UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES

SUBJECT: Biological Safety Manual

Instruction 6401

APR 22 2016

(EHS)

ABSTRACT

This Biosafety Manual was developed by the Center for Environmental Health and Occupational Safety in close cooperation with the Institutional Biosafety Committee (IBC) at the Uniformed Services University of the Health Sciences (USU). The manual is part of the USU Biosafety Program instituted to accomplish the following goals:

- Protect personnel from exposure to infectious agents.
- Prevent environmental contamination.
- Maintain a safe work place that facilitates an environment for high quality research.
- Comply with applicable Federal, state and local safety regulations.
- Institute a secure laboratory environment that prevents unauthorized access or use of biological agents.

This manual provides University-wide safety guidelines, policies and procedures for the use of biohazards, and should be used in the overall management of the USU Biosafety Program.

The Principal Investigator (PI) is primarily responsible for ensuring safe operating procedures are followed in the laboratory. His/her knowledge and judgment are critical in conducting risk assessments of biohazards and appropriately applying the recommendations in this manual. Safety is a shared responsibility among all of the laboratory staff. Many resources are available to assist the PI in their responsibilities, to include the Institutional Biosafety Committee (IBC), and the Center for Environmental Health and Occupational Safety (EHS). All researchers who are involved or working with biological agents are required to read and understand the contents of this manual, complete the required training, and seek additional advice when necessary. The IBC Chairperson and the Biosafety Officer (BSO) are available to assist researchers in this endeavor.

This instruction is effective immediately.

Charles L. Rice, MD
President

Uniformed Services University of the Health Sciences

Biological Safety Guide

Instruction 6401

Bethesda, Maryland

April 2016

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IMPORTANT TELEPHONE NUMBERS

EMERGENCY TELEPHONE NUMBERS

Fire, Police, Rescue,
Emergency Medical Service (Ambulance) 777
HAZMAT Team *1
(*1 HAZMAT Team for response to large spills or hazardous material situations that cannot be handled by the laboratory.)

Campus Security (301) 295-3033
Security Duty hours: 7:30am – 4:00pm
Security after duty hours: (301) 295-3038/3039

ASSISTANCE TELEPHONE NUMBERS

University Health Clinic	(301) 295-3630
Center for Environmental Health and Occupation Safety (EHS)	(301) 295-3531/3305
Biosafety Officer/EHS	(301) 295-3531
Occupational Health Nurse	(301) 295-9444
Radiation Safety Officer	(301) 295-3390
Safety Officer	(301) 295-9441

I. POLICY STATEMENT

Purpose
This is a statement of the official Uniformed Services University of the Health Sciences (USU) policy to establish the process for compliance with the following documents:

- ***NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines),*** current edition.
- ***Biosafety in Microbiological and Biomedical Laboratories (BMBL),*** current edition.
- ***42 CFR, Parts 72 & 73, Possession, Use, and Transfer of Select Agents and Toxins.***

Policy
USU is actively committed to preserving the health and safety of its students, staff, and faculty, and to protecting the environment and the community. It is recognized that use of potentially pathogenic microorganisms and organisms containing recombinant DNA (rDNA) is necessary in university research and teaching laboratories. To ensure the safe handling of these organisms, the University requires compliance with the *NIH Guidelines*, the recommendations in the *BMBL*, and the rules of *42 CFR, Parts 72 & 73*. Compliance with all other applicable Federal, state, and local regulations is also required.

II. ROLES AND RESPONSIBILITIES

The President, USU
The President, USU is responsible for ensuring that hazardous biological agents used at the University are secured, handled and disposed of in accordance with Federal law and their use in laboratories does not pose a health risk to workers or the community.

Institutional Biosafety Committee (IBC)
The IBC is a University-wide committee charged with reviewing policy and procedures related to the use of biological agents, including: human pathogens, oncogenic viruses, other infectious agents, and recombinant DNA (rDNA). The committee membership is established by the National Institutes of Health (NIH) through the *NIH Guidelines for Research Involving Recombinant DNA Molecules*. The Committee must:

- Review rDNA research conducted at, or sponsored by, the University for compliance with the *NIH Guidelines,* and approve those research projects that are found to conform with the *NIH Guidelines*.
- Review research involving infectious agents conducted at, or sponsored by, the University for compliance with the guidelines in *BMBL*, and approve those research projects that are found to conform with the recommendations in *BMBL*.
- Notify the PI of the results of the IBC's review and approval or disapproval; seek clarifying information as necessary in making approval/disapproval decisions.
- Recommend bio-containment levels in accordance with NIH and CDC guidelines, and adopts emergency plans covering accidental spills and personnel contamination.

- Report any significant problems with or violations of the *NIH Guidelines* and any significant research-related accidents or illness to the appropriate Institutional Official and to the NIH Office of Biotechnology DNA Activities (OBA) within 30 days.
- Follow the guidelines for membership defined by NIH.

Center for Environmental Health and Occupational Safety (EHS)
The EHS is the operational arm of the IBC. EHS provides instruction and training on safe work practices, conducts routine inspections of laboratories and work areas, investigates accidents and recommends preventative measures and corrective actions, reviews research protocols involving hazardous materials, reviews construction design for safety features and responds to emergencies. EHS must:

- Prepare and distribute this Biosafety Manual, with revisions as necessary.
- Receive and dispose of Biological Waste (also referred to as Biological, Pathological and Medical Waste -BPMW) or Medical Regulated Waste.
- Coordinate and provide routine laboratory safety, hazard communication and blood borne pathogen training to effected workers. (Laboratory specific/agent specific training is provided by the Principal Investigator.)
- Provide assistance to investigators in performing work hazard risk assessments as necessary.
- Conduct biosafety inspections of all laboratories at USU.
- Inspect laboratory-specific Biosafety Plans/documentation and review PI training records for currency of training during annual laboratory health and safety inspections as periodically as necessary.
- Provide medical surveillance services as required by the OSHA Bloodborne Pathogens Standard (CFR 1910.1030), and as recommended in the *BMBL* and *NIH Guidelines*.
- Provide necessary vaccinations (as may be required) to occupational workers.

Facilities Director
The Facilities Director will:

- Ensure facilities personnel are properly trained, vaccinated as necessary, or are able to wear appropriate personnel protective equipment (PPE) to enter laboratory spaces and perform required maintenance in all laboratories. EHS offers laboratory safety and hazard communication training for ancillary personnel who in the course of their work have occasion to enter laboratory spaces.
- Provide locksmith services to assist the Security Division with securing biological agents and associated laboratories and storage areas.
- Coordinate with EHS all non-emergency maintenance work requests involving structural, electrical, or mechanical equipment, or plumbing alterations or movements that would effect or alter the effectiveness of the engineering controls in place that protect the integrity of Biosafety operations in the laboratories. This includes the connection and disconnection of safety equipment such as fume hoods, biosafety cabinets, or any other related equipment or service.

Security Office
The Security Office will:

- Immediately notify EHS of any breach of security, incident or reported unsafe condition that involves biological agents or other hazardous materials.
- Be responsible for the physical security of buildings containing hazardous biological agents.
- Advise PIs in matters pertaining to the physical security of hazardous biological agents, including procedures for key custodian duties.

Logistics Division
The Logistics Division will:

- Establish written procedures for the ordering, receiving, and delivering of hazardous biological agents, within the University.
- Monitor all incoming and outgoing shipments of biological agents for compliance with Federal and state shipping regulations and that the shipments are properly received, stored and delivered to authorized personnel.
- Ensure applicable Logistics personnel receive the necessary training to conduct their work.
- Maintain constant security of hazardous biological agents until delivered to an authorized user/requestor.
- The Technical Service Branch (TSB) is responsible for ensuring manufacturer warranties to include recommended routine maintenance procedures are scheduled and repairs are performed on all Biosafety equipment such as autoclaves, glass washers, and biosafety cabinets.

    a) Records for the past three years of maintenance and repairs must be kept on file for review.

    b) The Department of Environment Health and Safety (EHS) will assist TSB in the tracking and management of annual BSC certification testing.

Biological Safety Officer (BSO)
The BSO is responsible for implementation and maintenance of the Biosafety Program. The BSO duties include, but are not necessarily limited to:

- Consultation with faculty, staff, and the IBC regarding development and implementation policies and procedures to reduce the risks of work with biohazardous materials with consideration given to having minimal interference with the conduct of research and teaching.
- Providing technical advice and training as necessary to the IBC and researchers on laboratory containment concerning biosafety and biosecurity procedures.
- Developing emergency plans for inclusion in this manual regarding the handling of spills and personnel contamination.
- Developing and reviewing the Biosafety Manual and other related documents.

- Reviewing infectious waste disposal policies and procedures to comply with state, Federal, and DoD regulations.
- Investigating laboratory accidents/incidents and reporting details and violations (if found) to the IBC per NIH Guidelines.

Departmental Chairpersons
The Departmental Chairpersons are responsible for providing support to researchers which ensures that appropriate facilities are available to contain biohazardous materials and to enable the PI to comply with pertinent USU policies. The Chairpersons are responsible for assuring that the PI has the training commensurate with the proposed project and that the project design and monitoring methods meet institutional safety standards.

Principal Investigator (PI)
The PI is responsible for full compliance with approved research protocols, the NIH Recombinant DNA Guidelines, the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogen Standard (human-derived materials), this Biosafety Manual, and other local, state and Federal regulations that apply to research. In addition the PI will:

- Submit an Institutional Biosafety Committee Project Registration (Appendix A; also known as USU Assurance Form 3208 Appendix 4) for all research projects. This must be completed and approved by the IBC before any research can occur.
- Register the following experiments with the IBC, as required:

  a) Recombinant DNA activities.

  b) Work with infectious agents.

  c) Experiments involving the use of human blood or other potentially infectious materials, such as unfixed human tissues, primary human cell lines, and certain body fluids.

  d) Animal and plant pathogens.

- Assess the risks of experiments and investigate all safety aspects of planned experimental work.
- Inform all individuals participating in the experiment of all potential hazards associated with the work.
- Prepare and maintain a lab-specific biosafety manual for laboratories that includes records of training for laboratory staff. Laboratories working with materials requiring BSL-2 or higher containment are required to have a lab-specific manual. Contact the BSO for a template.
- Ensure personnel working under an approved protocol obtain any necessary immunizations, if required. (Refer to Occupational Medicine/ EHS.)

- Ensure the safe operation of laboratories, proper maintenance and care of equipment, safe handling of bio-hazardous material and waste disposal.

- Train laboratory personnel in safe work practices.

Employee
An employee is to:

- Follow the University's health and safety policies, follow laboratory specific procedures, and the instructions of the responsible PI and BSO.
- Complete all required laboratory safety and biosafety training.
- Immediately report any hazardous or potentially unsafe condition to his/her Supervisor and/or Safety Officer.

III. REGISTRATION OF POTENTIALLY INFECTIOUS AGENTS

Procedures and facilities involved in protecting laboratory workers, the public, and the environment from laboratory biological hazards are governed by Federal and state regulations and prescribed guidelines. Many granting agencies require grant recipients to certify that they adhere to both the guidelines and the regulations. It is the policy at USU that all laboratories adhere to the NIH and CDC (Centers for Disease Control and Prevention) guidelines. Research with or possession of CDC or USDA (United States Department of Agriculture) Select Agents (See Appendix C) requires registration with the CDC and/or USDA. Currently, USU is not registered with the CDC (human pathogens) or USDA (plant and livestock pathogens) and work with Select Agents or Toxins is not permitted. Storage and work with toxins under permissible limited amounts is allowed (see below).

Microorganisms
NIH and the CDC both publish guidelines for working with infectious microorganisms. Publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* recommends work be completed using one of four levels of containment: Biosafety Level 1 (BSL-1), Biosafety Level 2 (BSL-2), Biosafety Level 3 (BSL-3), and Biosafety Level 4 (BSL-4). Appendix B in the *NIH Guidelines* classifies pathogenic agents into one of four risk groups according to specific criteria.

Investigators must register any project involving the use of a pathogenic agent with the IBC and obtain IBC approval before work is begun (See Appendix A). If the laboratory has not been classified for a specific containment level it must be surveyed by the Biosafety Officer (BSO) to ascertain that it meets the containment requirements listed in *BMBL* for the agent being studied. If the lab meets the requirements, the work will be reviewed and approved or disapproved by the IBC.

Genetically Engineered Microorganisms
Work with all genetically engineered organisms must comply with the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*. These guidelines also classify recombinant DNA experiments into four levels of containment (BSL-1, BSL-2, BSL-3, and BSL-4) based on the hazard of the microorganism and the procedures and quantities being

used. Additionally, the USDA requires a permit for field testing of genetically engineered plants. It is the policy at the USU that all laboratories follow these guidelines.

Toxins
Work with biological toxins is regulated through the NIH and the CDC. Some toxins are classified as Select Agents by the CDC (addressed below). Because USU is not certified as a Select Agent Facility, the use of such toxins must be limited to quantities below Select Agent threshold.

HHS Toxins [§73.3(d)(3)]	Amount
Abrin	100 mg
Botulinum neurotoxins	0.5 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	1000 mg
Ricin	100 mg
Saxitoxin	100 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	5 mg
T-2 toxin	1000 mg
Tetrodotoxin	100 mg

Specific guidance for safe handling of toxins is provided in Section VIII-G of the *BMBL.* Additionally, Appendix F of the *NIH Guidelines* provides specific guidance for containment conditions for cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. As with microorganisms, investigators must register any project involving the use of a biological toxin with the IBC and obtain IBC approval before work is begun.

Registration Document
Each PI is responsible for registering all recombinant DNA experiments, including those exempt from *NIH Guidelines.* Online registration forms are available at the Office of Research web site http://www.USU.edu/research/assuranceforms.html. An example of the registration form can be found in Appendix A. The BSO will conduct an audit of all laboratories.

Review and Approval of Experiments
The IBC, or at a minimum, the Chair of the IBC and the BSO will review the registration.

a) **Experiments covered by the *NIH Guidelines***
Many experiments involving rDNA molecules require registration and approval by the IBC before work may be initiated. Experiments that require IBC approval before initiation include those that involve:

- Risk Group 2, 3, 4, or Restricted Agents as host-vector systems.

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

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

- The formation of recombinant DNA molecules containing no more than 2/3 of the genome of any eukaryotic virus propagated in tissue culture.
- 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-E of the *NIH Guidelines*.
- The generation of transgenic rodents that requires BSL-1 containment.

b) **Experiments exempt from the *NIH Guidelines***

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

- Use rDNA molecules that are not in organisms or viruses.
- Consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.
- Consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.
- Consist entirely of DNA from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).
- Consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent.
- Do not present a significant risk to health or the environment as determined by the NIH Director, with the advice of the Recombinant DNA Advisory Committee (RAC), and following appropriate notice and opportunity for public comment.
- Contain less than one-half of any eukaryotic viral genome propagated in cell culture.
- Use *E. coli* K12, *Saccharomyces cerevisiae*, or *Bacillus subtilis* host/vector systems, unless genes from Risk Group 3 or 4 pathogens or restricted animal pathogens are cloned into these hosts.

- Involve the purchase or transfer of transgenic rodents for experiments that require BSL-1 containment.

Human Clinical Materials

Please refer to the *Bloodborne Pathogens Exposure Control Plan* (Visit the EHS Occupation Medicine website; https://sites.google.com/a/USU.edu/ehs/occupational-medicine) for detailed information on handling human material.

Work with human material is regulated by the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard, 29 CFR, Part 1910.1030. Human blood, unfixed tissue, cell culture, and certain other body fluids are considered potentially infectious for bloodborne pathogens such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). All human clinical material should be presumed infectious and handled using BSL-2 work practices. This concept is called Standard (Universal) Precautions. Investigators are responsible for notifying EHS of their use of human materials so training and immunization can be provided as required by OSHA.

Plant and Animal Pathogens

USU requires investigators to register their campus use of animal and plant pathogens with Institutional Animal Care and Use Committee (IACUC). The registration form for animal pathogens is available at http://www.USU.mil/iacucforms/iacforms.html. Registration of plant pathogens may be completed by forwarding a copy of the USDA/APHIS permit to the Chair of the IBC.

CDC Select Agents

CDC Select Agents are microorganisms and/or toxins that have potential for use in a terrorist act. The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 restricts the possession and use of select agents (including select agent toxins in excess of their published threshold limits, see above and Appendix B).

Laboratories possessing, using, and/or transferring select agents and certain levels of select agent toxins must comply with the regulations established in the 42 CFR, Part 73. These regulations contain detailed information pertaining to laboratory registration, personnel security risk assessments, safety plans, security plans, emergency response plans, training, record keeping, inspections, and notifications.

Additionally, institutions that plan to use such agents must meet strict registration requirements prior to acquisition of Select Agents. Because significant manpower and resources are required for certification for Select Agent use, approval to apply for such status must be granted by The Office of Research, the Office of the President and the Institutional Biosafety Committee. Such a request should be directed first to the BSO (EHS; 301-295-3531; Rm A2020). Acquisition of Select Agents without prior certification may result in civil or criminal liability and substantial fines for individual researchers and/or the University. If you have questions about what constitutes a Select Agent or Select Agent Registration, contact the USU Select Agent Responsible Official, the BSO, or visit the CDC's Select Agent website at http://www.selectagents.gov/index.html.

IV. RISK ASSESSMENT FOR INFECTIOUS MATERIAL

Classification of Infectious Agents on the Basis of Hazard
Worldwide there are several systems for classifying human and animal pathogens according to the hazard they present to an individual and the community. Although these classifications differ from each other, they all are based on the notion that some microorganisms are more hazardous than others. In general, the pathogenicity of the organism, mode of transmission, host range, availability of effective preventive measures and/or effective treatment are only some of the criteria taken into consideration when classifying infectious agents. In the United States, the most current classification is found in the *NIH Guidelines*. The human etiologic agents addressed in these guidelines are classified into four risk groups with Risk Group 1 (RG-1), of low or no hazard, through Risk Group 4 (RG-4) representing highly infectious agents:

Basis for the Classification of Biohazardous Agents by Risk Group

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

A comprehensive list of biological agents in Risk Groups 1, 2, 3, and 4 can be found in Appendix B of the *NIH Guidelines*. Please note that these lists are not all-inclusive, and agents not listed in RG-2, RG-3, and RG-4 are not automatically assumed to be classified in RG-1. Any unlisted agent needs to be subjected to a risk assessment based on the known and potential properties of the agent and its relationship to other agents that are listed.

RISK GROUPS AND BIOSAFETY LEVELS

Determining the risk group of a biological agent is part of the biosafety risk assessment that assists 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 containment. 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 in a BSL-2, under certain conditions. For more information, contact the BSO or the IBC Chairperson.

V. BIOHAZARDOUS MATERIALS

Biohazardous materials are defined as materials of biological origin that have the capacity to produce deleterious effects on humans or animals including:

- Recombinant DNA (rDNA) molecules.
- Microorganisms containing rDNA molecules.
- Microorganisms classified as risk group RG-1, RG-2, RG-3, or RG-4.
- Biological products derived from RG-1, RG-2, RG-3, or RG-4 microorganisms.
- Diagnostic specimens known or reasonably expected to contain pathogens in RG-1, RG-2, RG-3, or RG-4.
- Clinical/medical waste derived from the medical treatment of humans or animals or from biomedical research.

All studies using rDNA or infectious agents or biological toxins must undergo IBC review. Experiments using RG-2 or RG-3 agents must be reviewed and approved prior to the initiation of experiments. Experiments using RG-1 materials must also undergo IBC review. However, this review process is expedited and research may commence simultaneously with submission.

VI. HUMAN GENE TRANSFER EXPERIMENTATION

NIH Guidelines Section III-C describes "Experiments that Require Institutional Biosafety Committee (IBC) and Institutional Review Board Approvals and Recombinant DNA Advisory Committee (RAC) Review before Research Participant Enrollment". In this section is listed one subsection entitled, "Experiments Involving the Deliberate Transfer of Recombinant DNA, or DNA or RNA Derived from Recombinant DNA, into One or More Human Research Participants" (Section III-C-1). The general process to obtain approval for human gene transfer experimentation is to submit for review the following:

1. RAC [adhere to the NIH Guidelines that are outlined in Appendix M, "Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into One or More Human Research Participants"].

2. IBC.

3. IRB.

For human gene transfer experimentation, it is the responsibility of the IBC to ensure that:

1. A RAC review has been conducted.

2. All issues raised by the RAC in a summarization letter to the PI and the sponsoring institution have been considered.

3. No participant is enrolled until a RAC review has been completed and IBC and IRB approval have been obtained.

VII. RULES, REGULATIONS, AND GUIDELINES

The following is a brief summary of the regulatory authorities that either regulate or provide guidelines for the use of biological materials, infectious agents and recombinant DNA molecules. Copies of these documents are available by access to the appropriate website.

1. *National Institute of Health (NIH): NIH Guidelines for Research Involving Recombinant or Synthetic Acid Molecules (NIH Guidelines)*, November 2013 (http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines).

These guidelines address the safe conduct of research that involves construction and handling of recombinant DNA (rDNA) molecules and organisms containing them. In 1974, a recombinant DNA Advisory Committee (RAC) was established to determine appropriate biological and physical containment practices and procedures for experiments that potentially posed risks to human health and the environment. Because of the committee's activity, the initial version of the *NIH Guidelines* was published in 1976. It has been amended and revised many times since then. Included in the *NIH Guidelines* is a requirement for the institution to establish an Institutional Biosafety Committee (IBC) with authority to approve or disapprove proposed rDNA research using the *NIH Guidelines* as a minimum standard.

2. *Centers for Disease Control and Prevention (CDC) on Biosafety in Microbiological and Biomedical Laboratories,* 5th Edition, 2007 (BMBL manual) (http://www.cdc.gov/biosafety/publications/bmbl5/). This manual describes combinations of standard and special microbiological practices, safety equipment, and facilities that constitute Biosafety Levels 1 through 4, which are recommended for working with a number of infectious agents in various laboratory settings. This manual is commonly seen as the standard for biosafety.

3. *Occupational Safety and Health Administration: Bloodborne Pathogens Standard* (https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=10051&p_table=STANDARDS). In 1992, the Occupational Safety and Health Administration (OSHA) promulgated a rule to deal with the occupational health risk caused by exposure to human blood and other potentially infectious materials. OSHA's rule includes a combination of engineering and work practice controls, personal protective clothing and equipment, training and medical follow-up of exposure incidents, vaccination, and other provisions.

4. Packaging, shipment and transportation requirements for infectious substances, diagnostic specimens and biological products are addressed in the following rules and guidelines:

   International Air Transport Association (IATA)
   Dangerous Goods Regulations
   http://www.iata.org
   U.S. Department of Transportation
   49 CFR

http://hazmat.dot.gov/regs/rules.htm
U.S. Postal Service
39 CFR Part 111
U.S. Department of Labor, OSHA
29 CFR 1910.1030

5. Importation permits are required for infectious agents, biological materials and animals as outlined in U.S. Public Health Service, 42 CFR Part 71, *Foreign Quarantine*. In addition, the Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) requires permits for importation and transportation of controlled materials, certain organisms or vectors. This includes animal and plant pathogens, certain tissue cultures and live animals. APHIS also regulates the importation, interstate movement, or environmental release of genetically engineered organisms as regulated under 7 CFR Part 340.

6. *Select Agent Regulation* (42 CFR Part 73)
   This regulation became a final rule on March 18, 2005. It implements provisions of the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. A copy of the most current list of select agents and toxins covered under this rule is included in Appendix B.
   (http://www.selectagents.gov/SelectAgentsandToxinsList.html)

7. The Department of Commerce also regulates shipping certain agents.
   (http://www.bis.doc.gov/index.php/regulations/export-administration-regulations-ear)

VIII. BIOSAFETY CONTAINMENT LEVELS

Four levels of biosafety are defined in the publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, published by the CDC and NIH. The levels, designated in ascending order by degree of protection provided to personnel, the environment, and the community, are combinations of laboratory practices, safety equipment, and laboratory facilities. Most microbiological work at the USU is conducted at BSL-1 or BSL-2 containment. There are no BSL-4 laboratories at the University. A comprehensive description of each of the BSL containment levels can be found in the BMBL, which is free to download from the CDC web site at http://www.cdc.gov/biosafety/publications/bmbl15/index.htm

Biosafety Level 1 (BSL-1)
BSL-1 is appropriate for undergraduate and secondary educational training and teaching laboratories, and for other facilities in which work is done with well-characterized agents not known to cause disease in healthy adult humans. The laboratory is not necessarily separated from the general traffic patterns in the building. BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing. The Standard Microbiological Practices listed in the Administrative Controls paragraph of this manual apply to all Biosafety Levels.

Biosafety Level 2 (BSL-2)
BSL-2 is similar to BSL-1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment. With good microbiological techniques, work at BSL-2 can be conducted safely on the open bench, provided the potential for producing splashes or aerosols is low. Primary hazards to personnel working with BSL-2 agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. BSL-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.

Biosafety Level 3 (BSL-3)
BSL-3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents, and are supervised by competent scientists who are experienced in working with these agents. Primary hazards to personnel working at BSL-3 relate to autoinoculation, ingestion, and exposure to infectious aerosols. A comprehensive description of BSL-3 requirements can be found in USU Instruction 6403, Biohazard Suite Management.

Biosafety Level 4 (BSL-4)
USU does not conduct work requiring BSL-4 containment. BSL-4 is required for work with dangerous and exotic agents which pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. The facility is usually in a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building.

Biosafety Level 1 (BSL-1)	
Agents:	Not known to cause disease in healthy adult humans.
Practices:	Standard microbiological practices.
Safety Equipment: (Primary barriers)	None required.
Facilities: (Secondary barriers)	Open bench top with sink available.

Biosafety Level 2 (BSL-2)	
Agents:	Moderate risk agents that are present in the community and associated with human disease of mild to moderate severity.
Practices:	BSL-1 practice plus limited access, biohazard warning signs, "SHARPS" precautions, and an SOP defining any needed waste decontamination or medical surveillance policies.
Safety Equipment: (Primary barriers)	Primary barriers include a Class I or II Biological Safety Cabinet (BSC) or other physical containment devices used for the manipulations of agents that cause splashes or aerosols of infectious materials; Personal Protective Equipment (PPEs) including laboratory coats, gloves, face and eye protection as needed.
Facilities: (Secondary barriers)	BSL-1 plus the availability of an autoclave for decontamination.

Biosafety Level 3 (BSL-3)	
Agents:	Indigenous or exotic agents with a potential for aerosol transmission; and which may cause serious or potentially lethal infection.
Practices:	BSL-2 practice plus controlled access, decontamination of all waste, and decontamination of lab clothing before laundering.
Safety Equipment: (Primary barriers)	Primary barriers include a Class II BSC or other physical containment device used for the manipulations of agents, PPEs to include protective lab clothing, gloves, face and eye protection, and respiratory protection as needed.
Facilities: (Secondary barriers)	BSL-2 plus physical separation from access corridors, self-closing and double door access, exhausted air not recirculated with negative airflow into laboratory.

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

Clinical Laboratories
The initial processing of clinical specimens and identification of most bacterial and viral isolates can be done safely within a biosafety cabinet using BSL-2 practices. Note that BSL-2 is the recommended level for work with bloodborne pathogens such as HBV and HIV. Isolation and identification of respiratory and systemic infectious agents such as *Mycobacterium tuberculosis, Francisella, Brucella, and Coccidiodes immitis* and should be done in BSL-3 containment. BSL-3 is also required for the handling of clinical specimens that may contain the hemorrhagic fever viruses.

IX. GENERAL BIOSAFETY PRACTICES

Routes of Infection
Working in a biological research environment, it is reasonable to expect that a laboratory person working with infectious materials is more likely to become infected than members of the general community. An infection occurs when disease-causing microorganisms enter the human body in sufficient numbers and by a particular route and overcome the body's defense system. The following routes of infection have been reported for laboratory-acquired infections:

1. Through the mouth by:

   -Eating or drinking in the laboratory.
   -Mouth pipetting.
   -Transfer of microorganisms to the mouth by contaminated fingers or articles.

2. Through the skin by:

   -Accidental inoculation with a hypodermic needle, another SHARPS instrument, or glass.
   -Cuts or scratches.

3. Through the eyes by:

   -Splashes of infectious material into the eyes.
   -Transfer of microorganisms to the eyes by contaminated fingers.

4. Through the lungs by:

   -Inhalation of airborne microorganisms.

The general laboratory procedures outlined in this manual provide for guidance in handling infectious or potentially infectious materials.

ADMINISTRATIVE CONTROLS

Biohazard Warning Sign

A biohazard label is required for all areas or equipment in which RG-2 or RG-3 agents are handled or stored, or where BSL-2 or higher procedures are required. The appropriate place for posting the label is at the main entrance door(s) to laboratories and animal rooms, and on equipment such as refrigerators, incubators, transport containers, and/or lab benches. Requests for standardized biohazard warning signs can be submitted to EHS, Room A2020.

Training

Good microbiological and laboratory practices are essential for a safe work environment. Training and education on these practices and procedures needs to be implemented before laboratory duties are undertaken. All personnel working with RG-1, RG-2 or RG-3 agents are required to receive laboratory specific training from the PI or laboratory supervisor in addition to the required basic training provided by the Institution. Training should include at a minimum:

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

This Manual and the USU Exposure Control Plan (Visit the EHS Occupation Medicine website; https://sites.google.com/a/USU.edu/ehs/occupational-medicine) describe the training requirements for personnel whose research involves recombinant DNA or biohazards. The BSO and EHS staff conducts basic biosafety training in accordance with applicable local and Federal requirements. PIs are responsible for biohazard specific training in their laboratories.

Laboratory Audits

As part of the EHS audit program, the Environmental staff participates in routine laboratory inspections on an annual basis. Significant deficiencies are reviewed with the BSO and the Director, EHS. An example of the annual Biosafety Audit can be found in Appendix C.

Recordkeeping

The IBC project approval records are maintained by the Executive Secretary IBC (301-400-6019 Rm A2048) in accordance with Federal standards. Laboratory inspection results and training attendance records are maintained by EHS in accordance with applicable Federal regulations. PIs are responsible for updating IBC approved projects with EHS, and providing current listings of personnel involved in IBC approved projects.

Standard Microbiological Practices:

1. Access to the laboratory is limited or restricted at the discretion of the PI when experiments or work with cultures and specimens are in progress.

2. Personnel wash their hands after they handle viable materials and animals, after removing gloves, and before leaving the laboratory.

3. Eating, drinking, handling contact lenses, and applying cosmetics are not permitted in the work areas where there is reasonable likelihood of exposure to potentially infectious materials. Personnel that wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.

5. All procedures are performed carefully to minimize creation of splashes or aerosols.

6. Work surfaces are decontaminated at least daily and after any spill of viable material.

7. 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 to be placed in a durable, leak proof container and closed for transport from the laboratory. Materials to be decontaminated off-site are packaged in accordance with applicable state and Federal regulations before removal from the facility.

8. An insect and rodent control program is in effect. Contact Facilities (301-295-3045) if a need for pest control is identified.

Bloodborne Pathogen (BBP) Program
In accordance with OSHA requirements, USU has established an Exposure Control Plan (Visit the EHS Occupation Medicine website; https://sites.google.com/a/USU.edu/ehs/occupational-medicine) covering the potential exposure to bloodborne pathogens (e.g., HIV, Hepatitis B virus) found in human blood, serum and tissue, as well as in other potentially infectious materials. BBP training is required on an annual basis and available online at https://sites.google.com/a/USU.edu/ehs/training.

Institutional Biosafety Committee (IBC)
The IBC gives oversight on all projects involving biohazardous agents (RG-1, RG-2, and RG-3) and certain toxins on the campus. Membership is governed by the NIH Guidelines and includes the USU Biosafety Officer.

ENGINEERING CONTROLS

Biological Safety Cabinets (BSCs)
BSCs are designated to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three types of BSCs, designated as Class I, II and III, have been developed to meet various research and clinical needs. The common element to all classes of biological safety cabinets is the high efficiency particulate air (HEPA) filter. This filter removes particles of 0.3 microns with an efficiency of 99.97%. However, it does not

remove vapors or gases. The biosafety cabinet requires regular maintenance and certification by a professional technician to assure that it protects you, your experiments, and the environment. Each cabinet should be certified when it is installed, each time it is moved or repaired, and at least annually. TSB administers a program for annual certification of all BSCs at the University. Contact TSB at (301) 295-3612 to confirm that your cabinet is included in this program.

Laboratory personnel must be trained in the correct use and maintenance of BSCs to ensure that personnel and product protection (where applicable) are maintained. Before selecting any biosafety cabinet for purchase, contact the EHS Industrial Hygiene Officer (301) 295-9442 for a work-specific assessment and selection criterion.

1. **Class I BSC**
Protects personnel and the environment, but not research materials. They provide an inward flow of unfiltered air, similar to a chemical fume hood, which protects the worker from the material in the cabinet. The environment is protected by HEPA filtration of the exhaust air before it is discharged into the laboratory or ducted outside via the building exhaust. The Class I BSC is suitable for work involving low to moderate risk agents, where there is a need for containment, but not for product protection (e.g., sterility).

2. **Class II BSC**
(Types A1, A2, B1, B2, and B3) provide personnel, environment, and product protection. Air is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air within the cabinet provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air passes through the exhaust HEPA filter, it is contaminant-free (environmental protection), and may be recirculated back into the laboratory (Type A) or ducted out of the building (Type B).

3. **Class III BSC** (sometimes called Class III glove boxes)
This type of cabinet provides the highest level of product, environmental, and personnel protection, and is designed for work with infectious agents that require BSL-4 containment. The cabinet is gas-tight, maintained under negative air pressure, with a non-opening view window, and has rubber gloves attached to ports in the cabinet that allow for manipulation of materials in the cabinet. Air is filtered through one HEPA filter as it enters the cabinet, and through 2 HEPA filters before it is exhausted to the outdoors.

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

OPERATING INSTRUCTIONS FOR CLASS II BSCs:

1. Turn on cabinet fan 15 minutes before beginning work.

2. Disinfect the cabinet work surface with 70% ethanol or other disinfectant.

3. Place supplies in the cabinet. Locate container inside the cabinet for disposal of pipettes. (Movement of hands in and out of the cabinet to discard pipettes into a container located outside of the cabinet creates turbulence and disrupts the air barrier that maintains sterility inside the cabinet.) Work as far to the back (beyond the air split) of the BSC work space as possible. Always use mechanical pipetting aids. Avoid using open flames inside BSCs. If a flame is necessary, use a burner with a pilot light and place it to the rear of the work space. Flames disrupt the airflow and contribute to the heat load inside the BSC. Flames have burned holes through HEPA filters and have caused explosions in BSCs. Do not work in a BSC while a warning light or alarm is signaling.

4. Locate liquid waste traps inside cabinet and use a hydrophobic filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container (plastic trays capable of holding the container's contents) to prevent spilling.

5. Always wear gloves when there is potential for skin contact with infectious material.

6. Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper air flow and the level of protection provided. Also, keep the front and rear grilles clear.

7. When work is completed, remove equipment and supplies from the cabinet. Wipe the work area with 70% ethanol (or a suitable disinfectant for the agent in use, e.g. 1:10 household bleach, followed by ethanol) and allow the cabinet to run for 15 minutes.

8. Some BSCs are equipped with ultraviolet (UV) lights. However, if good procedures are followed, UV lights are not needed. If one is used, due to the limited penetrating ability of UV light the tube should be wiped with alcohol every two weeks, while turned off, to remove dust. UV radiation should not take the place of suitable disinfection of the cabinet interior.

9. The UV lamp should never be on while an operator is working in the cabinet.

10. Minimize traffic around the BSC and avoid drafts from doors and air conditioning.

Negative Pressure Rooms

All laboratories having a ducted exhaust air ventilation system where the directional airflow draws air into the laboratory (i.e. negative pressure), must incorporate a method to monitor the direction of airflow to ensure that it is properly working. It is recommended that a visual monitoring device be used to confirm directional inward airflow at the lab entry.
In special containment laboratory areas (BSL-3 labs), an electronic quantitative monitoring of the air flow should be continually maintained and tested at least annually.

Exhaust systems with HEPA filters require a mechanism to monitor proper functioning of the filter to determine when replacement is needed.

Protection of Vacuum Lines
All vacuum lines used to aspirate supernatants, tissue culture media, and other liquids that may contain microorganisms should be protected from contamination by the use of a collection flask and overflow flask. In addition, at BSL-2 containment and higher, a hydrophobic vacuum line filter should be used. Hydrophobic filters such as the Gelman Vacushield are available from several scientific supply companies (i.e. Fisher Scientific-and VWR).

Collection and Overflow Flasks:

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

Other Safety Equipment

Safety Showers
Safety showers provide an immediate water drench of an affected person. Standards for location, design and maintenance of safety showers are available from Facilities Maintenance.

Eyewash Stations
Eyewash stations are required in all laboratories where injurious or corrosive chemicals are used or stored and where employees perform tasks that might result in splashes of potentially infectious materials. Eyewash stations need to be checked and flushed periodically (weekly) to ensure their operability. A log should be kept to track periodic inspections.

Ventilation Controls
Ventilation controls are intended to minimize employee exposure to infectious substances by removing air contaminants from the work site. The two types of ventilation controls are:

a. General (Dilution) Exhaust - Laboratory air must be continually replaced, preventing the increase of air concentration of toxic substances during the work. General exhaust systems are inadequate for RG-3 agents or BSL-3 work.

b. Local (Removal) Exhaust - Local exhaust systems capture or contain contaminates at their source before they escape into the workroom environment. Typical systems consist of one or more hoods, ducts, an air cleaner, if needed, and a fan.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

PPE is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may also protect the experiment from contamination. Personal protective devices and safety equipment must be provided to all employees under the appropriate circumstances and employees have the responsibility of properly using the equipment. The following PPE is recommended for regular use:

Face Protection

Splash goggles or safety glasses with solid side shields in combination with masks, or chin length face shields or other splatter guards are required for anticipated splashes, sprays or splatters of infectious or other hazardous materials to the face

Laboratory Clothing

This category includes laboratory coats, smocks, scrub suits, and gowns. Long-sleeved garments should be used to minimize the contamination of skin. If splashes occurred, 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. At a minimum, a laboratory coat should be worn in all laboratories working at a BSL-2. 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 workers, and service workers in the event it is required. All protective clothing should be either discarded in the laboratory or laundered (USU Logistics section). 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. Protection from contact with toxic or corrosive chemicals may also be required. Powdered latex gloves should not be used at the USU.

Double gloving provides an extra barrier of protection. Loss of dexterity and the discomfort incurred should be considered during risk assessments, but use of double gloves may be appropriate when:

- Highly infectious or toxic agents, such as those requiring BSL-3 containment.
- Working with concentrated volumes of infectious or agents or toxins.
- Working with animals, especially those infected with infectious agents or toxins.
- Working with sharps, especially when also handling infectious agents or toxins.

Respirators
For certain protocols and projects, additional PPE like respiratory protection may be required. Respirator selection is based on the hazard and the protection factor required. Fit testing for HEPA-filtered masks is available through EHS.

RECOMMENDED WORK PRACTICES

Pipettes and Pipetting Aids
Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used. Confine pipetting of biohazardous or toxic fluids inside a BSC if possible. The following precautions should be followed:

- Always use cotton-plugged pipettes when pipetting biohazardous or toxic fluids.
- 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 in a pan or upright pipette beaker containing enough liquid disinfectant to completely cover the contaminated portion.
- Autoclave 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 while in use.

Syringes and Needles
Syringes and hypodermic needles are dangerous objects which need to be handled with extreme caution to avoid accidental injection and aerosol generation. Generally, the use of syringes and needles should be restricted to procedures for which there is no alternative. Do not use a syringe and needle as a substitute for a pipette. Use needle locking syringes or disposable syringe-needle units in which the needle is an integral part of the syringe. When using syringes and needles with biohazardous or potentially infectious agents:

- Work in a BSC whenever possible.
- Wear gloves. Double gloves recommended with a risk assessment considering the loss of dexterity and discomfort.
- Fill the syringe carefully to minimize air bubbles.
- Expel air, liquid and bubbles from the syringe vertically into a cotton pad moistened with a disinfectant.

Needles should not be bent, sheared, replaced in the sheath or guard (capped), or removed from the syringe following use. If it is essential that a contaminated needle be recapped or removed from a syringe, the use of a mechanical device or the one-handed scoop method must be used.

Always dispose of needle and syringe unit promptly into an approved SHARPS container. Do not overfill SHARPS containers (2/3 filled = full) before discarding.

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

Cryostats
Frozen sections of unfixed human 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 70% ethanol or any other 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.
- Defrost and decontaminate the cryostat with a tuberculocidal hospital type disinfectant once a week and immediately after tissue known to contain bloodborne pathogens, *M. tuberculosis* or other infectious agents is cut.
- Handle microtone 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. Users should be properly trained in operating instructions and safety precautions should be prominently posted on the unit. Aerosols are created by practices such as filling centrifuge tubes, removing supernatant and resuspending sediment pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation. To minimize the generation of aerosols when centrifuging biohazardous material, follow these procedures:

- 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 in 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 case 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 resuspending sediment 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. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatiguing resulting in disintegration of rotors and to use proper cleaning techniques and centrifuge components. Manufacturer's recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.
- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, appropriate chemical disinfectants are necessary for decontamination.

Blenders, Ultrasonic Disrupters, Grinders and Lyophilizers
The use of any of these devices results in considerable aerosol production. Blending, cell-disrupting and grinding equipment should be used in a BSC.

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. If blender containers are not leak-proof, they should be tested with sterile saline or dye solution prior to use with biohazardous material. The use of glass blender jars is not recommended because of the breakage potential. If they must be used, glass jars should be covered with a polypropylene jar to prevent spraying of glass and contents in the event the blender jar breaks. A towel moistened with disinfectant should be placed over the top of the blender during use. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle. The device should be decontaminated promptly after use.

Lyophilizer 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 a 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.

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 where electric incinerators or gas flames are not available or recommended. Continuous flame gas burners should not be used in BSCs. These burners can produce turbulence that disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter.

Laundry
All personal protective clothing must be cleaned, laundered and disposed of by the employer at no cost to employees. 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. Contact EHS at (301) 295-3531 for additional information concerning the handling of contaminated clothing.

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. Additional laboratory housekeeping concerns include:

- Keeping the laboratory neat and free of clutter. Surfaces should be clean and free of infrequently used chemicals, glassware and equipment. Access to sinks, eyewash stations, emergency showers and exits, and fire extinguishers must not be blocked.
- Proper disposal of chemicals and wastes. Old and unused chemicals should be disposed of promptly and properly.
- Providing a workplace that is free of physical hazards. Aisles and corridors should be free of tripping hazards. Attention should be paid to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment and avoidance of the creation of electrical hazards in wet areas.

All laboratory equipment needs to be cleaned and certified of being free of hazards before being released for repair or maintenance.

X. USE OF ANIMALS IN RESEARCH AND TEACHING

The use of animals in research and teaching is subject to state and Federal laws and guidelines including the Animal Welfare Act (AWA 1990), DoD Instruction: Use of Animals in DoD

Programs, (DoD Instruction 3216.01, 2010), USU Instruction 3203 and *The Guide for the Care and Use of Laboratory Animals*, Eight Edition 2011 (National Academies Press). Policy specifies that:

- All animals under the sponsorship of the Institution will be treated humanely.

- Prior to their inception, all projects in which vertebrate animals are used must receive approval by the Institutional Animal Care and Use Committee (IACUC).
- Researchers will comply with state and Federal regulations regarding the use and care of animals.
- Only workers trained and experienced in handling animals should be permitted to conduct operations with animals. This includes operations involving injection of toxins or infectious solutions using hollow-bore needles.

The IACUC should be contacted for questions regarding the use of animals for teaching and research. Principal Investigators planning to use animals for any USU activity must submit an application to the IACUC for review prior to the start of the project, regardless of the source of funding for the project. A copy of the application can be obtained from the IACUC [(301) 295-9719] at https://sites.google.com/a/USU.edu/iacuc/forms. The completed form will include descriptions of experimental protocols, plans for animal care, available facilities, and information on the use of hazardous materials including infectious agents. All animal protocols involving the use of rDNA and infectious or transmissible agents must be submitted to the IBC for review prior to final approval by the IACUC.

XI. EMERGENCY PROCEDURES

Biological Spills
A spill kit should be kept in each laboratory where work with microorganisms is conducted. Basic equipment for the kit contains: concentrated disinfectant (such as chlorine bleach), a package of paper towels, household rubber gloves, autoclave bags, a SHARPS container, and forceps to pick up broken glass.

General Spill Cleanup Guidelines

- Alert coworkers and mark off area if necessary.
- Wear gloves and a lab coat.
- Use forceps to pick up broken glass and discard into a SHARPS container.
- Cover spilled material with paper towels.
- Add diluted disinfectant in sufficient quantity to ensure effective microbial inactivation.
- Dispose of towels in biohazard waste container.
- Wipe spill area with diluted disinfectant.
- Wash hands with soap and water when finished.

Specific Spill Cleanup Guidelines

Spill inside of BSL-1

- Wearing gloves and a lab coat, pick up broken glass with forceps and place in a SHARPS container.
- Absorb the spill with paper towels or other absorbent material.
- Discard these contaminated materials into a biohazard waste container.

- Wipe the spill area with the appropriate dilution of a disinfectant effective against the organism.
- Autoclave all towels, gloves, and other materials worn or used to clean up the spill.
- Wash hands with soap and water.

Spill inside of BSL-2

- Keep other workers out of the area to prevent spreading spilled material. Post warning sign, if needed.
- Remove contaminated clothing and put it into a biohazard bag for decontamination later.
- Wash hands and exposed skin and inform the PI of the spill. Call the BSO at (301) 295-3058 for assistance, if necessary.
- Put on protective clothing (lab coat, gloves, and if needed, face protection and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, SHARPS container, and paper towels).
- Pick up broken glass with forceps and dispose it into a SHARPS container.
- Cover the spill with paper towels and add appropriately diluted disinfectant.
- After at least 20 minutes of contact time, pick up the paper towels and re-wipe the spill area with diluted disinfectant.
- Collect all contaminated materials into a biohazard waste container and autoclave.
- Wash hands with soap and water.

Spill inside of BSL-3

- Stop work immediately.
- Avoid inhaling airborne material while quickly leaving the room. Notify others to leave. Close the door, and post a warning sign.
- Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag. Wash hands with soap and water.
- Notify the PI. Call the BSO at (301) 295-3531 (after hours and weekends call the Security Desk at (301) 295-3038 or 911) for assistance, if necessary.
- Allow 30 minutes for aerosols to disperse before re-entering the laboratory to begin clean-up.
- Put on personal protective equipment (HEPA filtered respirator, gown, gloves, and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, SHARPS container, and paper towels).
- Contain the spill with absorbent paper towels or disposable pads. Carefully add 10% chlorine bleach to the spill; avoid creating aerosols when pouring the disinfectant. Leave the room and allow 30 minutes for the bleach to inactivate the material.
- Pick up broken glass with forceps and discard in a SHARPS container.
- Clean up liquid with paper towels and collect all contaminated materials into biohazard bag or container. Remove all spilled materials and decontaminate the area again with an appropriate disinfectant.

- Autoclave (or soak in 10% bleach solution) lab coat, gloves, and other protective equipment that was worn for clean up.
- Wash hands thoroughly with soap and water.

Spill of Human Blood

- Wear gloves and a lab coat to clean up a spill.
- If broken glass is present, use forceps to pick up and place in a SHARPS container.
- Absorb blood with paper towels and discard them in a biohazard waste container.
- Using a detergent solution, clean the spill site of all visible blood.
- Wipe the spill site with paper towels soaked in a disinfectant such as bleach diluted 1:10 (vol/vol).
- Discard all contaminated materials into a biohazard waste container.
- Wash hands with soap and water.

Spill inside a Biological Safety Cabinet

- Leave the cabinet fan running.
- Wearing gloves and a lab coat, spray or wipe cabinet walls, work surfaces, and equipment with disinfectant such as hospital type disinfectant or 1:10 dilution of household bleach. If necessary, flood work surface, as well as drain pans and catch basins below the work surface, with disinfectant. Allow at least 20 minutes contact time.
- Soak up the disinfectant and spill with paper towels, and drain catch basin into a container. Lift front exhaust grille and tray, and wipe all surfaces. Ensure that no paper towels or solid debris are blown into area below the grille.
- Surface disinfect all items that may have been spattered before removing them from the cabinet.
- Discard all clean-up materials into biohazard waste container. Wash hands and exposed skin areas with soap and water.
- The BSO (301-295-3130) should be notified if the spill overflows into the interior of the cabinet.
- It may be necessary to do a more extensive decontamination of the cabinet.

Spill of Combined Radioactive & Biological Material

A spill involving combined radioactive and biological materials requires emergency response procedures that are different from the procedures used for either material alone. As a general rule, disinfect the microorganism using a chemical disinfectant, then dispose of all clean-up materials in a separate bag/container labeled to indicate that a radioisotope is mixed with a chemically disinfected microorganism. Be sure to use procedures to protect yourself from the radionuclide while disinfecting the biological material. Before any clean-up, consider the type of radionuclide, the characteristics of the microorganism, and the volume of the spill. Contact the Radiation Safety Officer (RSO) [USU RSO (301) 295-3390/1906; AFRRI RSO (301) 295-2723] for specific radioisotope clean-up procedures. For biological spill procedures contact the BSO at (301) 295-3531.

Preparation for Clean-up

- Avoid inhaling airborne material, while quickly leaving the room. Notify others to leave. Close door, and post with warning sign.
- Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag.
- Wash all exposed skin with soap or hand washing antiseptic, followed by a three minute water rinse.
- Inform the PI and the RSO [USU RSO - (301) 295-3390/1906; AFRRI RSO – (301) 295-2723] of the spill, and monitor all exposed personnel for radiation.
- Allow aerosols to disperse for at least 30 minutes before reentering the laboratory. Assemble clean-up materials (diluted disinfectant, autoclavable containers, forceps, paper towels, SHARPS container).
- Confirm with the RSO that it is safe to re-enter the lab.

Clean-up of a Biological Spill Containing Radioactive Material

- Put on protective clothing (lab coat, surgical mask, gloves, and shoe covers). Depending on the nature of the spill, it may be advisable to wear a HEPA filtered respirator instead of a surgical mask. In setting up your spill plan, contact EHS for advice since the use of many types of respirators requires prior training, fit-testing, and medical approval.
- Pick up any sharp objects with forceps and put in a SHARPS container labeled according to Radiation Safety guidelines.
- Cover the area with paper towels, and carefully pour diluted disinfectant around and into the spill. Avoid enlarging the contaminated area. Use additional disinfectant as it becomes diluted by the spill. Allow at least 20 minutes contact time. **Do not use bleach solutions on iodinated materials: radioiodine gas may be released. Instead, use an alternative disinfectant such as an iodophor.**
- Wipe surrounding areas where the spill may have splashed with disinfectant.
- Absorb the disinfectant and spill materials with additional paper towels, and place into an approved radioactive waste container. Keep separate from other radioactive waste. **Do not autoclave contaminated waste without the approval of the RSO.**
- Disinfect contaminated protective clothing prior to disposal as radioactive waste.

    i. With the assistance of Radiation Safety Division personnel, place contaminated item(s) on absorbent paper and scan for radioactivity. **If no radiation is detected as cleared by the RSO, dispose of these items as biohazard waste.**
    ii. If radioactive contamination is detected, spray with disinfectant and allow a 20 minute contact time.
    iii. Wrap the item(s) inside the absorbent paper and dispose of as radioactive waste.

- Wash hands and exposed skin areas with soap and water, and monitor personnel and spill area for residual radioactive contamination. If skin contamination is detected, repeat decontamination procedures under the direction of the RSO. If spill area has residual activity, the RSO will determine if it is fixed or removable and handle it accordingly.

Injury Involving Biological Materials

Severe Injuries

- Call "777" for assistance and transportation to the nearest emergency room.
- Accompany the injured person to the medical facility and provide information to personnel about the accident/exposure.
- Report the accident to the PI and EHS.

Splash to the Eye

- Immediately flush the eye with a gentle stream of clean, temperate water for 15 minutes. Hold the eyelid open. Be careful not to wash the contaminant into the other eye. Use an emergency eyewash if one is accessible.
- Military personnel contact the University Health Clinic (UHC) at (301) 295-3630 to obtain care. Civilian personnel and contractors can visit the Occupational Health Office (A2024; 301-295-9444). If UHC or the Occupational Health Office is closed, military and civilian personnel should go to the emergency room at WRNMMC or to the most convenient local emergency room (Suburban Hospital, 8600 Old Georgetown Rd, Bethesda, MD 20814). Contractors should go to Suburban Hospital.
- Report the accident to the PI and EHS, and seek additional medical assistance if necessary.

Contamination to the Body

- Immediately remove contaminated clothing and drench skin with water. Wash with soap and water, and flush the area for 15 minutes.
- Military personnel contact the University Health Clinic at (301) 295-3630 to obtain care. Civilian personnel and contractors can visit the Occupational Health Office (A2024; 301-295-9444). If UHC or the Occupational Health Office is closed, military and civilian personnel should go to the emergency room at WRNMMC or to the most convenient local emergency room (Suburban Hospital). Contractors should go to Suburban Hospital.
- Report the injury to the PI and to EHS, and seek additional medical assistance if necessary.

Fires Involving Biological Materials

- **Without placing yourself in danger,** put biological materials in a secure location, such as an incubator or freezer.
- Activate the building's fire alarm.
- Leave the building at once.
- Call the fire department at 777 from a safe location.
- Meet the fire department outside and direct them to the fire.

XII. DECONTAMINATION AND DISPOSAL

Sterilization, disinfection, and antisepsis are all forms of decontamination. Sterilization implies the killing of all living organisms. Disinfection refers to the use of antimicrobial agents on inanimate objects; its purpose is to destroy all non-spore forming organisms. Antisepsis is the application of a liquid antimicrobial chemical to living tissue. All equipment must be appropriately decontaminated before repair, maintenance, or removal from the laboratory.

Chemical Disinfectants

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

1. Sterilizer or Sterilant - will destroy all microorganisms including bacterial and fungal spores on inanimate surfaces.

2. Disinfectant - will destroy or irreversibly inactivate specific viruses, bacteria, and pathogenic fungi, but not bacterial spores.

3. Hospital Disinfectant - agent shown to be effective against *S. aureus*, *S. choleresis* and P. *aeruginosa*. It may be effective against *M. tuberculosis*, pathogenic fungi or specifically named viruses.

4. Antiseptic – an agent formulated to be used on skin or tissue – it is not a disinfectant.

<u>Disinfectants Commonly Used in the Laboratory</u>

Iodophors

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

Hypochlorites (bleach)

- Working dilution is 1:10 to 1:100 household bleach in water.
- Effective against vegetative bacteria, fungi, most viruses at 1:100 dilution.
- Effective against bacterial spores at 1:10 dilution.
- Very corrosive.
- Rapidly inactivated by organic matter.
- Solutions decompose rapidly; fresh solutions should be made daily.

Alcohols (ethanol, isopropanol)

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

Important Characteristics of Disinfectants

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

Dilution Of Disinfectants

1. Chlorine compounds (Household Bleach)

Dilution in Water	% Available Chlorine	Available Chlorine mg/l or ppm
Not diluted	5.25	50,000
1/10	0.5	5,000
1/100	0.05	500

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

2. Iodophor
Manufacturer's recommended dilution is three ounces (90 ml) into five gallons water, or approximately 4.5 ml/liter. For porous surfaces, use six ounces per five gallons water.

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

Autoclaving Procedures
Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable system available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation.

Container Selection

- **Polypropylene bags.** Commonly called biohazard or autoclave bags, these bags are able to withstand autoclaving and are tear resistant, but can be punctured or may burst during autoclaving. Therefore, **place bags in a rigid container such as a polypropylene or stainless steel pan during autoclaving.** Bags are available in a variety of sizes, and some are printed with an indicator that changes color when processed.
- Polypropylene bags are impermeable to steam, and for this reason should not be twisted and taped shut, but gathered loosely at the top and secured with a large rubber band or autoclave tape. For dry materials, some water must be added into the bag to assure that steam is produced inside the bag.

- **Polypropylene containers and pans.** Polypropylene is a plastic capable of withstanding autoclaving, but resistant to heat transfer. Therefore, materials contained in a polypropylene pan will take longer to autoclave than the same materials in a stainless steel pan. To decrease the time required to sterilize material in these containers,
    a. Remove the lid (if applicable).
    b. Turn the container on its side when possible.
    c. Select a container with the lowest sides and widest diameter possible for the autoclave.
- **Stainless steel containers and pans.** Stainless steel is an efficient conductor of heat and is less likely to increase sterilizing time, though is more expensive than polypropylene.

Preparation and Loading of Materials

- Fill liquid containers only 50%.
- Loosen caps, or use vented closures.
- Always put bags of biological waste into autoclavable pans to catch spills.
- Position biohazard bags on their sides, with the bag neck taped loosely.

- Leave space between items to allow steam circulation.
- Household dishpans melt in the autoclave. Use autoclavable polypropylene or stainless steel pans.

Cycle Selection

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

Time Selection

- Take into account the size of the articles to be autoclaved. A 2-liter flask containing 1 liter of liquid takes longer to sterilize than four 500 ml flasks each containing 250 ml of liquid.
- Material with a high insulating capacity (animal bedding, high-sided polyethylene containers) increases the time needed for the load to reach sterilizing temperatures.
- Bags of biological waste should be autoclaved for 50 minutes to assure decontamination.

Removing the Load

- Check that the chamber pressure is zero.
- Wear a lab coat, eye protection, heat insulating gloves, and closed-toe shoes.
- Stand behind door when opening it.
- Slowly open door only a crack. Beware of a possible rush of steam.
- After the slow exhaust cycle, open autoclave door and allow liquids to cool for 20 minutes before removing.

Monitoring

Autoclaves shall be tested periodically to ensure effectiveness. Testing parameters include biological indicators (described below) which are used to monitor the sterilization process. Chemical indicators (autoclave tape) are used in conjunction with biological indicators and physical parameters (i.e., pressure and temperature readings). They provide instantaneous feedback to confirm that the load has been sterilized; however, they must not be used as the sole indicator of sterility. The results of biological indicator testing must be entered in the *Equipment Log Sheet (see attached example)* in the *Autoclave Log Book.*

Chemical Indicators

Periodicity:

- One strip is dated and included in each load of the autoclave.

Method:

- Tape indicates that time, temperature, and the presence of steam have been adequate to ensure sterilization. The strip must completely change color (colors vary by manufacturer) or reveal the word "autoclaved" to ensure effective operation.
- If the indicator has not fully turned color, the operation of the autoclave should be reviewed, an entry is made on the *Equipment Log Sheet* in the *Autoclave Log Book*, and the load ran again. If the strip fails to indicate adequate exposure after the second attempt, notify the USU Technical Services Branch (301-295-3612) for assistance and possible corrective action.

Biological Indicators

Periodicity:

- Every 40 hours of use (required for autoclaves that are used to deactivate human or non-human primate blood, tissues, clinical samples, or human pathogens), OR
- Every six months (required for autoclaves used to deactivate other material).

Method:

- A commercially available test indicator kit that uses bacterial spores such as *Bacillus stearothermophilus* that can be rendered unviable at 250 degrees F or 121 degrees C. For this type of test, ampoules of *B. stearothermophilus* are autoclaved along with a load of waste. Upon completion of the cycle, the ampoules need to be incubated for 48 hours and then observed for any sign of growth, which would indicate that the autoclave is not sterilizing properly.

If for any reason the integrity of the sterilization process is in question, the load should be considered contaminated and should be reprocessed.

When results of autoclave monitoring are unacceptable, an entry will be made on the *Equipment Log Sheet* stating the problem, the corrective action taken, and how it was resolved. This record is maintained in the *Autoclave Log Book.*

When autoclave equipment fails to operate properly, an entry is made on the *Equipment Log Sheet* stating the problem, the corrective action taken, and how it was resolved. This record is maintained in the *Autoclave Log Book.*

When autoclaves with the capability to generate printouts or temperature charts are utilized, these records of autoclave function should be reviewed from each load to assure that sterilization has occurred. The autoclave operator should make sure paper is in the printer at all times and replaced as needed.

Autoclave Record Keeping

The following records regarding autoclave operations must be maintained on site in the *Equipment Log Sheet* and kept in the *Autoclave Log Book*:

1. Dates when maintenance and/or repairs are performed on the unit.

2. Operations log (each load of deactivated material shall be logged as follows):

    - Date, time, and operator's name.
    - Type and approximate amount of waste (lbs.).
    - Confirmation of sterilization.

Record the temperature, pressure, and length of time the load is sterilized. Note that temperature sensitive autoclave tape is not sufficient to indicate that the load reached sterilization conditions because the tape will change color at lower temperatures, or save the autoclave printout if the autoclave has a working printer.

Autoclave Training and Operation
PI and/or supervisors must train and qualify their staff for operation of autoclaves. Qualified autoclave users should understand the time, temperature, pressure relationships required for proper materials decontamination. Additional training on handling materials to be decontaminated should also be provided. Supervisors should maintain a permanent record of training provided to their staff.

Autoclave Maintenance
Follow manufacturer recommended routine maintenance procedures. For repair, use manufacturer warranty if possible. For autoclaves out of warranty, call Technical Services Branch at (301) 295-3612.

AUTOCLAVE LOGSHEET

Model No:_____ **Series No.** _____ **Room No:** _____

Date	Time	Type Waste	Amount	Timer set (minutes)	Temp	Pressure	Bio-Indicator	Chem-Indicator	Corrective Action Taken
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	
							Pass / Fail	Pass / Fail	

AUTOCLAVE SAFETY

Caution - Autoclaves May Cause Serious Burns

To Prevent Injury:

- **Loosen screw caps on bottles and tubes of liquids before autoclaving.**
- **Check that chamber pressure has returned to zero before opening door.**
- **Wear eye and face protection.**
- **Stand behind door when opening it.**
- **Slowly open door only a crack. Beware rush of steam.**
- **Keep face away from door as it opens. Escaping steam may burn.**
- **Wait 5 minutes after opening door before removing liquids.**
- **Liquids removed too soon may boil up and out of container, burning operator.**

Use and Disposal of SHARPS
To prevent needle stick 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.
- Do not overfill SHARPS containers. Close completely when they are 3/4 full and request pickup from EHS at (301) 295-3531.
- Locate SHARPS containers in areas in which needles are commonly used. Make containers easily accessible.
- SHARPS containers must be purchased from laboratory supply distributors such as VWR and Fisher Scientific.

In the event of a needle stick injury:

- Wash thoroughly with soap and water. Notify supervisor and go immediately seek medical attention. Military personnel contact the University Health Clinic (UHC) at (301) 295-3630 to obtain care. Civilian personnel and contractors can visit the Occupational Health Office (A2024; 301-295-9444). If UHC or the Occupational Health Office is closed, military and civilian personnel should go to the emergency room at NNMC or to the most convenient local emergency room (Suburban Hospital, 8600 Old Georgetown Rd, Bethesda, MD 20814). Contractors should go to Suburban Hospital.

Biological Waste Disposal Procedures

Biohazardous Waste

- One time training (Regulated Medical Waste Training) is required for personnel who generate and/or biowaste. This includes waste that is autoclaved or placed in "burn boxes" as described below. This training is available at the EHS website https://sites.google.com/a/USU.edu/ehs/
- All biohazard waste containers must be conspicuously labeled "biohazardous waste" or with the international biohazard symbol and the word "biohazard."
- All bags must be tied to prevent leakage or expulsion of contents during future storage, handling, or transport. (Recommendation: **Bags should not be more than 2/3 full and use autoclave tape to seal bag.**)

- Bags must be placed for storage, handling or transport in a rigid secondary container, which may be disposable, reusable, or recyclable. Containers must be leak resistant. The secondary containers may be any color and labeled with the words "biohazardous waste" or the international biohazard symbol and the word "biohazard" on the lid and on the sides so as to be visible from any lateral direction.
- Full biohazard bags must not be stored **above 0°C (32°F)** for more than **seven days or below 0°C (32 °F) for more than 90 days** before treatment.
- Biohazardous waste must be separated from other waste at the point of origin in the producing facility. Decontamination of biohazardous waste can be accomplished through autoclaving or incineration.

  White/Clear Bag (autoclaving)

  EHS has approved the use of white/clear autoclave bags for BSL-1 and BSL-2 labs tissue culture materials, and non-sharp Risk Group 1 and 2 agent research waste. You may autoclave the white/opaque bags in any autoclave.

  This option allows disposal of non-sharp items in white/opaque autoclave bags. These shall be autoclaved, placed in opaque trash bags, and placed in the regular trash for custodial pick-up. Spill containment is still important both before and after autoclaving; do not set any waste bags directly on floors or counters at any point. Biohazardous bags must be placed in secondary containment.

  Red Bag/Biowaste/Regulated Medical Waste

  Laboratories can also accumulate human blood products and non-sharp infectious waste in double red biohazard bags. All SHARPS from all labs, regardless of agent's Risk Group, must be accumulated in SHARPS containers. These items must be placed in the medical waste boxes, or "burn boxes", for destruction off-site (incineration). EHS utilizes the CDC/NIH categorization system to determine an agent's Risk Group.

  Red bags and biowaste boxes can be obtained through EHS when dropping off (contact EHS at (301) 295-9443/9442).

Human Surgery Specimens or Tissue Waste
Biohazardous waste comprised of human surgery specimens or tissues that have been fixed with formaldehyde or other fixatives shall be segregated for storage, and then disposed of by incineration at an off-site facility.

Multi-hazard or Mixed Waste
Avoid generating mixed waste if possible. Keep volume to a minimum. Do not autoclave mixed waste. When discarding waste containing an infectious agent and radioactive material, inactivate the infectious agent first, and then dispose as radioactive waste. Seek advice from the RSO at (301) 295-3390 before beginning inactivation procedures. When discarding waste containing an

infectious agent and a hazardous chemical, inactivate the infectious agent first, and then dispose as chemical waste. Seek advice before beginning inactivation procedures. Contact EHS at (301) 295-3531 for instructions.

Disposal of Animal Tissues, Carcasses and Bedding
Disposal of animal carcasses/tissues is coordinated through the Laboratory Animal Medicine (LAM). Place animal carcasses/tissues into a plastic bag. Double-bag when carcass contains a zoonotic agent (transmissible from animals to humans). Place bag in freezer until pickup. Call LAM at (301) 295-1910 for pickup. Disposal of animal carcasses/tissues that are contaminated with radioactive materials or hazardous chemicals is performed through EHS. Disposal instructions are available by phoning (301) 295-3531.

Radioactive Biohazardous Waste
All radioactive biohazardous waste must be chemically disinfected and then disposed of through EHS as radioactive waste. See the EHS website and fill out the "Radioactive Waste Collection Form" or the "Chemical Waste Collection Form." EHS will pick up your waste within 1-3 days.

Pharmaceutical Waste

- Non-Controlled substances - Dispose of by placing the material in a cardboard box (no larger than 12in. x 12in. x 12in.). The box must be securely taped shut and labeled "Pharmaceutical Waste." Arrangements can be made to bring it to the Pharmacy. Contact EHS at (301) 295-3668.
- Controlled substance - Must be returned to the Pharmacy with a copy of the yellow sheet. The Pharmacy Officer and Controlled Substances Officer will arrange for controlled substance waste to be picked up and disposed of by an outside vendor. Controlled Substance Custodians must transport controlled substances.

Liquid or Semi-Liquid Biohazardous Waste
Waste such as blood or culture solutions that have been treated by chemical disinfection with bleach may be discharged into the public sewage system. No other disinfectants may be discharged into the sanitary sewers. Other disinfectants must be collected and disposed of by EHS. An example of proper decontamination of liquid waste is adding bleach solution and allowing at least 30 minutes of contact time. Make sure that suction flasks always have the appropriate disinfectant inside the flask before suctioning off the media. The NIH, the CDC, or the American Biological Safety Association must recognize the disinfecting method used.

SHARPS Containers
Full SHARPS containers must be tightly sealed or taped to ensure that contents will not spill. **Do not overfill SHARPS containers!** SHARPS containers should be closed and disposed when ¾ full in order to minimize risk of puncture.

- SHARPS Contaminated with Infectious Materials - Contaminated needles, syringes, scalpels, blades, broken glass, etc. must be placed in rigid, puncture- and leak-resistant containers, which are labeled with the words "SHARPS Waste" and with the international

biohazard symbol or the word "Biohazard" (Appropriate SHARPS containers can be ordered). The containers are picked up by a vendor for off-campus incineration.

- Broken glass NOT Contaminated with Infectious Materials - Broken glass can be placed in rigid, puncture- and leak- resistant containers and taped shut before disposal in the regular trash. Broken glass can be placed in a broken glass container or rigid box, taped shut, and disposed as regular trash. Syringes and razor blades should be disposed in a biohazard SHARPS container with the biohazard symbol defaced.
- SHARPS Contaminated with Radioactive Materials - SHARPS can be contained in rigid, puncture-resistant, non-biohazardous containers, and then disposed through EHS as dry radioactive waste. Label containers "Radioactive Waste." The composition would be dry SHARPS.
- SHARPS Contaminated with Hazardous Materials - SHARPS must be contained in rigid, puncture resistant non-biohazardous containers, and disposed of through EHS. Label containers as "Hazardous Waste." The composition would be solid SHARPS.

XIII. TRANSPORT OF BIOLOGICAL MATERIALS

All biological materials should be transported in a way that maintains the integrity of the material during normal transport conditions, as well as prevents any accidental release and endangerment of the public and the environment.

On-Campus Transport between Laboratories or Buildings
When moving infectious substances between labs or buildings on campus, the following minimum procedures must be followed:

- Sample must be in sealed primary container (i.e. vials, tubes, and other containers that are sealed). Utilize plastic containers whenever possible.
- Place primary container in sealed secondary container, with absorbent (paper towels) between primary and secondary container suitable for the volume transported.
- If dry ice is needed, the secondary container should be placed in an outer container, with the dry ice placed between the secondary and tertiary container (never place dry ice in a sealed container)
- Place biohazard label with agent name, lab address, and phone number on outer container.

XIV. SHIPMENT OF BIOLOGICAL MATERIALS

General Information
Shipment of infectious agents, biological products, and diagnostic specimens is regulated by many agencies, and requirements are not always uniform. In addition, regulations are continually modified and new ones are added. A summary of current requirements is presented here, but it is required that the investigator consult with the USU BSO, Logistics (LOG), and the various agencies before shipping any material that may be regulated. In general, **first** determine whether

the material you wish to ship requires a permit and documented training before you begin the application process. **Second,** decide on a carrier, and learn the packaging and labeling requirements of that carrier. **Third,** contact the BSO (301-295-3531) and/or Logistics (301-295-3060) to determine if a certifying official's signature is needed for the shipment. The investigator must provide a telephone number to LOG and must ensure there is available staff to respond to telephone calls from LOG or any other agency, regarding the specific shipment 24 hours per day until the shipment is delivered to the final destination. LOG will provide the investigator with a copy of the Emergency Response Guidebook.

Permits

- Permits are required from CDC to import or transport:

    1. Any microorganism that causes disease in humans.

    2. Biological materials, such as blood and tissues, when known or suspected to contain an infectious agent.

    3. Live insects, such as mosquitoes, known or suspected of being infected with any disease transmissible to humans.

    4. Any animal known or suspected of being infected with any disease transmissible to humans.

    Importation permits are issued only to the importer, who must be located in the U.S. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the U.S. Public Health Service Division of Quarantine and release by U.S. Customs. Transfers of previously imported material within the U.S. also require a permit. Application for the permit should be made at least 10 working days in advance of the anticipated shipment date. Further information and application forms may be through the CDC web site at http://www.cdc.gov/od/eaipp/.

- Permits are required from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) for importation or transport infectious to livestock; and of biological reagents containing animal, particularly livestock, material (this includes tissue culture media containing growth stimulants of bovine origin such as calf serum). Further information and application forms may be obtained by calling the USDA/APHIS at (301) 734-4401, or through the APHIS website at http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/importexport.
- Permits are also required from the USDA/APHIS for interstate movement, importation, or release into the environment (i.e., field tests) of genetically engineered organisms that are plant pests, or that contain portions (plasmids, DNA fragments, etc.) of plant pests. Application should be made at least 120 days in advance of the anticipated release or shipment date. Information and application forms may be obtained by calling the

USDA/APHIS at (877) 770-5990, or through the APHIS web site at http://www.aphis.usda.gov/wps/portal/footer/topicsofinterest/applyingforpermit

- Facility registration and completion of the APHIS/CDC Form 2 are required by the CDC prior to transfer of select agents and toxins (42 CFR Part 73). Select agents are listed in Appendix G, and a copy of the regulation is available at http://www.selectagents.gov/Regulations.html. Please contact the Biosafety Officer at (301) 295-3531 if your work might include any of the agents listed in Appendix C.
- A validated license is required by the Department of Commerce for **export** of certain microorganisms and toxins to all countries outside of the United States, except Canada. Most agents in this category are included in the List of Select Agents found in Appendix C. As a general rule, the USU will not export biohazardous agents or material. Questions concerning this may be directed to the Biosafety Officer in EHS ((301) 295-3531).

Packaging

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

- *Infectious Substance (Category A)*: Substances known, or reasonably expected, to contain pathogens such as microorganisms, or its toxin and are transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease to humans or animals. (DOT requires shippers of infectious substances to attend training every two years.)
- *Infectious Substance (Category B)*: An infectious substance which does not meet the criteria for inclusion in Category A.
- *Biological Product*: Products derived from living organisms such as vaccines and investigational new drugs.

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

- A primary container that contains the specimen.
- A secondary container that contains the primary container and packaging capable of absorbing the specimen.
- An outer rigid shipping container that contains the secondary container and other material.

Genetically Modified Microorganisms
The *NIH Guidelines for Experiments Involving Recombinant DNA Molecules* (January 2011) states that:

- Host organisms should be shipped as etiologic agents, regardless of whether they contain recombinant DNA (rDNA), if they are regulated as human pathogens, animal pathogens, or plant pests.
- Host organisms should be shipped as etiologic agents if they contain: (1) rDNA that includes the complete genome of an organism that is a human or animal pathogen or plant pest; (2) rDNA that codes for a toxin involved in eliciting human, animal, or plant disease, and that is carried on an expression vector or within the host chromosome; or (3) rDNA from an organism regulated as a human or animal pathogen or a plant pest that has not been adequately characterized.

Human Clinical Materials
The OSHA Bloodborne Pathogens Standard requires that all packages containing human blood and other potentially infectious materials be labeled with the universal biohazard symbol or color-coded. Various carriers may have additional requirements.

XV. BIOSECURITY

Biosecurity is defined as protection of high-consequence microbial agents and toxins, or critical relevant information against theft or diversion by those who intend to pursue intentional misuse. The following biosecurity issues should be considered by all laboratories handling biohazardous agents:

- Risk and threat assessment.
- Facility security plans.
- Physical security.
- Data and electronic technology systems
- Security policies for personnel.
- Policies regarding accessing the laboratory and animal areas.
- Specimen accountability.
- Receipt of agents into the laboratory.
- Transfer or shipping of biohazardous agents from the laboratory.
- Emergency response plans.
- Reporting of incidents, unintentional injuries, and security breaches.

As part of a biosecurity program and to comply with Federal legislation, special procedures are required for the possession and transfer of specific biohazardous agents called CDC Select Agents (see Appendix B for a listing of these agents). As part of the Institutional biosecurity program, the PI should address the following issues in the

conduction of research activities: (1) personnel suitability and reliability (including student access), (2) pathogen accountability (both on-site and through the transfer process), and (3) response to biosecurity incidences

XVI. USEFUL WEB SITES

NIH Guidelines for Research Involving Recombinant DNA Molecules:
http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines

Biosafety in Microbiological and Biomedical Laboratories:
http://www.cdc.gov/biosafety/publications/bmbl5/

NIH Office of Biotechnology Activities:
http://osp.od.nih.gov/office-biotechnology-activities

CDC Select Agents Program:
http://www.selectagents.gov/

USDA/APHIS Select Agents Program:
http://www.aphis.usda.gov/programs/ag_selectagent/

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

Selection, Installation, and Use of Biological Safety Cabinets:
http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_appendixA.pdf

USU Environmental Health and Occupational Safety:
http://www.USU.edu/ehs/

Public Health Agency of Canada Pathogen Safety Data Sheet (PSDS) and Risk Assessments:
http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php

XVII. ADDITIONAL USU INSTRUCTIONS & SAFETY REFERENCES

1020 Proper wearing of Laboratory Coats and Name Tags by USU Personnel.
4120 Infectious Waste Decontamination Quality Control Program
6002 USU Occupational Health & Safety Program
6402-M Radiation Safety Guide
6404 Management of Controlled Substances and Regulated Chemicals
6407 USU Chemical Hygiene Plan

USU Institutional Biosafety Committee (IBC) Project Registration

Please use the attached application for requesting a review and approval of activities involving recombinant DNA (rDNA) and biohazardous agents by the Uniformed Services University Institutional Biosafety Committee (IBC). This application should be used for all activities including research, teaching, and testing. The IBC requests the information in accordance with its charge. This information is required by the Occupational Safety and Health Administration's *Occupational Exposure to Hazardous Chemicals in Laboratories Standard*, its *Blood-Borne Pathogen Standard*, the *Biosafety in Microbiological and Biomedical Laboratories, 5th Edition* and/or the *NIH Guidelines for Research Involving Recombinant DNA (rDNA) Molecules*.

Principal investigators are required to take the Introduction to the NIH Guidelines for Principal Investigators before review of any submitted project application occurs. If you have not had this initial training, or if you have any questions or need assistance with completing this form, please contact the USU Biosafety Officer (BSO) in A2020 (295-3531).

Project Title

Check all that apply.

__ This application is an initial registration for Grant/Project Number: _____

__ This is an amendment/modification to a previous registration using this form. *(Please fill out and attach all relevant Sections.)* Grant/Project Number: _____

__ All materials and techniques fall under a previous registration using this form. *(Please fill out Section A).* Grant/Project Number(s): _____

Principal Investigator Information

PI Name: _____

Department: _____ Office (Bldg/Room): _____

Phone: _____ Email: _____

Statement of Responsibility: I accept responsibility for the safe conduct of work with the agents described in this application. The information in this application is accurate and complete.

_____ Date: _____

(Signature of Principal Investigator)

Please contact the BSO if there are materials that are considered proprietary.

Part A: Location of Research and Overview

1.) List all areas at USU/AFRRI where work will be performed:

2.) Please briefly describe the experimental design, highlighting the recombinant DNA methodology used and/or the use of infectious microorganisms if either are part of the study. Write for non-specialists.

PART B: Recombinant DNA

Will this project involve recombinant DNA? Yes No

If yes, please complete the following question. If no, proceed to the Section C.

1) Please describe the source of the rDNA, vectors used for cloning, and host(s) if applicable. *(Use the* **Supplemental Recombinant DNA Table** *and attach if necessary.)*

Recombinant DNA (source)	**Plasmid/vector(s) cloned into**	**Host(s)**	**Gene Expressed**
Example:GRP78 – glucose regulated proteins 78; The chaperone molecule required to maintain ER function during toxic insults (Human)	*pIRES-GFP*	*Dh5α E. coli (K12 strain), HeLa cells, NIT-1 cells, Mice*	*Yes/No*

2) Will the cloning or expression of any genes result in production of a known toxin or hazard?

Yes No If yes, please explain below:

3) Are any of the vectors listed above considered viral vectors? Yes No

If yes, are there any changes/additions involving viral vectors on most recent Biosafety Audit that need to be made as a result of this new project? Yes No

If yes, please attach or contact the Biosafety Officer.

PART B: Recombinant DNA (Continued)

1) Please specify the relevant category or categories listed below from the *NIH Guidelines* that cover the proposed work. Refer to http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html for complete details or you can contact the Biosafety Officer for help (A2020; 295-3531). *[The descriptions in parentheses are provided only as a guide. Keep in mind that more than one category may apply.]*

__ **Section III-A** (Transfer of drug resistance genes into microorganisms that are not known to acquire the trait naturally, if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture)

__ **Section III-B** (Cloning of toxins with LD50 < 100 ng/kg body weight)

__ **Section III-C** (Transfer of rDNA, DNA, or RNA derived from rDNA ***into human subjects***)

__ **Section III-D** (rDNA from Risk Group 2, 3, 4, or restricted agents as vector systems; Infectious or defective DNA or RNA viruses; Whole animals; Volumes > 10 L)

__ **Section III-E** (rDNA involving < 2/3 of the genome of any eukaryotic virus in the absence of helper virus or plasmids; transgenic rodents)

__ **Section III-F** (Exempt Experiments, see below and circle any that apply)

1. Those that are not in organisms (*example: identification by PCR*).

2. Those that consist entirely of DNA segments from a single non-chromosomal or viral DNA source.

3. Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or related strain).

4. Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (excluding viruses) when propagated only in that host (or related strain)

5. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes. *Lists of exchangers are provided in Appendix A.*

6. Those that do not present a significant risk to health or the environment (*See Appendix C*). Including the following:

a. rDNA in Tissue Culture (< ½ of viral genome; no genes coding for molecules toxic to vertebrates; no DNA from RG 3, 4, or restricted agents).

b. E. coli K-12 Host-Vector Systems (no conjugation proficient plasmids or generalized transducing phages; no DNA from RG 3, 4, or restricted agents).

c. Saccharomyces Host-Vector Systems (no DNA from RG 3, 4, or restricted agents).

d. Bacillus subtilis or *Bacillus licheniformis* Host-Vector systems (no DNA from RG 3, 4, or restricted agents).

e. Extrachromosomal Elements of Gram Positive Organisms

f. The Purchase or Transfer of Transgenic Rodents.

PART C: Infectious Agents and Biological Toxins Use

Does this project involve the use of human or animal pathogens or biological toxins? Yes No
If yes, please complete the following section. If no, proceed to Section D.

1) Has an Annual Biosafety Audit been conducted within the last year? Yes No

2) Please list all new infectious agents or biological toxins not identified in the latest Biosafety Audit to be used in this project and identify the Risk Group. *[See Appendix B of* NIH Guidelines; *Example: Staphylococcus aureus (RG2)]*

3) Does this project involve agents or toxins considered Select Agents or Toxins by the Center for Disease Control?

Yes No If yes, contact the CDC Responsible Official in EHS (295-3531).

PART D: Projects Involving Animal Studies

Will this project involve work with animals?

Yes No *If yes, please complete the following questions. If no, proceed to Section E.*

1) Please list the type of animal(s) to be used: _____
All research with animals must be properly registered with IACUC. All animals must be identified and housed appropriately to ensure safety of the animals and laboratory personnel.

2) Will this project involve the inoculation of animals with infectious agents or toxins? Yes No

Please specify if and how inoculated animal species may shed the infectious agent or toxin.

Part E: Containment and Safety Procedures

1) Please indicate the proposed biosafety containment level(s) to be used in the project. You can refer to the *NIH Guidelines*, Appendix G or the CDC publication *Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition* for guidance or contact the USU Biosafety Officer (301-295-3531) for help.

__ Biosafety Level 1 (BSL-1)
__ Biosafety Level 2 (BSL-2)
__ Biosafety Level 3 (BSL-3)*

__ Animal Biosafety Level 1 (ABSL-1)
__ Animal Biosafety Level 2 (ABSL-2)
__ Animal Biosafety Level 3 (ABSL-3)*

*For BSL-3 containment requirements and use of BSL-3 laboratory space in AFRRI, please contact the Biosafety Officer, Mr. Peter Bouma (pbouma@USU.mil, 301-295-3531).

2) All laboratory personnel are required to have **General Laboratory Safety Training** and **Hazardous Communications Training** (both required every two years). Please check additional training which is required for work within this protocol:

__ Blood Borne Pathogen *(Annual)*
__ Regulated Medical Waste Handling *(One Time)*
__ CDC Select Agent Safety & Security *(Annual)*
__ Site Specific Training (Required for BSL-2 or higher laboratories; *PI monitored)*

3) Please list any special precautions *(Mouth/nose/respiratory protection, eye wear/face shield, special sharps precautions, decontamination methods, aerosol containment, etc.)*, in addition to the personal protective equipment and the biosafety guideline requirements that may be employed to the laboratory for safety and waste handling. You may attach your **Laboratory Specific Biosafety Manual** and or **Experimental SOPs** if applicable.

HHS (CDC) and USDA Select Agents and Toxins
7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

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
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

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Biosafety Laboratory Annual Audit Checklist

Principal Investigator		**Department**	
Lab Phone #		**Lab Room(s)**	
Name & Title of Person Providing Information			

I. Biological Agent Information

1. Please mark all agents that apply to research within the laboratory.

Y	N	**a.** ***Bacteria*** *(not to include K12 or other non-pathogenic E. coli forms for cloning)*
Y	N	**b.** ***Virus*** *(not to include bacteriaphages or viral vectors for expression, see Section II - rDNA.)*
Y	N	**c.** ***Parasite***
Y	N	**d.** ***Biological Toxin***
Y	N	**e.** ***Fungal Agent***
Y	N	**f.** ***Other Biological Agent*** *(archaea, rickettsial agents, prions, etc.)*

Provide name and information (*strains, serotypes, etc.*) of agents identified above. For agents for which there are multiple strains (>5), give a general description of inventory.

Y	N		**2. Does research in the lab incorporate the use of animals?**
Y	N	NA	**3. Are any animals inoculated with infectious agents or toxins? If yes…**
			a. Please list the species inoculated:
			b. Please identify if and how the animal(s) may shed the infectious agent or toxin.
Y	N		**4. Is a Lab Specific Biosafety Manual readily available?** *(Required for BSL-2 laboratories)*
Y	N		**5. Are potential health effects of agents/hazards known to staff?**
Y	N		**6. Do personnel have specific experience/training to work in the lab?** *(This does not include general training provided by EHS)*
Y	N		***a. Is experience/training updated and documented?***
Y	N		**7. Is medical surveillance recommended or vaccine available?** *(check each if applicable)*

Animal Handling Surveillance — **Bloodborne Pathogen Surveillance**

Use of Respirator Surveillance — **Base line Serum sample recommended**

Vaccination *(please describe):*

Y N	8. Are any procedures/equipment used that may generate aerosols with infectious/ biohazardous agents?

Aspirators	*Sonicators*	*Blenders*
Shakers	*Centrifuges*	*Pressurized Vessels*
Homogenizers	*Vortexers*	*Pipetting Infectious Liquids*
Other, please list:		

II. Recombinant DNA

Y N	1. Does research in the lab involve the use of recombinant DNA or transgenic animals?
Y N NA	2. Are viral vectors used to produce viral particles for gene transfer/expression? If yes, please complete *Appendix A* – Viral Vectors Information for each viral vector used.
Y N NA	3. Has any research since the last Biosafety Audit incorporated the use of new plasmids/vectors or cloning/expression of new genes?

III. Tissues, blood, and cell culture

Y N	1. Does the research involve human or non-human primate blood, blood products (*sera*), tissues, or primary cells? *If "Yes", Please specify:*
Y N	a. Are staff current with Bloodborne Pathogen Training?
Y N	b. Is handling of blood/blood product waste described in the Lab Specific Manual? If not, please describe:
Y N	2. Does the research involve human or non-human primate tissue culturing other than primary cell lines (i.e. – continuous or immortalized cell lines)? *If "Yes", Please specify:*
Y N NA	*a.* Are at risk personnel familiar with Appendix H of *Biosafety in Microbiological and Biomedical Laboratories – BMBL* (Working with human or non-human primate cells and tissues)?
Y N NA	b. Is handling of tissue culture waste described in the Lab Specific Manual? If not, please describe:
Y N NA	3. Are universal precautions practiced?
Y N NA	4. Have all personnel working with materials listed above been offered the Hepatitis B vaccination?
Y N	5. Does the research involve other animal/insect tissue culturing not identified above?
Y N NA	6. Have at risk personnel taken Regulated Medical Waste Training?

IV. Work Practices	
Y N	1. Are food and beverages stored or consumed in the lab?
	2. What disinfectant is used to clean or decontaminate surfaces after work with infectious agents or after a spill?
	3. Please identify how broth cultures are disposed? Bleach/Decontaminant Autoclave Not Applicable
Y N	4. Are biohazardous materials transported outside of the lab?
Y N	*a. Are secondary containers used to transport biohazardous materials?*
Y N	5. Is staff informed to report all injuries and accidents to the PI (*Principal Investigator*)?
Y N	6. Are glass Pasteur pipettes, needles, syringes, razor blades and scalpels placed in a rigid SHARPS disposal container and disposed as Regulated Medical Waste?
V. Safety Equipment/ PPE	
Y N	1. Are lab coats/protective clothing worn while working?
Y N	2. Are gloves worn and hands washed after removing gloves and after exiting the laboratory? (*Disposable gloves are not reusable*)
Y N	3. Does research require use of a respirator?
Y N	4. Are emergency shower and eyewash areas easily accessible? (*Access not blocked*)
Y N NA	5. Is a biosafety cabinet (*Tissue culture hood*) used whenever there is potential for splashes or creations of aerosols?

Comments

Principal Investigator Review: _____

(Signature)

Date: _____

When complete, please return to the University Biosafety Officer (EHS, A2020). Laboratories working with materials requiring BSL-2 containment or higher are also required to have laboratory specific safety manual accessible to laboratory personnel for review.

Viral Vectors – Fill out the following questions for each viral vector used in research within your laboratory

Identify (*commercial name, virus species, etc.*) viral vector used to produce viral particles for gene transfer/expression?	
Y N	1. Are the viral particles produced competent?
	a. Number of plasmids use to create viral particles? 1 2 3 4 5
Y N	2. Have any essential genes been deleted in the vector/packaging system? List if known:
	3. List the viral envelope(s) used in packaging system(s):
Y N	a. Are any envelopes used non-native?
Y N	b. Do any envelopes increase species or cell type tropism? If yes, please describe:
Y N	4. Do the viral particles incorporate a transgene that is oncogenic or toxic in nature? If yes, please identify: