arenaviruses
--Guanarito virus
--Lassa virus

--Junin virus (except the candid #1 vaccine strain listed in Appendix B-II-D, Risk Group 2 (RG2) - Viruses - -Machupo virus
--Sabia

Bunyaviruses (Nairovirus) --Crimean-
Congo hemorrhagic fever virus

Filoviruses
--Ebola virus
--Marburg virus

Flaviruses - Group B Arboviruses
--Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses

Herpesviruses (alpha)
--Herpesvirus simiae (Herpes B or Monkey B virus)

Paramyxoviruses --
Equine morbillivirus

Hemorrhagic fever agents and viruses as yet undefined

This list was adapted from the NIH Guidelines for Research Involving Recombinant DNA Molecules Appendix B, October 2011. The reader should always check the most current listing or refer to the ABSA Risk Group Database:
https://my.absa.org/tiki-index.php?page=Riskgroups

Appendix I- Equipment Release Form

Equipment Release Form

Date:_____ Location of Origin:

Principal Investigator: _____

Destination/Service Department: _____

Service to Be Performed: _____

_____

_____

Type of Equipment:

_____

_____

Contaminated (Yes/No):_____

Contaminants Identified/Suspected:

_____

_____

_____

_____

Method of Decontamination:_____

_____

_____

_____

_____

Name of Person Decontaminating:

I certify that the above listed equipment is free of contamination or hazardous agents, and that it is safe to release to unrestricted areas and perform the work described above on this equipment.

_____

Signature of Responsible Person

Appendix J- Exposure Response Procedures

Exposure Response Procedure:
Potentially Infectious Materials

Potentially infectious materials in the lab include items such as: cell culture, serum, environmental specimens that may contain pathogens, or any items contaminated with such material.

A potentially infectious material exposure incident occurs when potentially infectious materials:

- Come into contact with a worker's mucous membranes (eyes, nose, or mouth)
- Enter the body through possible breaks in the skin
- Are accidentally ingested

Example incidents include:

- Splashing cell culture waste into your eye
- Puncturing your finger with a piece of glass that is contaminated with blood
- Spilling liquids that may contain pathogens onto an open wound on your hand

What to Do In The Event of an Exposure

When an exposure incident occurs, immediate response is the key to reducing your risk of getting a laboratory-acquired infection. Take these 3 actions:

1. **Flush the exposed area with water.** If your eyes, nose, or mouth were exposed to blood or other potentially infectious materials, flush these areas for 15 minutes. If your skin was exposed, thoroughly wash these areas with soap and water. Bandage the affected area if needed to control the bleeding;

2. **Notify your supervisor if he or she is available;**

3. **If medical attention is needed, call 911 or 765-285-1111.** Take a copy of the *Biological Safety Data Sheet*, or other information on the infectious agent with you to the emergency room.

4. **Report for post-exposure follow-up as soon as possible (immediately if exposure is to human-derived materials).** Refer to the Emergency Contact Information page at the front of this *Biological Safety Manual*; and,

5. **Report the incident to the EHS Office** at 5-2807 or tlrussell@bsu.edu for investigation, follow-up and any necessary reporting to regulatory agencies.

For further information on potentially infectious materials exposures, contact the EHS at 765-285-2807.

Appendix K- Example Inventory Log

Inventory Log

	BSU #	Organism name	Characteristics	Source	Quantity Received	Received From	Date Received	Storage location	Logged By	Date of last Activity	Activity Reference #
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											

Appendix L: Biosafety Practices for Handling Prions

Recommended Biosafety Practices for Handling Prions and Prion-Infected Tissues

Updated May 2007

Introduction

Research-related activities involving prions or tissues containing prions have been on the rise at BSU in both the animal health and human health arenas. Because the infectious nature of prions is not well characterized and destruction of these particles goes beyond the techniques typically required for biohazard inactivation, work with these agents requires special considerations for biocontainment to minimize both occupational and environmental exposure risk.

Prions & General Biosafety Recommendations

Prions (proteinaceous infectious particles, an abnormal isoform of a normal cellular protein) cause Creutzfeldt-Jakob disease (CJD), scrapie and other related human and animal neurodegenerative diseases. Human prions are manipulated at Biosafety Level (BSL) 2 or 3, depending on the activity, with most human prions treated as BSL-3 under most experimental conditions. In many instances, BSE prions can also be manipulated at BSL-2, however due to the high probability that BSE prions have been transmitted to humans, certain circumstances may require the use of BSL-3 facilities. All other animal prions are considered BSL-2 pathogens. However, when a prion from one species is inoculated into another the resultant infected animal should be treated according to the guidelines applying to the source of the inoculum. Please see the following table adapted from the BMBL for a list of common mammalian prions and general BSL recommendation.

Note: *Biosafety level assignment should be established using a risk assessment that accounts for the nature and host range of the agent, as well as the nature of the procedures and concentration and quantity of the agent.*

Table: The Prion Diseases

Disease (abbreviation)	Natural Host	Prion	Pathogenic PrP Isoform	Biosafety Level
Scrapie	sheep, goats and mouflon	scrapie prion	$OvPrP^{Sc}$	2
Transmissible mink encephalopathy (TME)	mink	TME prion	$MkPrP^{Sc}$	2
Chronic wasting disease (CWD)	mule deer, elk and white tail deer	CWD	$MdePrP^{Sc}$	2
Bovine spongiform encephalopathy (BSE)	cattle	BSE prion	$BoPrP^{Sc}$	2/3
Feline spongiform encephalopathy (FSE)	cats	FSE prion	$FePrP^{Sc}$	2

Exotic ungulate encephalopathy (EUE)	nyala, greater kudu and oryx	EUE prion	$UngPrP^{Sc}$	2
Kuru	humans	kuru prion	$HuPrP^{Sc}$	2/3
Creutzfeldt-Jakob disease (CJD)	humans	CJD prion	$HuPrP^{Sc}$	2/3
Gerstmann-Sträussler-Scheinker syndrome (GSS)	humans	GSS prion	$HuPrP^{Sc}$	2/3
Fatal familial insomnia (FFI)	humans	FFI prion	$HuPrP^{Sc}$	2/3

BMBL, 5th ed., 2007

The highest concentration of prions is found in the central nervous system (CNS), and extreme caution must be exerted when handling CNS samples. However prions can also be found in the CSF, lung, liver, kidney, spleen/lymph nodes, placenta. Unfixed samples of brain or spinal cord, as well as other tissues known to contain human prions should be handled at BSL-3. With regards to BSE prions, it is also recommended that animal tissue samples (e.g., brain, spinal cord) known or strongly suspected to contain prions be handled at BSL-3 (BMBL 2007). For other samples, the level of containment will depend on the type of tissue handled, the nature of the manipulation and the amount of material handled (MSDS 1997).

Formaldehyde or formal in-fixed, glutaraldehyde-fixed and paraffin-embedded tissues, particularly of the brain, remain infectious for long periods, if not indefinitely (BMBL 2007, WHO 2000). They should be handled cautiously as fresh materials from fixation through embedding, sectioning, staining and mounting on slides, unless treated with 95% formic acid (WHO 2000).

Although there are no documented laboratory-acquired prion infections, the primary hazard is from accidental parenteral inoculation or ingestion. Cuts and punctures should be avoided and the use of sharp knives, scalpels, blades and needles should be minimized. If the use of sharps cannot be avoided, cut-resistant gloves should be worn (CFIA 2005).

Wherever possible, the laboratory and equipment used for work with prions should be dedicated to that task alone. All employees should be informed and aware that prion research is being conducted in the lab. The entrance to the lab should allow for the separation of PPE/lab clothing and staff clothing. An exposure protocol should be developed, posted and communicated to all employees (CFIA 2005, UCSD 2002). Procedures should be in place for the effective decontamination of all waste, re-usable equipment, surfaces and other lab space (CFIA 2005, UCSD 2002).

Working with Prion-Risk Materials at BSU

At this time, work with prion-risk materials at BSU is limited to research and diagnostic laboratory applications. Therefore, this guidance document applies to these procedures only. Guidelines for use of prion-risk materials in conjunction with live animals will be developed if needed. Therefore, if future project plans call for use of live animals and prion-risk materials, please notify the BSU Biosafety Officer at the proposal-writing stage to perform a risk assessment and identify containment requirements.

Procedures involving the manipulation of animal tissues that are from known or suspected scrapie or CWD cases must be handled under BSL-2 conditions as a minimum standard. Procedures involving manipulation of human tissues that are known or suspected cases of CJD must typically be handled at BSL-3 conditions, unless a risk assessment completed in conjunction with an EHS Biosafety Professional allows for BSL-2 facilities and procedures. In general, procedures that involve aerosolization or vigorous disruption of the material (i.e., centrifugation, sonication, laser dissection) bear the greatest risk to personnel and the environment and will require special consideration for containment at both biosafety levels.

A summary of BSL-2 and BSL-3 facility and procedural requirements as outlined in the BMBL is attached at the end of this document. Additionally, the following specific measures should be implemented for all work with prion-risk materials:

1. Access to the laboratory must be restricted to trained personnel when work is being conducted on tissue.
2. Personnel working with prion-risk materials must complete Biosafety Principles for Animal Users through the EHS, as well as complete on-site training relative to the nature of the prion in use, routes of transmission, and specific hazards of the tissue handling process. Written procedures and training records should be kept as outlined in the BMBL.
3. Personnel must wear gloves and gowns while handling tissues that are potentially contaminated. All protective clothing must be removed before leaving the laboratory.
4. All fixed, non-fixed, or frozen tissues must be contained within watertight containers. Containers must be individually labeled with the universal biohazard symbol or placed in a secondary container (i.e., a tray with sides) that is labeled with the universal biohazard symbol.
5. Sonication or homogenization of tissues must be performed in a properly certified Class II biosafety cabinet.
6. Microtome blades and knives used for cutting tissue must be cleaned with an instrument that does not put the hand or finger of the operator in or near contact with the blade.
7. Disposable, absorbent pads or disposable trays should be used whenever possible to help confine contamination and to facilitate cleanup and disinfection.
8. The following practices should be followed when using reusable instruments:
    - Instruments should be kept wet until cleaned and decontaminated;
    - Instruments should be cleaned as soon as possible to prevent drying of material;
    - Do not mix instruments used on materials potentially infected with prions with those instruments used for other purposes;
    - Instruments that will be cleaned in a dishwasher must be decontaminated first and the washer must be run through an empty cycle before being used for other instruments
9. The following provisions for decontamination of wastes, reusable instruments and contaminated surfaces must be followed to assure effective inactivation of prions:
    - **Liquid waste** Liquid waste may be treated

    in the following ways:

- Mix with NaOH for a final concentration of 1.0 N NaOH and hold at room temperature for 1 hour; or
- Mix with bleach for a final concentration of 20,000 ppm available chlorine and hold at room temperature for 1 hour

This waste should be stored in a chemical fume hood for the duration of the treatment period. After the treatment period, liquid waste may be neutralized and discharged to the sewer by way of the lab sink, or disposed of through the EHS as liquid chemical waste.

- **Contaminated surfaces**

Contaminated surfaces may be treated in the following ways:

- Bleach solution (20,000 ppm available chlorine) for 1 hour; or
- 1N NaOH for 1 hour

After treatment, surfaces should be thoroughly rinsed with clear water.

- **Contaminated reusable instruments**

Contaminated reusable instruments may be treated in the following ways:

- Immerse in 1N NaOH or sodium hypochlorite (20,000 ppm available chlorine) for 1 hour, transfer to water, autoclave (gravity displacement) at 121°C for 1 hour (BMBL 2007, WHO 2000);
- Immerse in 1N NaOH or sodium hypochlorite (20,000 ppm available chlorine) 1 hour, rinse with water, autoclave at 121°C for 1 hour (gravity displacement) or at 134 °C for I hour (porous load) (BMBL 2007, WHO 2000); or
- Immerse in sodium hypochlorite solution with 20,000 ppm available chlorine (preferred) or 1N NaOH (alternative) for 1 hour (WHO 2000)

- **Contaminated dry waste**

All contaminated dry waste should be picked up for incineration. Prion-contaminated sharps waste must be identified as “prion contaminated sharps- for incineration only” on the hazardous waste pickup request to assure incineration of these materials. Contact the EHS Biosafety Staff for further assistance regarding treatment and disposal.

10. Intact skin exposure to prion-risk materials should be followed by washing with 1N NaOH or 10% bleach for two to three minutes, followed by extensive washing with water. For needle sticks or lacerations, gently encourage bleeding, wash with warm soapy water, rinse, dry and cover with a waterproof dressing. In the event of a splash to the eye, rinse the affected eye with copious amounts of water or saline **only**. In the instance of a splash or puncture, the exposed individual should then report to Olin Urgent Care for follow-up through BSU Occupational Health.
11. The Principal Investigator (PI) must assure that all spills or exposures involving prion-risk materials are managed with the proper procedures. Additionally, these events should be reported to the BSU Biosafety Officer as soon as possible for follow-up and assistance with actions to reduce future occurrences.
12. Prion-risk materials may be subject to permit requirements for shipment and receipt. USDA permits apply to interstate and international shipment of animal-related materials capable of transmitting infection. CDC permits apply to import of materials that are

potentially infectious to humans. Additionally, shipment of these materials requires specific training for the shipper. Contact the EHS Biosafety Staff for further information.

Notes on chemical disinfection

Sodium hydroxide (NaOH, or soda lye): Be familiar with and observe safety guidelines for working with NaOH. 1N NaOH is a solution of 40 g NaOH in 1 liter of water. 1 N NaOH readily reacts with CO2 in air to form carbonates that neutralize NaOH and diminish its disinfective properties. 10 N NaOH solutions do not absorb CO2, therefore, 1N NaOH working solutions should be prepared fresh for each use either from solid NaOH pellets, or by dilution of 10 N NaOH stock solutions.

Sodium hypochlorite (NaOCl solution, or bleach): Be familiar with and observe safety guidelines for working with sodium hypochlorite. Household or industrial strength bleach is sold at different concentrations so a standard dilution cannot be specified. Efficacy depends upon the concentration of available chlorine and should be 20,000 ppm available chlorine.

These solutions are corrosive and appropriate personal protective equipment must be worn when preparing and using them.

Appendix M-Requirements for Handling Exempt Strains of Select Agents

CONTAINMENT AND SECURITY REQUIREMENTS FOR HANDLING EXEMPT STRAINS OF SELECT AGENTS

Introduction:

The United States Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA) have established regulations for the possession, use and transfer of select agents and toxins (see 42 CFR Part 73, 7 CFR Part 331 and 9 CFR Part 121). These regulations have also established a procedure by which an attenuated strain of a select agent that does not pose a severe threat to public health and safety, animal health, or animal products may be excluded from the requirements of the regulations when used for specific purposes. Please note that if an excluded attenuated strain is manipulated in such a way that virulence is restored or enhanced, or if factors associated with virulence are reintroduced, it will then be subject to the regulations. Because of the nature of these exempt strains and the potential for them to be manipulated for use as a biological weapon, the Office of Environmental Health and Safety has implemented the following containment and security requirements for handling exempt strains of select agents.

Applicability:

The containment and security requirements apply to the following exempt strains of select agents:

- *Bacillus anthracis* strains devoid of both plasmids pX01 and pX02
- *Bacillus anthracis* strains devoid of the plasmid pX02 (e.g., Bacillus anthracis Sterne, $pX01^{+}pX02^{-}$)
- *Brucella abortus* strain RB51 (vaccine strain)
- *Brucella abortus* strain 19
- *Coxiella burnetii* Phase II, Nine Mile Strain, plaque purified clone 4
- *Francisella tularensis* subspecies *novicida* (also referred to as *Francisella novicida*) strain, Utah 112 (ATCC 15482)
- *Francisella tularensis* subspecies *holartica* LVS (live vaccine strain; includes NDBR 101 lots, TSI-GSD lots, and ATCC 29684)
- *Francisella tularensis* ATCC 6223 (also known as strain B38)
- Rift Valley fever virus, MP-12 vaccine strain
- Venezuelan equine encephalitis virus, TC-83 strain
- Venezuelan equine encephalitis virus vaccine candidate strain V3526
- Highly pathogenic avian influenza virus, recombinant vaccine reference strains of the H5N1 and H5N3 subtypes
- Japanese encephalitis virus, SA-14-14-2 strain

Requirements:

After conducting a risk assessment, EHS has determined that biosafety level 2 precautions in addition to specific security measures are not only appropriate, but prudent practice for handling exempt strains of select agents. Therefore the following requirements have been implemented:

All biosafety level 2 practices, safety equipment and facility requirements must be followed. For specific information on those requirements please contact Dr. Jamie Sue Willard-Smith (353-1877) or Amber Bitters (432-5262):

A. *Standard Microbiological Practices*

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when

experiments are in progress.

2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
9. An insect and rodent control program is in effect.

B. *Special Practices*

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.
2. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate signage will be provided by the EHS.
3. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).
4. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
5. Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
6. The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
7. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

    a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.

    b. Only needle-locking syringes or disposable syringe needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in

conveniently located puncture- resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

c. Syringes which re-sheathe the needle, needleless systems, and other safety devices are used when appropriate.

d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

9. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
10. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.
11. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
12. Animals not involved in the work being performed are not permitted in the lab.

C. *Safety Equipment* (Primary Barriers)

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
    e. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.
    f. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.
2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.
3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching “clean” surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

D. *Laboratory Facilities* (Secondary Barriers)

1. Provide lockable doors for facilities that house restricted agents (as defined in 42 CFR 72.6).

2. Consider locating new laboratories away from public areas.
3. Each laboratory contains a sink for handwashing.
4. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
5. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
6. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
7. Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.
8. An eyewash station is readily available.
9. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
10. There are no specific ventilation requirements. 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. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

- The following security measures must be adhered to:
  - o An accurate and up-to-date inventory must be maintained. The following information must be included in the inventory:
    - Date of use
    - Name of person using the materials
    - Beginning amount of material
    - Amount of material used for procedure
    - End amount of material
    - Procedure the material was used for
  - o All exempt strains of select agents (i.e., stock solutions, working solutions, etc.) must be stored in a lockable storage unit;
  - o Storage units that house exempt strains of select agents must be kept locked when not actively being used; and
  - o Only those people approved by the principal investigator and the EHS may have access to the strains.

- Please notify the Biosafety Officer at 285-2807 or ORI at 285-5070 if you possess or plan to possess any of the exempted select agent strains. A lab inspection must be conducted prior to working with these agents.

- If inconsistencies exist with the inventory please contact the EHS at 285-2807.

Contacts: Any questions regarding these requirements should be directed to the EHS Office (285-2807)

Appendix N: Dual Use Chemicals

United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern

Section I: Purpose and Principles

1) The purpose of this Policy is to establish regular review of United States Government funded or conducted research with certain high-consequence pathogens and toxins for its potential to be dual use research of concern (DURC) in order to: (a) mitigate risks where appropriate; and (b) collect information needed to inform the development of an updated policy, as needed, for the oversight of DURC. The fundamental aim of this oversight is to preserve the benefits of life sciences research while minimizing the risk of misuse of the knowledge, information, products, or technologies provided by such research.
2) This Policy complements existing United States Government regulations and policies governing the possession and handling of pathogens and toxins. Currently, the Select Agent Regulations ensure appropriate oversight of biosafety and biosecurity of the possession and handling of pathogens and toxins that have the potential to pose a severe threat to human, animal, or plant health, or to animal and plant products. In addition, recommendations from Federal advisory bodies such as the National Science Advisory Board for Biosecurity (NSABB) have helped inform United States Government policies for identifying and managing DURC. This Policy will be updated, as needed, following domestic dialogue, engagement with our international partners, and input from interested communities including scientists, national security officials, and global health specialists.
3) The following principles guide implementation of this Policy:
    a) Life sciences research is essential to the scientific advances that underpin improvements in the health and safety of the public, agricultural crops and other plants, animals, the environment, materiel, and national security. Despite its value and benefits, some research may provide knowledge, information, products, or technologies that could be misused for harmful purposes.
    b) Accordingly, some degree of Federal and institutional oversight of DURC is critical to reducing the risks to public health and safety, agricultural crops and other plants, animals, the environment, materiel, and national security.
    c) Measures that mitigate the risks of DURC should be applied, where appropriate, in a manner that minimizes, to the extent possible, adverse impact on legitimate research, is commensurate with the risk, includes flexible approaches that leverage existing processes, and endeavors to preserve and foster the benefits of research.
    d) The United States Government will facilitate the sharing of the results and products of life sciences research conducted or funded by United States Government agencies, and honor United States Government obligations within relevant international frameworks and agreements, while taking into account United States' national security interests.
    e) In executing this Policy, the United States Government will abide by and enforce all relevant Presidential Directives and Executive Orders, all applicable laws and regulations, and support the implementation of legally binding treaties, commitments, and United Nations Security Council resolutions prohibiting the development and use of biological agents as weapons.

Section II: Definitions

1) For the purpose of this Policy, DURC is 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[1].
2) "Life sciences" pertains to living organisms (e.g., microbes, human beings, animals, and plants) and their products, including all disciplines and methodologies of biology such as aerobiology, agricultural science, plant science, animal science, bioinformatics, genomics, proteomics, synthetic biology, environmental science, public health, modeling, engineering of living systems, and all applications of the biological sciences. The term is meant to encompass the diverse approaches for understanding life at the level of ecosystems, organisms,

organs, tissues, cells, and molecules.

3) Extramural research is that which is funded by a department or agency under a grant, contract, cooperative agreement, or other agreement and not conducted directly by the department or agency.
4) Intramural research is that which is directly conducted by a department or agency.

Section III: Scope

Under this Policy, review will focus on research that involves one or more of the agents or toxins listed in Section (III.1) below, which pose the greatest risk of deliberate misuse with most significant potential for mass cBSUalties or devastating effects to the economy, critical infrastructure, or public confidence, and produces, aims to produce, or is reasonably anticipated to produce one or more of the effects listed in Section (III.2) below:

1) Agents and toxins[2]:
   a) Avian influenza virus (highly pathogenic)
   b) Bacillus anthracis
   c) Botulinum neurotoxin
   d) Burkholderia mallei
   e) Burkholderia pseudomallei
   f) Ebola virus
   g) Foot-and-mouth disease virus
   h) Francisella tularensis
   i) Marburg virus
   j) Reconstructed 1918 Influenza virus
   k) Rinderpest virus
   l) Toxin-producing strains of *Clostridium botulinum*
   m) Variola major virus
   n) Variola minor virus
   o) Yersinia pestis
2) Categories of experiments:
   a) Enhances the harmful consequences of the agent or toxin;
   b) Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification;
   c) Confers to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies;
   d) Increases the stability, transmissibility, or the ability to disseminate the agent or toxin;
   e) Alters the host range or tropism of the agent or toxin;

[1] This definition of DURC is derived from the NSABB definition, but is modified for purposes of this Policy.

[2] These agents and toxins are regulated by the Select Agent Program under Federal Law (7 C.F.R. part 331, 9 C.F.R. part 121, and 42 C.F.R. part 73), and have the potential to pose a severe threat to human, animal, or plant health, or to animal and plant products.

   f) Enhances the susceptibility of a host population to the agent or toxin; or
   g) Generates or reconstitutes an eradicated or extinct agent or toxin listed in Section (III.1) above.

Section IV: Department and Agency Responsibilities

1) Federal departments and agencies that conduct or fund life sciences research should implement the following actions:
   a) Conduct a review to identify all current or proposed, unclassified intramural or extramural, life sciences research projects that fall within the scope of Section III. This review will include, at a minimum, initial proposals and any progress reports.
   b) Determine which, if any, of the projects identified in Section (IV.1.a) meet the definition of DURC in Section (II.1) of this document.
   c) Assess the risks and benefits of such projects, including how research methodologies may generate risks and/or whether open access to the knowledge, information, products, or technologies generates risk.

d) Based on the risk assessment, in collaboration with the institution or researcher, develop a risk mitigation plan to apply any necessary and appropriate risk mitigation measures. In addition:

i) For DURC that is proposed and not yet funded, departments and agencies will assess whether to incorporate risk mitigation measures in the grant, contract, or agreement.

ii) For currently funded DURC, funding departments and agencies will consider modifying the grant, contract, or agreement to incorporate risk mitigation measures. If such modifications are not possible or desirable, departments and agencies will seek voluntary implementation of mitigation measures by the institution.

e) A risk mitigation plan may include, but not be limited to, the following risk mitigation measures:

i) Modifying the design or conduct of the research.

ii) Applying specific or enhanced biosecurity or biosafety measures.

iii) Evaluating existing evidence of medical countermeasures (MCM) efficacy, or conducting experiments to determine MCM efficacy against agents or toxins resulting from DURC, and where effective MCM exist, including that information in publications.

iv) Referring the institution to available DURC educational tools such as: http://oba.od.nih.gov/biosecurity/biosecurity.html

v) Regularly reviewing, at the institutional level, emerging research findings for additional DURC.

vi) Requesting that institutions notify funding departments or agencies if additional DURC is identified, and propose modifications to the risk mitigation plan, as needed.

vii) Determining the venue and mode of communication (addressing content, timing, and possibly the extent of distribution of the information) to communicate the research responsibly.

viii) Reviewing annual progress reports from Principal Investigators to determine if DURC results have been generated, and if so, flagging them for institutional attention and applying potential mitigation measures as described above, as necessary.

ix) If the risks posed by the research cannot be adequately mitigated with the measures above, Federal departments and agencies will determine whether it is appropriate to:

(a) Request voluntary redaction of the research publications or communications [3];

(b) Classify the research:

(i) In accordance with National Security Decision Directive/NSDD-189, departments and agencies will make classification determinations withinthe scope of their classification authorities and appropriate classification guidelines or may consult with other departments and agencies to make these determinations.

(ii) Departments and agencies may consider whether to refer classified research to another department or agency for funding.

(c) Not provide or terminate research funding.

2) Federal departments and agencies are requested to report the following to the Assistant to the President for Homeland Security and Counterterrorism:

a) Within 60 days of issuance of this Policy, the following results of the review conducted in response to Section (IV.1.a):

i) Aggregate number of current and proposed unclassified, intramural, and extramural research projects identified that include work with one or more of the agents and toxins in Section (III.1).

ii) Aggregate number of current and proposed unclassified, intramural, and extramural research projects that include work with one or more of the agents and toxins in Section (III.1) and produces, aims to produce, or are reasonably anticipated to produce one or more of the effects listed in Section (III.2).

b) Within 90 days of issuance of this Policy, the following results of the review conducted in response to Sections (IV.1. b. c. and d):

i) Number of unclassified current and proposed DURC projects. [4]

ii) Number of current projects identified as DURC through initial proposals versus progress reports. [5]

iii) Summary of risks, mitigation measures already in place that address those risks, any additional mitigation measures that have been proposed or implemented, and number of projects to which each mitigation measure would be applied.

3) Following completion of the reporting requirements in Section (IV.2), Federal departments and

agencies are requested to submit periodic reports on items in Section (IV.2.a. and b) biannually.

4) Federal departments and agencies should implement Section IV in accordance with their relevant and applicable authorities, regulations, and statutes.

5) For additional guidance on how to conduct the risk assessment identified in Section (IV. 1.c), departments and agencies may refer to the "Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information," which identifies useful assessment tools and is available at: http://oba.od.nih.gov/biosecurity/biosecuritydocuments.html .

Section V: Consultation

As necessary and appropriate, the United States Government will continue to consult with the NSABB (in compliance with provisions of the Federal Advisory Committee Act) or convene the Countering Biological Threats Interagency Policy Committee for guidance on matters relating to the review and conduct of DURC and the mitigation of DURC risks.

[4,5] Report the number of projects by agent and/or toxin plus the category of experiment.

Appendix O: Site-Specific Training Checklist

Required on-site training	Required for: Chemical	Biological	Bloodborne	Complete
Location and Review of Safety Protocol Guides				
Emergency Contacts	X	X	X	
Chemical Hygiene Plan	X	X	X	
MSDS	X	X	X	
Hazardous Waste Guide	X	X	X	
Standard Operating Procedures (task specific)	X	X	X	
Emergency Response Procedures	X	X	X	
Biological Safety Manual		X	X	
Biohazardous Waste Plan		X	X	
Exposure Incident Response Procedure		X	X	
Exposure Control Plan			X	
Source Protocol			X	
Inventory, Storage, Labeling, and Proper Use of:				
Chemical Storage	X	X	X	
Hazardous Chemicals	X	X	X	
Biohazardous Materials		X	X	
Location, Proper Use, and Maintenance of:				
Personal Protective Equipment	X	X	X	
Emergency Eyewash/Shower	X	X	X	
Fume Hood	X	X	X	
Compressed Gasses	X	X	X	
Chemical Spill Kit	X	X	X	
Biological Spill Kit		X	X	
Biosafety Cabinet		X	X	
Laminar Flow Hood		X	X	
Autoclaves		X	X	
Disinfectants		X	X	
Safer Sharps			X	
Waste Segregation, Storage, Transport, and Treatment				
Sharps Waste	X	X	X	
Glass Waste	X	X	X	
Solid Waste	X	X	X	
Liquid Waste	X	X	X	
Waste Tags	X	X	X	
90 day Disposal	X	X	X	
Transport	X	X	X	
Treatment		X	X	
Security				
Laboratory Security	X	X	X	
Inventory	X	X	X	

Emergency Contacts. Same as posted on door signs
Chemical Hygiene Plan. Online or hard copy in lab and present upon inspection
MSDS. know location and present upon inspection
Hazardous Waste Guide. Online or hard copy in lab and present upon inspection
Standard Operating Procedures. Online or hard copy in lab and present upon inspection

Emergency Response Procedures. Post in prominent place in lab or near phone
Biological Safety Manual. Hard copy in lab and present upon inspection
Biohazardous Waste Plan. Hard copy in lab and present upon inspection
Exposure Incident Response Procedure. Post in prominent place
Exposure Control Plan. Hard copy in lab and present upon inspection
Source Protocol. Hard copy in lab and present upon inspection
Chemical Storage. Know what types are stored where and how to label
Hazardous Chemicals. Know what types are stored where and how to label
Biohazardous Materials. Know what types are stored where and how to label
Personnel Protective Equipment. know what types, when to use, and how to maintain them
Emergency Eyewash/Shower. Know location and maintenance
Fume Hood. Know when and how to use
Compressed Gasses. Know how and when to use
Chemical Spill Kit. Location and maintenance
Biological Spill Kit. Location and maintenance
Biosafety Cabinet/Laminar Flow Hood. Location, use and maintenance including certification
Autoclaves. Location, use and maintenance including certification
Disinfectants. Location, use, concentration, MSDS, expiration and disposal
Safer Sharps. Use, annual review, and evaluation
Sharps/Glass/Solid/Liquid Waste. Location, labeling, use and disposal of container
Waste Tags. Use
90 day Disposal. which wastes fall under this law
Transport. secondary container use
Treatment. how to treat each type of waste
Laboratory Security. Aware of security plan for BSU, department policies, and lab policy
Inventory. Online or hard copy of hazardous/biohazardous material, present upon inspection

_____
(Print Employee's/Student Name)

_____
(Manager/Precept/Trainer signature. Date) (Faculty/Student/Employee Signature. Date)

I certify that the site specific training items were reviewed and understood as required by the BSU EHS.
(This must be completed and signed at each facility the student or employee is working in)

Appendix P: Biohazard Registration Document

BALL STATE UNIVERSITY. University/Institutional Biosafety Committee	**Biological Agent Document of Registration**

(Added by BSO) *[**Credit**: Form developed by Northern Arizona University]*

Directions:

- The content of this IBC application should describe all work with biohazardous materials (including rDNA and synthetic nucleic acids placed in a biological system) to be conducted by a Principal Investigator (PI). Therefore, it may cover multiple projects as long as all the work (e.g. biological materials, personnel, & locations) are similar and all the procedures involving biohazardous materials are described comprehensively on this application.
- There is no limit to the number of applications a single investigator can submit, nor are there limitations as to how often this application is updated to incorporate an increase or change in scope.
- If you need assistance filling out this form, please contact Tom Russell, the BSU Biological Safety Officer (BSO), at (765) 285-2807 or tlrussell@bsu.edu.
- Submit the completed application electronically to tlrussell@bsu.edu, or mail (Tom Russell, NS112, BSU, 3401 N. Tillotson Ave., Muncie IN 47306. Retain a copy of your completed application for your records.

Section 1: General Information

Principal Investigator: _____ Title: _____

Department: _____ Campus Address: _____

Primary work phone #: _____ Email: _____

Laboratory Contact: _____ Title: _____

Department: _____ Campus Address: _____

Primary work phone #: _____ Email: _____

Project Title: **Location of Project** Bldg. #: Project Room(s):	**Will animals* be used?** ☐ Yes ☐ No Housing & Procedure Room(s): IACUC Protocol #: Approval Date:
Will human subjects be used? ☐ Yes ☐ No IRB Protocol #: Approval Date:	**Will isotopes be used?** ☐ Yes ☐ No RSC Protocol #: Approval Date:

* If using or creating transgenic animals, please complete the rDNA – Transgenic Animals Registration Form

Project Funding Status (check all that apply):

	Status	Name of Granting Agency (or other source)	Title of Award
☐	Fully or partly funded by federal grant		
	Pending funding by federal grant		

☐			
☐	Funding to be provided by BSU		
☐	Funding provided by other organization		

Section 2: Biological Agents

List each agent and the biosafety level/risk category associated with it.

Agent (genus, species, & common name {if applicable})	**Biosafety level/Risk category**

Section 3: Description of Project

Provide a brief description and overview of your research goals and objectives involving biohazardous agents, recombinant DNA (rDNA), and/or synthetic nucleic acids placed in a biological system. This should be the general area under study, not the detailed experimental methods; however, sufficient information must be presented so that the IBC may understand the general scope of the work. Research descriptions should be readily comprehensible by a lay-person and free from technical jargon (unless unavoidable; then briefly define/describe the term). Try to include: overall objectives; the source(s) of biological material, describe host(s) and vector(s) to be used. If research involves rDNA: what is the nature of the inserted DNA sequences (genetic modification); will a deliberate attempt by made to express a foreign gene; indicate the protein or transcript that will be produced.

Section 4: Scope of Research

1. A) Will you transport or ship biological agents/infectious substances/diagnostic specimens?
   ☐ Yes ☐ No
   B) Have you and your staff taken a Shipping & Transport of Biohazardous Materials class in the last 2 years?
   ☐ Yes ☐ No If yes, please provide the class date:

2. *If you answer 'yes' to any question below, please fill out a **Risk Assessment** for the agent(s), the Biological Safety Officer is available for assistance**

   A) Will you manipulate a naturally occurring pathogen(s), one that has not been altered by rDNA, which is infectious to humans, animals, or plants?
   ☐ Yes ☐ No

   B) Will you work with non-human primate body fluids, tissues, or cell cultures?
   ☐ Yes ☐ No

   C) Will you work with human body fluids, human tissues, and/or human cell cultures?
   ☐ Yes ☐ No If yes, please describe:

   If yes, have the researchers been registered in the bloodborne pathogen program?
   ☐ Yes ☐ No

If yes, please provide the names of personnel:

3. Will you acquire, store, generate, or manipulate animal (other than human and non-human primate) cells and/or tissue samples?
   ☐ Yes ☐ No

4. Will you acquire, store, generate, or manipulate any regulated agents, select agents, and/or biotoxins?
   ☐ Yes ☐ No

5. Permits and Licenses:

   A) If your project will involve a human pathogen or human material that originated outside of the USA, a CDC Etiologic Agent Import Permit may be required. Please visit their website for information.

   B) If your project uses infectious agents of livestock and biological materials containing animal material, a USDA/APHIS permit may be required. Visit their website.

   C) U.S. Fish and Wildlife Service permits are required for certain live animals, including bats. Visit their website or call 1-800-344-WILD for more information.

   D) If your project will involve the import of select agents and toxins, you must be registered with CDC's Select Agent Program. Contact the BSU BSO, Tom Russell, for more information.

   E) If your project will involve the export of a wide variety of etiologic agents of human, plant, and animal diseases, a license from the Department of Commerce may be required. Visit their website or call (202) 482-4811 for more information.

   F) If the appropriate permit(s) have already been obtained, please list the applicable permit numbers:

Section 5: Biosafety Considerations & Procedures

Biosafety cabinet: ☐ Yes ☐ No Model: Location: Certification date:	Autoclave available: ☐ Yes ☐ No Location: QA/QC monitored by:
Personal protective equipment used (list): Method of inactivation of agent(s): Disinfection of surfaces procedure:	How do you dispose of biohazardous waste? Are Standard Operating Procedures (SOPs) prepared for work with the agent(s)? ☐ Yes ☐ No Hand washing sink available? ☐ Yes ☐ No

1. Will you work with biological agents with/in any of the following aerosol-producing devices/procedures?

   ☐ Centrifuges
   ☐ Sonicators
   ☐ Intranasal inoculation of animals
   ☐ Flow Cytometer
   ☐ Vortexers
   ☐ Tissue homogenizers
   ☐ Pressurized vessels (besides autoclaves)
   ☐ Shakers
   ☐ Infected animal necropsy
   ☐ Large volumes (≥ 10L) of infectious material

2. Have staff received immunizations and/or tests for agents in use or that are potentially present in the lab (e.g. hepatitis B vaccine, Vaccinia vaccine, TB skin testing)?
   ☐ Yes ☐ No If yes, please list:

3. Will the protocols require the use of sharps, such as, but not limited to, needles, scalpels, and/or razor blades?

☐ Yes ☐ No If yes, please visit the EHS website to learn about the BSU Bloodborne Pathogens/Universal Precautions Program, then complete the evaluation form(s) as necessary.

Section 6: NIH Regulated Recombinant DNA and Synthetic Nucleic Acids Molecules Research

The NIH guidelines define recombinant and synthetic nucleic acids as:

i. Molecules that are constructed by joining nucleic acid molecules and can replicate in a living cell (i.e. recombinant nucleic acids);

ii. Nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acids (i.e. synthetic nucleic acids).

iii. Molecules that result from the replication of those described in (i) or (ii) above.

☐ A) My research does **not** involve rDNA or synthetic nucleic acids placed in a biological system.
If checked, proceed to Section 7

☐ B) My research does involve rDNA or synthetic nucleic acids placed in a biological system. (This includes plasmids, viral vectors, creation of transgenic organisms, gene therapy, etc.)
If checked, complete the rest of Section 6

Please select the classification for each type of recombinant/synthetic nucleic acid research performed in your laboratory below. Use the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules to help classify your research.

☐ **Section III-A-1-a:** The deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (see Section V-B, *Footnotes and References of Sections I-IV*), if such acquisition could compromise the ability to control disease agents in humans, veterinary medicine, or agriculture, will be reviewed by the RAC. (Requires IBC approval, RAC review, and NIH Director approval before research may begin).

☐ **Section III-B-1:** Experiments involving the cloning of toxin molecules with LD_{50} of less than 100 ng/kg of body weight (Requires NIH/OBA and IBC approval before research may begin).

☐ **Section III-C-1:** Experiments involving the deliberate transfer of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, into one or more human research participants (Requires IBC and RAC review before research may begin).

☐ **Section III-D-1:** Experiments using Risk Group 2, Risk Group 3, Risk Group 4, or restricted agents as host-vector systems. Please select risk group below:

- ☐ **Risk Group 2 (RG2):** Agents are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are often available.
- ☐ **Risk Group 3 (RG3):** Agents are associated with serious or lethal human disease for which preventative or therapeutic interventions may be available.

☐ **Section III-D-2:** Experiments in which DNA from Risk Group 2, Risk Group 3, or restricted agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems. Please select risk group below:

- ☐ **Risk Group 2 (RG2):** Agents are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are often available.
- ☐ **Risk Group 3 (RG3):** Agents are associated with serious or lethal human disease for which preventative or therapeutic interventions may be available.

☐ **Section III-D-3:** Experiments involving the use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems.
  ☐ **Risk Group 2 (RG2):** Agents are associated with human disease which is rarely serious and for which preventative or therapeutic interventions are often available.
  ☐ **Risk Group 3 (RG3):** Agents are associated with serious or lethal human disease for which preventative or therapeutic interventions may be available.

☐ **Section III-D-4:** Experiments involving whole animals that cannot be done at BSL-1

☐ **Section III-D-5:** Experiments involving whole plants; Experiments to genetically engineer plants by recombinant or synthetic nucleic acid molecule methods, to use such plants for other experimental purposes (e.g. response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant or synthetic nucleic acid molecules that cannot be done at BSL-1.

☐ **Section III-D-6:** Experiments involving more than 10 liters of culture.

☐ **Section III-D-7:** Experiments involving Influenza viruses.

☐ **Section III-E-1:** Experiments involving the formation of recombinant or synthetic nucleic acid molecules containing no more than 2/3 of the genome of any eukaryotic virus **(BSL-1 experiments only)**.

☐ **Section III-E-2:** Experiments involving recombinant DNA-modified whole plants, and /or experiments involving recombinant or synthetic nucleic acid molecule-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-D, or III-F **(BSL-1 experiments only)**.

☐ **Section III-E-3:** Experiments involving transgenic rodents modified by the stable introduction of recombinant or synthetic nucleic acid molecules into their genome, or nucleic acids derived therefrom, into the germ-line (transgenic rodents). **(BSL-1 experiments only)**.

NIH Exempt Experiments (subject to review by the IBC Chair and Biological Safety Officer):

☐ **Section III-F-1:** Experiments using synthetic nucleic acids that: (1) can neither replicate nor generate nucleic acids that can replicate in any living cell, (2) are not designed to integrate into DNA, and (3 do not produce a toxin that is lethal for vertebrates at an LD_{50} of <100 ng/kg.

☐ **Section III-F-2**: Recombinant/synthetic molecules are not in organisms, cells, or viruses, and that have not been modified or manipulated to make cellular membrane penetration possible.

☐ **Section III-F-3**: Recombinant/synthetic molecules that consist entirely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists in nature.

☐ **Section III-F-4:** Recombinant/synthetic molecules that consist entirely of DNA from a prokaryotic host including its indigenous plasmids, or viruses when propagated only in that host (or closely related strain of the same species), or when transferred to another host by well-established physiological means.

☐ **Section III-F-5:** Recombinant/synthetic molecules that consist entirely of DNA from an 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).

☐ **Section III-F-6:** Those that consist entirely of DNA segments form different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers is prepared and periodically revised by the NIH Director and can be found in the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules.

☐ **Section III-F-7:** Those genomic DNA molecules that have acquired a transposable element, provided the transposable element does not contain any recombinant and/or synthetic DNA.

☐ **Section III-F-8:** Those exemptions as determined by the NIH Director to not present a significant risk to health or the environment are listed in the appendices below. Please check all categories that apply:
  ☐ **Appendix C-I:** Recombinant or synthetic nucleic acid molecules in tissue culture.
  ☐ **Appendix C-II:** *Escherichia coli* K-12 host-vector systems.

☐ **Appendix C-III:** *Saccharomyces* host-vector systems.
☐ **Appendix C-IV**: *Kluyveromyces* host-vector systems.
☐ **Appendix C-V:** *Bacillus subtilis* or *Bacillus licheniformis* host-vector systems.
☐ **Appendix C-VI:** Extrachromosomal elements of gram positive organisms.
☐ **Appendix C-VII:** The purchase or transfer of transgenic rodents.
☐ **Appendix C-VIII:** Generation of BL1 transgenic rodents via breeding.

Recombinant or Synthetic Nucleic Acid Molecules Project Summary	
1. Does this research involve "the deliberate transfer of a drug resistance trait to microorganisms if such acquisition could compromise the ability to treat or manage disease agents in human and veterinary medicine, or agriculture?" ☐ Yes ☐ No If yes, explain:	2. Will the research involve the use of antibiotic selection markers? ☐ Yes ☐ No If yes, list the markers and the microbial agents used (e.g., kanamycin resistance marker in *E. coli*).
3. Are you increasing the pathogenicity of a pathogen? ☐ Yes ☐ No If yes, explain:	4. Will you be working with >10 liters of recombinant material? ☐ Yes ☐ No If yes, explain:
5. Are you working with genetic material coding for a vertebrate toxin as defined in the NIH Guidelines? ☐ Yes ☐ No If yes, explain:	6. List all cell lines to be used in the research, including the source species (Note that work with human or primate cell lines requires BSL2 containment.):
7. Purpose of the project (one sentence):	
8. Project goals/intent:	
9. Expected outcome:	
10. What will be analyzed or measured?	
11. What effect would transgene expression have in an accidental host?	
12. What are possible safety hazards associated with the recombinant/synthetic nucleic acid molecules component of this project, and how will you address them?	

13. Use the **table below** to detail recombinant or synthetic nucleic acid molecules used in this project. Please use one column per construct, using additional sheets as necessary. Additionally, please attach **vector/construct maps** (electronic versions are preferred, e.g. .bmp, .tif, .jpg, .pdf, but hard copies are acceptable)

	Construct 1	Construct 2	Construct 3	Construct 4	Construct 5	Example
DNA/Gene Source						Green fluorescent protein (GFP)
Gene Function						marker
Selectable Marker						Antibiotic resistance gene
Vector Name (*provide maps*)						pTR-UF12
Vector Type/ Origin						Viral/ Adeno-associated virus (AAV)

Expression control elements (promoter, enhancer, regulatory elements, etc)						MCMV enhancer, Chicken B-actin promoter
Conc / titer of rDNA (i.p./ml)						1x 10^8 to 1x10^{12} infectious particles/ml
Host & Strain						*E. coli*, Sure™, Mouse heart cells, in vivo
Host Range (including any genetic alterations to host range)						Human, other mammalian cells
Is recombinant or synthetic DNA made in your lab? If not, where?						UF Powell Gene Therapy Center
What (approx.) % of original vector genome has been deleted or substituted?						2/3

Section 7: Dual Use Research of Concern (DURC)

1. Does your research involve one or more of the agents or toxins listed below? ☐ Yes ☐ No
   - Avian influenza virus (highly pathogenic)
   - *Bacillus anthracis*
   - Botulinum neurotoxin
   - *Burkholderia mallei*
   - *Burkholderia pseudomallei*
   - Ebola virus
   - Foot-and-mouth disease virus
   - *Francisella tularensis*
   - Marburg virus
   - Toxin-producing strains of *Clostridium botulinum*
   - Variola major virus
   - Variola minor virus
   - *Yersinia pestis*
2. Please indicate whether your research project produces, aims to produce, or can be reasonably anticipated to produce any of the following experimental effects. The IRE should review descriptions of the research in question, the PI's assessment of the applicability of the categories of experiments, and other relevant information, as warranted. Examples of materials to consider include the project proposal, any project reports, any outcomes of previous reviews for dual use, and examples of similar research in the literature.
   - Enhances the harmful consequences of the agent or toxin ☐ Yes ☐ No
   - Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification ☐ Yes ☐ No

- Confers to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies ☐ Yes ☐ No
- Increases the stability, transmissibility, or the ability to disseminate the agent or toxin ☐ Yes ☐ No
- Alters the host range or tropism of the agent or toxin ☐ Yes ☐ No
- Enhances the susceptibility of a host population to the agent or toxin ☐ Yes ☐ No
- Generates or reconstitutes an eradicated or extinct agent or toxin listed above in Question 1 ☐ Yes ☐ No

3. If you checked yes for any of the experimental effects listed in Question 2, please provide more information:

Section 8: Personnel

Identify all personnel that will be involved in the project described above. By signing, they agree that they are familiar with and agree to abide by the current BSU and federal guidelines.

Name	NIH Guidelines rDNA Training/Quiz Date	Biosafety and/or Bloodborne Pathogens Training/Quiz Date	BBP Hepatitis B Vaccination Status (Circle One)	Signature	Date Signed
			Declination form or vaccinated		
			Declination form or vaccinated		
			Declination form or vaccinated		
			Declination form or vaccinated		

Section 9: Principal Investigator's Acknowledgement of Responsibilities

By signing below, I certify that I have read the following statements and agree that I and all listed personnel on my IBC protocol (including all personnel added to the protocol in the future) will abide by the statements, as well as all policies and procedures governing the use of infectious agents, recombinant DNA, and other biohazardous materials, as outlined by BSU policies and applicable federal regulations. I recognize that:

- I have a responsibility for ensuring the information provided in this application is complete, accurate, and thorough by participating in the development of the IBC application and conducting a review of the protocols.
- I have read and understand my responsibilities as Principal Investigator outlined in Section IV-B-7 of the NIH Guidelines and agree to comply with these responsibilities.
- I have responsibilities for ensuring that anyone who enters my laboratory practices appropriate biosafety precautions.
- I have responsibilities for ensuring that all listed participants conducting this work have received or will receive appropriate training in safe laboratory practices and procedures for this protocol before any work begins on this project. Also, I have a responsibility for ensuring that anyone working in or having access to

spaces where this project is conducted must be instructed on the hazards associated with this project. The UBC, IBC or EHS staff may review my records documenting the training or instruction of personnel.

- I have a responsibility for complying with the requirements pertaining to the shipment and transfer of biohazardous materials.
- I have a responsibility for reporting to the Biosafety Officer immediately any spill of biohazardous material, and containment equipment of facility failure, any permitted decontamination of equipment, and/or any breakdown in procedures, which may result in potential exposure of laboratory personnel and/or the public to the biohazardous material.
- I have a responsibility for reporting to the Biosafety Officer immediately should an employee become ill and/or exhibit symptoms and signs consistent with an infection caused by an organism associated with my research.
- I have a responsibility for following all the applicable guidelines as approved for this protocol.
- I have a responsibility for submitting in writing a request for approval from the IBC of any significant modifications to the protocol.
- I must not carry out the work described in this application until it has been approved by the IBC.
- I attest that this application is accurate and complete.

Electronic Signature of the
Principal Investigator: **Date:**

By typing your name you are submitting an electronic signature that confirms your understanding and adherence to the above statements and IBC policies. This is considered legal documentation and confirmation of your agreement to execute all activities as approved.

Appendix Q: Section IV-B-2, Institutional Biosafety Committee

Section IV-B-2. Institutional Biosafety Committee (IBC)

The institution shall establish an Institutional Biosafety Committee whose responsibilities need not be restricted to recombinant or synthetic nucleic acid molecule research. The Institutional Biosafety Committee shall meet the following requirements:

Section IV-B-2-a. Membership and Procedures

Section IV-B-2-a-(1). The Institutional Biosafety Committee must be comprised of no fewer than five members so selected that they collectively have experience and expertise in recombinant or synthetic nucleic acid molecule technology and the capability to assess the safety of recombinant or synthetic nucleic acid molecule research and to identify any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the Institutional Biosafety Committee) and who represent the interest of the surrounding community with respect to health and protection of the environment (e.g., officials of state or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community). The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P, *Physical and Biological Containment for Recombinant or Synthetic Nucleic Acid Molecule Research Involving Plants*, require prior approval by the Institutional Biosafety Committee. The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q, *Physical and Biological Containment for Recombinant or Synthetic Nucleic Acid Molecule Research Involving Animals*, require Institutional Biosafety Committee prior approval. When the institution conducts recombinant or synthetic nucleic acid molecule research at BL3, BL4, or Large Scale (greater than 10 liters), a Biological Safety Officer is mandatory and shall be a member of the Institutional Biosafety Committee (see Section IV-B-3, *Biological Safety Officer*). When the institution participates in or sponsors recombinant or synthetic nucleic acid molecule research involving human research participants, the institution must ensure that: (i) the Institutional Biosafety Committee has adequate expertise and training (using *ad hoc* consultants as deemed necessary); (ii) all aspects of Appendix M have been appropriately addressed by the Principal Investigator; (iii) no research participant shall be enrolled (see definition of enrollment in Section I-E-7) in a human gene transfer experiment until the NIH protocol registration process has been completed (see Appendix M-I-B, *Selection of Individual Protocols for Public RAC Review and Discussion*); and (iv) final IBC approval is granted only after the NIH protocol registration process has been completed (see Appendix M-I-B, *Selection of Individual Protocols for Public RAC Review and Discussion*). Institutional Biosafety Committee approval must be obtained from the clinical trial site.

Note: Individuals, corporations, and institutions not otherwise covered by the *NIH Guidelines*, are encouraged to adhere to the standards and procedures set forth in Sections I through IV (see Section IV-D, *Voluntary Compliance*. The policy and procedures for establishing an Institutional Biosafety Committee under *Voluntary Compliance*, are specified in Section IV-D-2, *Institutional Biosafety Committee Approval*).

Section IV-B-2-a-(2). In order to ensure the competence necessary to review and approve recombinant or synthetic nucleic acid molecule activities, it is recommended that the Institutional Biosafety Committee: (i) include persons with expertise in recombinant or synthetic nucleic acid molecule technology, biological safety, and physical containment; (ii) include or have available as consultants persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment, and (iii) include at least one member representing the laboratory technical staff.

Section IV-B-2-a-(3). The institution shall file an annual report with NIH OSP which includes: (i) a roster of all Institutional Biosafety Committee members clearly indicating the Chair, contact person, Biological Safety Officer (if applicable), plant expert (if applicable), animal expert (if applicable), human gene therapy expertise or *ad hoc* consultant (if applicable); and (ii) biographical sketches of all Institutional Biosafety Committee members (including community members).

Section IV-B-2-a-(4). No member of an Institutional Biosafety Committee may be involved (except to provide information requested by the Institutional Biosafety Committee) in the review or approval of a project in which he/she has been or expects to be engaged or has a direct financial interest.

Section IV-B-2-a-(5). The institution, that is ultimately responsible for the effectiveness of the Institutional Biosafety Committee, may establish procedures that the Institutional Biosafety Committee shall follow in its initial and continuing review and approval of applications, proposals, and activities.

Section IV-B-2-a-(6). When possible and consistent with protection of privacy and proprietary interests, the institution is encouraged to open its Institutional Biosafety Committee meetings to the public.

Section IV-B-2-a-(7). Upon request, the institution shall make available to the public all Institutional Biosafety Committee meeting minutes and any documents submitted to or received from funding agencies which the latter are required to make available to the public. If public comments are made on Institutional Biosafety Committee actions, the institution shall forward both the public comments and the Institutional Biosafety Committee's response to the Office of Science Policy, National Institutes of Health, preferably by e-mail to: NIHGuidelines@od.nih.gov; additional contact information is also available here and on the OSP website (www.osp.od.nih.gov).

Section IV-B-2-b. Functions

On behalf of the institution, the Institutional Biosafety Committee is responsible for:

Section IV-B-2-b-(1). Reviewing recombinant or synthetic nucleic acid molecule research conducted at or sponsored by the institution for compliance with the *NIH Guidelines* as specified in Section III, *Experiments Covered by the NIH Guidelines*, and approving those research projects that are found to conform with the *NIH Guidelines*. This review shall include: (i) independent assessment of the containment levels required by the *NIH Guidelines* for the proposed research; (ii) assessment of the facilities, procedures, practices, and training and expertise of personnel involved in recombinant or synthetic nucleic acid molecule research; (iii) ensuring that all aspects of Appendix M have been appropriately addressed by the Principal Investigator; (iv) ensuring that no research participant is enrolled (see definition of enrollment in Section I-E-7) in a human gene transfer experiment until the NIH protocol registration process has been completed (see Appendix M-I-B, *Selection of Individual Protocols for Public RAC Review and Discussion*), Institutional Biosafety Committee approval (from the clinical trial site) has been obtained, Institutional Review Board approval has been obtained, and all applicable regulatory authorizations have been obtained; (v) for human gene transfer protocols selected for public RAC review and discussion, consideration of the issues raised and recommendations made as a result of this review and consideration of the Principal Investigator's response to the recommendations; (vi) ensuring that final IBC approval is granted only after the NIH protocol registration process has been completed (see Appendix M-I-B, *Selection of Individual Protocols for Public RAC Review and Discussion*); and (vii) ensuring compliance with all surveillance, data reporting, and adverse event reporting requirements set forth in the *NIH Guidelines*.

Section IV-B-2-b-(2). Notifying the Principal Investigator of the results of the Institutional Biosafety Committee's review and approval.

Section IV-B-2-b-(3). Lowering containment levels for certain experiments as specified in Section III-D-2-a, *Experiments in which DNA from Risk Group 2, Risk Group 3, Risk Group 4, or Restricted Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems*.

Section IV-B-2-b-(4). Setting containment levels as specified in Sections III-D-4-b, *Experiments Involving Whole Animals*, and III-D-5, *Experiments Involving Whole Plants*.

Section IV-B-2-b-(5). Periodically reviewing recombinant or synthetic nucleic acid molecule research conducted at the institution to ensure compliance with the *NIH Guidelines*.

Section IV-B-2-b-(6). Adopting emergency plans covering accidental spills and personnel contamination resulting from recombinant or synthetic nucleic acid molecule research.

Note: The *Laboratory Safety Monograph* describes basic elements for developing specific procedures dealing with major spills of potentially hazardous materials in the laboratory, including information and references about decontamination and emergency plans. The NIH and the CDC are available to provide consultation and direct assistance, if necessary, as posted in the *Laboratory Safety Monograph*. The institution shall cooperate with the state and local public health departments by reporting any significant research-related illness or accident that may be hazardous to the public health.

Section IV-B-2-b-(7). Reporting any significant problems with or violations of the *NIH Guidelines* and any significant research-related accidents or illnesses to the appropriate institutional official and NIH OSP within 30 days, unless the Institutional Biosafety Committee determines that a report has already been filed by the Principal Investigator. Reports to NIH OSP shall be sent to the Office of Science Policy, National Institutes of Health,

preferably by e-mail to: NIHGuidelines@od.nih.gov; additional contact information is also available here and on the OSP website (www.osp.od.nih.gov).

Section IV-B-2-b-(8). The Institutional Biosafety Committee may not authorize initiation of experiments which are not explicitly covered by the *NIH Guidelines* until NIH (with the advice of the RAC when required) establishes the containment requirement.

Section IV-B-2-b-(9). Performing such other functions as may be delegated to the Institutional Biosafety Committee under Section IV-B-2, *Institutional Biosafety Committee*.

Appendix R: USE AND DISPOSAL OF SHARPS

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 2 persons are working together. Locate sharps container between the workers when possible.
- Do not overfill sharps containers. Close completely when they are 3/4 full and request pickup by EHS.
- Locate sharps containers in areas in which needles are commonly used. Make containers easily accessible.
- Sharps containers may be provided by EHS, as well as from laboratory supply distributors such as VWR and Fisher Scientific, or through suppliers such as Grainger.
- Occasionally needles must be filled, recapped, and set aside for use later. In these cases, recapping may only be performed by the one-handed scoop technique as demonstrated below, or by placing the needle in a sterile conical tube or other safety device.


In the event of a needle stick injury:

- Wash thoroughly with soap and water. Notify supervisor and go immediately to the BSU Health Center. If the BHC is closed, go to the most convenient local emergency care room.

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.