# Table of Contents

1.0 PURPOSE ...................................................................................................................... 4
2.0 SCOPE ............................................................................................................................ 4
3.0 RESPONSIBILITIES..................................................................................................... 5
   - Institutional Biosafety Committee (IBC)................................................................................. 5
   - Biosafety Officer (BSO) .......................................................................................................... 5
   - Principal Investigator (PIs) .................................................................................................... 6
   - Department Chairpersons, Research Institute/Center Directors ........................................... 7
   - Employees and Lab Workers .................................................................................................. 8
4.0 RISK GROUPS AND BIOSAFETY LEVELS .............................................................. 9
   - Risk Groups............................................................................................................................. 9
   - Biosafety Levels ...................................................................................................................... 9
5.0 PROHIBITED ACTIVITIES ....................................................................................... 11
6.0 PHYSICAL CONTAINMENT .................................................................................... 12
   - Infectious Organisms ............................................................................................................ 12
   - Oncogenic Viruses ................................................................................................................ 12
   - Plant and Animal Pathogens ................................................................................................ 12
   - Recombinant DNA Studies.................................................................................................... 12
   - In-Vitro Cell Cultures or Tissues.......................................................................................... 13
   - Human Blood and Body Fluids............................................................................................. 14
7.0 STANDARD MICROBIOLOGICAL PRACTICES .................................................... 14
   - All Laboratories (BSL 1 & BSL 2). ...................................................................................... 14
   - Additional Practices for BSL 2 Laboratories ....................................................................... 16
8.0 EXPERIMENTAL ANIMAL WORK ......................................................................... 17
9.0 LABELING OF FACILITIES OR WORK AREAS ..................................................... 18
10.0 BIOLOGICAL WASTE.............................................................................................. 18
    - Classification ....................................................................................................................... 18
    - Disposal of Infectious Wastes ............................................................................................ 19
    - Disposal of Non-Regulated Biological Wastes .................................................................. 21
    - Multihazard Wastes ............................................................................................................ 22
11.0 BIOLOGICAL SAFETY CABINETS............................................................................. 22
12.0 TRANSPORTATION OF BIOLOGICAL MATERIALS (INCLUDING IMPORT/EXPORT PERMITS) ................................................................. 23

   Personal Transport ........................................................................................................... 23
   Shipping and Receiving .................................................................................................... 24
   Permits ............................................................................................................................. 24
   Shipping Regulations ...................................................................................................... 24

13.0 BIOLOGICAL EMERGENCIES AND SPILLS .......................................................... 25

   Emergency Incidents ........................................................................................................ 25
   Biohazardous Agent Spills .............................................................................................. 26
   Personnel Exposure to Biohazardous Agents ................................................................. 28

14.0 TRAINING AND INFORMATION ............................................................................. 29

15.0 PERSONAL PROTECTIVE EQUIPMENT (PPE) ...................................................... 30

APPENDIX A: EMERGENCY PHONE NUMBERS ................................................................. 31
APPENDIX B: SELECT AGENTS & TOXINS LIST ............................................................... 33
APPENDIX C: REFERENCE WEBSITES ............................................................................. 35
APPENDIX D: RISK GROUPS AND BIOSAFETY GUIDELINES ........................................ 38
APPENDIX E: OVERVIEW OF BIOLOGICAL SPILL PROCEDURES AND .................. 40
THE INFECTIOUS WASTE SPILL PROCEDURE ................................................................ 40
APPENDIX F: DISINFECTANT INFORMATION ................................................................. 43
APPENDIX G: LABORATORY SPECIFIC INFORMATION .................................................. 45
The Biosafety Policy and the Biosafety Program at Ohio University (OU) were established to prevent infection, promote safety in research studies, ensure safe handling of biological agents, provide for safe disposal of infectious laboratory wastes, and maintain compliance with applicable institutional policies and regulatory requirements. This manual provides the details of how Ohio University policy, 44.107 Biohazards will be implemented.

1.0 PURPOSE

The purpose of this manual is to describe the operation of the Biosafety Program and to provide guidelines for the safe operation of laboratories and the performance of experiments involving potentially hazardous biological agents. The goals of the Biosafety Program are to:

- protect personnel from exposure to biohazardous agents,
- prevent release of biohazardous agents,
- provide an environment for quality research, and
- comply with applicable government regulations and guidelines.

This manual is also available on the Environmental Health and Safety Department (EHS) web site (http://www.ohio.edu/ehs/docs/Biosafety_Manual_2007.pdf).

2.0 SCOPE

A biohazardous agent is defined as any agent of biological origin that has the capacity to produce deleterious effects on humans, animals, plants or the environment. This includes:

a. Infectious organisms and materials: some bacteria, viruses, rickettsia, fungi, parasites, prions, etc.

b. Harmful metabolic products of microorganisms, e.g., bacterial exotoxins and mycotoxins (aflatoxins, sterigmatocystin, luteoskyrin, rugulosin, cyclochlorotine, patulin, etc.)

c. Oncogenic viruses

d. Recombinant DNA molecules
e. Infectious materials of animal or plant origin (such as cell cultures or tissues)

f. Invertebrate vectors of human diseases

g. Any infectious agents or toxins regulated by the "Anti-Terrorism and Effective Death Penalty Act", commonly called the Agent Transfer Law, or designated by CDC or USDA as a “Select Agent”. See Appendix B.

h. Human blood, and other potentially infectious material (OPIM) as defined in the OSHA Bloodborne Pathogens standard:

1. Human Blood

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

3. Human Cell Lines

3.0 RESPONSIBILITIES

Institutional Biosafety Committee (IBC)

The IBC, in coordination with the Biosafety Officer, is responsible for monitoring and oversight of the use of biohazardous agents to safeguard the health and safety of Ohio University personnel, students, the community, and the environment. The IBC and Biosafety Officer must also ensure compliance with applicable federal regulations and guidelines, granting agency guidelines, as well as Ohio University policies and procedures, as listed in this manual. For details of the structure and operations of the IBC, see the IBC Bylaws and Procedures, which are available on the EHS website.

Biosafety Officer (BSO)

The BSO is appointed from the EHS staff by the Director of EHS and is primarily responsible for implementation of the Biosafety Program. Major duties or activities are as follows:
1) Assist in the preparation and periodic updating of a biosafety manual.

2) Provide consultation to investigators on matters relating to laboratory safety, appropriate handling and containment of biohazardous agents, decontamination, and disposal of infectious wastes.

3) Aid investigators in the development of appropriate emergency measures for dealing with accidental spills and personnel contamination.

4) Surveillance of laboratories in which biohazardous agents are employed to ensure compliance with prescribed safety guidelines and rectification of any deficiencies.

5) Investigate incidents involving biohazardous agents to determine causes and necessary corrections. Upon completing the investigation, the Biosafety Officer will prepare a written report of findings for review and action, if any, by the IBC.

6) Monitor intra-campus transport and provide information for off-campus shipment of biohazardous materials.

7) Review plans for new facilities and modifications of existing structures where etiologic agents or recombinant DNA materials will be used.

8) Develop, arrange, or conduct training programs for laboratory personnel using biohazardous agents.

9) Serve as liaison between OU and outside regulatory agencies concerned with the use of biohazardous agents.

10) Perform other duties, as outlined in the IBC Bylaws and Procedures.

11) The Biosafety Officer, upon concurrence by the Vice President for Research or chairperson of the IBC (or in his/her absence, by at least three other technically qualified members of the Committee), may temporarily stop any work with biohazardous agents that create a recognized hazard to personnel, the public or environment, or involves experiments prohibited by the institution. The entire IBC then will review the problem and forward written recommendation(s) to the Vice President of Research and Director of EHS for final action.

**Principal Investigator (PIs)**

1) The principal investigator is responsible for following all applicable procedures described in this manual. If the investigator wants to use procedures different than those listed in this manual or procedures in addition to what is listed in this manual, it is the responsibility of the investigator to have the alternate...
procedures approved by the IBC, to maintain written documentation of those procedures, and to train laboratory workers in those procedures.

2) The investigator is responsible to personally monitor and be responsible for the day-to-day operation of the laboratory, and to take all necessary steps for the protection of staff, students, and the general public against undesirable consequences of experimental work conducted. The investigator is responsible for thoroughly informing all persons directly involved in hazardous experiments of the potential health risk presented and the safety procedures necessary to minimize exposure.

3) The investigator is responsible for compliance with government and granting agency regulations appropriate to their work.

4) Investigators must obtain written approval from the IBC to begin work with biohazardous materials, prior to initiating those activities. Documentation of what activities require approval and procedures for obtaining approval are found in the IBC Bylaws and Procedures.

5) Investigators must establish specific emergency procedures for their lab. The recommended procedures are included in this manual; the investigator must determine which specific laboratory materials will be used in emergency procedures, and which specific decontamination methods will be employed. See Appendix G for a laboratory specific information sheet that the investigator may use to document this information and make it available to all lab workers.

6) Investigators must arrange for health surveillance of laboratory personnel if deemed appropriate for the research project.

7) Investigators must cooperate with the Biosafety Officer and regulatory personnel during inspection visits and other biosafety projects, such as biosafety cabinet certification, etc.

8) Investigators must keep appropriate records for all biosafety activities including but not limited to: IBC protocol approval, any laboratory-specific procedures, and training records.

Department Chairpersons, Research Institute/Center Directors

1) The chief administrator of each Department, Research Institute or Center is responsible for the general safety of faculty, staff, and students working with biohazardous agents in his/her overall area of jurisdiction.

2) The chief administrator shall ensure that each principal investigator in his/her area of jurisdiction is provided with a copy of the Ohio University Biosafety
Manual. The chief administrator is responsible for stressing the importance of compliance with the Biosafety Manual.

3) The chief administrator is jointly responsible, with the principal investigator, for informing the Institutional Biosafety Committee of work involving biohazardous agents and reporting incidents involving such agents to the Biosafety Officer.

4) The chief administrator is jointly responsible, with the faculty members supervising teaching laboratories, for informing students of proper precautions to be taken when working with biohazardous materials.

Employees and Lab Workers

1) All employees and lab workers are required to follow the procedures listed in this manual, to follow alternate procedures that have been approved by the IBC for their specific laboratory or specific project, and to follow any additional safety procedures that have been adopted by the relevant Principle Investigator.

2) Employees and lab workers are directed to seek guidance from their PI, Department Chair, Center Director, the Biosafety Officer or the IBC if they are unclear about any biosafety procedures.
4.0  RISK GROUPS AND BIOSAFETY LEVELS

Risk Groups

Biohazardous agents are classified into Risk Groups by regulating agencies such as the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH). The classification of many biohazardous agents can be found in the CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL), the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guide), or from the American Biological Safety Association (ABSA) Risk Group Database. The risks are generically defined as follows; see Appendix D for specific definitions from several agencies.

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>RG 1</td>
<td>Well characterized agents that are not known to cause disease in healthy adults, or agents that present a minimal risk to the environment.</td>
</tr>
<tr>
<td>RG 2</td>
<td>Agents that are known to cause disease in healthy adults which is rarely serious and for which prevention or treatment options are available, or agents that pose a moderate risk to the environment.</td>
</tr>
<tr>
<td>RG 3</td>
<td>Agents that are known to cause serious or fatal disease, particularly by the inhalation route, and for which there may be preventive or treatment options available, or agents that pose a high risk to the environment.</td>
</tr>
<tr>
<td>RG 4</td>
<td>Agents that are likely to cause serious or fatal disease for which prevention or treatment options are not usually available, or those agents that pose a serious threat to the environment.</td>
</tr>
</tbody>
</table>

Biosafety Levels

The same regulatory groups have created Biosafety Levels (BSL) that define a set of laboratory practices, facilities and equipment that are appropriate to contain and safely work with the different Risk Groups. The determination of appropriate biosafety level for a project or for a laboratory is made by evaluating the agents in use and the specific procedures and experiments being performed with those agents. Unless specified by regulation, the determination of Biosafety Level for a project or laboratory is made by the Ohio University IBC in conjunction with the PI. In general, the Biosafety Level will be the same as the highest Risk Group for the agents involved. The following table gives an overview of Biosafety Levels; it is taken from the CDC BMBL 5th Edition, 2007. For a detailed description of Biosafety Levels, see the BMBL.
TABLE I
SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

<table>
<thead>
<tr>
<th>BSL</th>
<th>AGENTS</th>
<th>PRACTICES</th>
<th>PRIMARY BARRIERS AND SAFETY EQUIPMENT</th>
<th>FACILITIES (SECONDARY BARRIERS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not known to consistently cause disease in healthy adults (RG1)</td>
<td>Standard Microbiological Practices</td>
<td>None required</td>
<td>Laboratory bench and sink required</td>
</tr>
</tbody>
</table>
| 2   | • Agents associated with human disease  
     • Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure (RG2) | BSL-1 practice plus:  
     • Limited access  
     • Biohazard warning signs  
     • “Sharps” precautions  
     • Biosafety manual defining any needed waste decontamination or medical surveillance policies | Primary barriers:  
     • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials  
     • Biosafety manual defining any needed waste decontamination or medical surveillance policies | BSL-1 plus:  
     • Autoclave available |
| 3   | • Indigenous or exotic agents with potential for aerosol transmission  
     • Disease may have serious or lethal consequences (RG3) | BSL-2 practice plus:  
     • Controlled access  
     • Decontamination of all waste  
     • Decontamination of laboratory clothing before laundering  
     • Baseline serum | Primary barriers:  
     • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials  
     • Biosafety manual defining any needed waste decontamination or medical surveillance policies  
     • Protective laboratory clothing; gloves; respiratory protection as needed | BSL-2 plus:  
     • Physical separation from access corridors  
     • Self-closing, double-door access  
     • Exhaust air not recirculated  
     • Negative airflow into laboratory |
| 4   | • Dangerous/exotic agents which pose high risk of life threatening disease  
     • Aerosol-transmitted laboratory infections have occurred; or related agents with unknown risk of transmission (RG4) | BSL-3 practices plus:  
     • Clothing change before entering  
     • Shower on exit  
     • All material decontaminated on exit from facility | Primary barriers:  
     • All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit | BSL-3 plus:  
     • Separate building or isolated zone  
     • Dedicated supply and exhaust, vacuum, and decontamination systems  
     • Other requirements outlined in the text |

* PPE – Personal Protective Equipment
5.0 **PROHIBITED ACTIVITIES**

Ohio University does not have appropriate facilities or regulatory approvals to conduct the following activities. These activities are prohibited.

1) Work with RG 3 and RG 4 agents.

2) Work that requires BSL 3 or BSL 4 facilities or procedures.

3) Work with [Select Agents](#) that are regulated by the CDC and the U.S. Department of Agriculture (USDA). Except for some work, with small quantities of select agent toxins, that is exempted from the federal regulations. Contact the BSO for more details.

4) Experiments with animal pathogens that are forbidden from entry into the United States.

5) Experiments with plant pathogens or vectors for which the U.S. Department of Agriculture may refuse to issue a permit for importation or interstate movement.

6) Experiments with non-human primates.

7) The following recombinant DNA experiments:
   
   a. Formation of recombinant DNAs derived from pathogenic organisms classified as Risk Group 3 or 4 or from cells known to be infected with such agents, regardless of the host-vector system used.
   
   b. Large scale propagation (more than 10 liters of culture) of recombinant DNA containing organisms that require BSL 3 or higher containment conditions and practices.
   
   c. Creation by the use of recombinant DNA of a plant pathogen with increased virulence and host range beyond that which occurs by natural genetic exchange.
   
   d. Deliberate release into the environment of any organism containing recombinant DNA, unless specifically approved in writing by NIH and/or EPA.
   
   e. Transfer of a drug-resistant trait to microorganisms that are not known to acquire it naturally, if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.
6.0 PHYSICAL CONTAINMENT

Experiments with infectious agents and other potentially hazardous substances are expected to be performed under containment conditions which minimize the possibility of dissemination both within and outside the laboratory area. Final decisions on required containment will be by the IBC and the PI. Typical containment requirements for various types of experiments are listed below. Refer to the BMBL or NIH Guide for more detailed descriptions.

Infectious Organisms

1) Except for situations where the nature of the work with a particular agent may dictate a higher level of containment than that generally deemed acceptable, experiments involving infectious organisms must be performed under the following containment conditions:

   a. RG 1 agents require BSL 1 physical containment and practices. RG 2 agents require BSL 2 physical containment and practices.

2) Detailed documentation of significant reduction in pathogenicity must accompany all written requests made to the IBC to lower the specified physical containment and practices for "attenuated" etiologic agents.

Oncogenic Viruses

All studies with viruses capable of inducing tumors in animals or transformation of cells in culture will be performed according to current safety standards. Refer to the appropriate NIH and CDC sources to determine the risk group for each specific agent and the biosafety level recommended.

Plant and Animal Pathogens

Refer to the appropriate NIH and CDC sources to determine the risk group for each specific agent and the biosafety level recommended. Contact the Biosafety Officer if a plant or animals pathogens cannot be found in the NIH and CDC references.

Recombinant DNA Studies

1) Experiments involving recombinant DNA molecules must be conducted in accordance with the NIH Guide and any recommendations that may be issued on a case-by-case basis by the NIH Office of Recombinant DNA Activities. This is required of all studies regardless of the source of funds used to support the work.
2) Questions concerning recombinant experiments that require prior NIH approval should be directed to the Biosafety Officer at EHS for review with the NIH Office of Biotechnology Activities. While the BSO will provide assistance, it is ultimately the responsibility of the investigator concerned to obtain an official appraisal from the NIH Office of Recombinant DNA Activities.

3) The recombinant DNA guidelines exempt certain experiments from the procedures of the NIH Guide. However, these experiments must be submitted to the OU IBC so that the IBC can verify that the proposed research is exempt.

In-Vitro Cell Cultures or Tissues

The following minimal containment conditions will be required for experimental cell culture or tissue work not involving purposeful infection with human or animal pathogens:

1) Primary cell cultures or tissues of nonmammalian origin - BSL 1 physical containment and practices may be used for primary cell cultures of non-mammalian origin. However, BSL 2 conditions must be used for primary cell cultures of malignant avian tissue.

2) Established cell cultures or tissues derived from normal or malignant mammalian origin (not including cells of primate origin) – BSL 1 containment conditions may be used for established cell cultures derived from normal or malignant mammalian tissue provided it has been clearly demonstrated that they do not carry or release endogenous microbial agents; otherwise, at least BSL 2 containment conditions are required.

3) Primary cell cultures or tissues of rodent origin - BSL 2 containment conditions may be used for primary cell cultures of rodent origin if the animals are from breeding colonies known to be free of infection with lymphocytic choriomeningitis virus (Class 3 agent). Work with cell cultures from a breeding colony known to be infected with LCMV or from a breeding colony of unknown status must be approved by the IBC prior to bringing material on campus, as some work with LCMV requires BSL 3 conditions which are not available at Ohio University.

4) All cell lines or tissues of primate origin – A minimum of BSL 2 containment conditions are required for all cell lines of primate origin.
Human Blood and Body Fluids

BSL 2 procedures must be used for all human blood, tissues or body fluids. Compliance with the OSHA Bloodborne Pathogens Standard is also required. See the Ohio University Bloodborne Pathogen Program for details.

7.0 STANDARD MICROBIOLOGICAL PRACTICES

Standard microbiological practices must be used in all laboratories with biohazardous agents, regardless of risk group or biosafety level. These practices are standard safety behaviors for every biological laboratory. See the BMBL section on Laboratory Biosafety Level Criteria for the most current requirements. Listed below are the Standard Microbiological Practices required for all labs and additional requirements for BSL 2 labs, these are taken from the BMBL 5th Edition, 2007.

All Laboratories (BSL 1 & BSL 2)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

10) The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual’s susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution’s healthcare provider for appropriate counseling and guidance.
Additional Practices for BSL 2 Laboratories

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

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

3) When appropriate, a baseline serum sample should be stored.

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

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

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

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

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

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

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

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

12) All procedures involving the manipulation of infectious materials that may generate an aerosol must be conducted within a BSC or other physical containment devices.
8.0 **EXPERIMENTAL ANIMAL WORK**

1) The Institutional Animal Care and Use Committee (IACUC) and the IBC must be fully informed of studies involving use of infectious agents, or other biohazardous agents in animals. These committees and the principal investigator must concur on all aspects of containment conditions and practices essential for protection of personnel and other experimental animals. It is imperative that no hazardous work is started until complete agreement is reached. In general, submission of separate forms to each committee and written approval from each committee is required.

2) Work involving animals and biohazardous agents must follow all CDC recommendations for Animal Biosafety Level (ABSL) work. See the [BMBL](#) for current recommendations.

3) Persons performing work that involves both biohazardous agents and animals are expected to follow the applicable Standard Microbiological Practices and BMBL recommended ABSL practices.

4) Wastes from animals or waste animals must be handled according to the requirements for either EPA infectious waste regulations, procedures for non-regulated biological wastes as outlined in this manual, or according to the procedures developed by the Laboratory Animal Resources (LAR) department.

5) Transportation of infected or infectious animals must be conducted in a manner that does not spread contamination. Contact the Director of LAR or the BSO for situation specific requirements.

6) Work with captive wild animals and certain domestic animals with unknown health history are potentially dangerous even though no experimental infectious agent or hazardous substance is used. This is because the animals may harbor zoonotic disease capable of infecting other animals or humans. Unless the absence of pathogens is determined by appropriate screening procedures, it is best to regard questionable animals as potentially infectious. Appropriate biosafety precautions must be developed by the PI that are specific to the work conducted.

7) Transplantable rodent tumors are of particular concern since it has been shown that they frequently harbor a variety of indigenous viruses, such as lymphocytic choriomeningitis virus, a Class 3 human pathogen. Before arranging to obtain tumor-bearing rodents of unknown health history from sources outside Ohio University, it is imperative that the LAR and EHS be informed to assure availability of a suitable isolation room. Screening procedures for detection of indigenous viruses in transplantable rodent tumors should be performed before use.
9.0 LABELING OF FACILITIES OR WORK AREAS

1) All biological safety cabinets, incubators, refrigerators, freezers, and liquid nitrogen storage containers used for biohazardous materials of RG 2 must be posted with "BIOHAZARD" signs; RG 1 materials may be posted. This is the minimum signage requirement. Where regulations require higher levels of warning signage, this must be done. The equipment must be kept locked at all times when located in an area accessible to persons other than laboratory personnel.

2) The entrance to work areas used for RG 2 agents or recombinant DNA activities or designated BSL2 must be posted with "BIOHAZARD" signs. RG 1 agents and BSL1 may be posted.

3) A sign or label giving the name(s) and telephone number(s) of responsible person(s) who may be contacted in case an emergency occurs when the laboratory is not in operation, must be affixed to BIOHAZARD signs posted at the entrance to work areas and on biohazard storage facilities located in unrestricted corridors and rooms that use RG 2 agents or rDNA or are designated BSL2. RG 1 agents and BSL1 areas may be posted.

10.0 BIOLOGICAL WASTE

Classification
Biological wastes are classified either as Infectious Waste or Non-Regulated Biological Waste.

Infectious Waste - is regulated by the Ohio Environmental Protection Association (OH EPA). The Ohio Administrative Code defines infectious waste as follows:

1) Cultures and stocks of infectious agents and associated biologicals. This includes specimen cultures, cultures and stocks of infectious agents, wastes from the production of biologicals, and discarded live and attenuated vaccines.

2) Laboratory wastes that were, or were likely to have been, in contact with infectious agents that may present a substantial threat to public health if improperly managed.

3) Pathological wastes, including human and animal tissues, organs, and body parts, and body fluids and excreta that are contaminated with or are likely to be contaminated with infectious agents, removed or obtained during surgery or autopsy or for diagnostic evaluation, provided that, with regard to pathological waste from animals, the animals have or are likely to have been exposed to a zoonotic or infectious agent.
4) Waste materials from the rooms of humans, or the enclosures of animals, that have been isolated because of diagnosed communicable disease that are likely to transmit infectious agents. Such waste materials from the rooms of humans do not include any system established by the Centers for Disease Control, unless specific wastes generated under the universal precautions system have been identified as infectious wastes by the Public Health Council in rules adopted in accordance with ORC Chapter 119.

5) Human and animal blood specimens and blood products that are being disposed of, provided that, with regard to blood specimens and blood products from animals, the animals were or are likely to have been exposed to a zoonotic or infectious agent. "Blood products" does not include patient care waste such as bandages or disposable gowns that are lightly soiled with blood or other body fluids, unless the generator determines that they are soiled to the extent that they should be managed as infectious wastes.

6) Contaminated carcasses, body parts, and bedding of animals that were intentionally exposed to infectious agents from zoonotic or human diseases during research, production of biologicals, or testing or pharmaceuticals, and carcasses and bedding of animals otherwise infected by zoonotic or infectious agents that may present a substantial threat to public health if improperly managed.

7) Sharp wastes used in the treatment or inoculation of human beings or animals. Also sharp wastes that have or are likely to have, come into contact with infectious agents in medical, research, or industrial laboratories. Sharp wastes include, but are not limited to, hypodermic needles, syringes, scalpel blades, and glass articles that have been broken.

8) Any other waste materials generated in the diagnosis, treatment, or immunization of human beings or animals, research pertaining to the immunization of human beings or animals, or in the production or testing or biologicals, that the public health council identifies as infectious wastes after determining that the wastes present a substantial threat to human health when improperly managed because they are, or may be, contaminated with infectious agents.

9) Any other waste materials the generator designates as infectious wastes.

Non-Regulated Biological Wastes - are any waste materials with a biological origin that do not meet the definition of an Ohio EPA Infectious Waste.

Disposal of Infectious Wastes
Infectious Waste may be disposed of by two methods: removal and disposal by a contract infectious waste company or cultures may be treated with hypochlorite solution. These are the only two treatment options that Ohio University is licensed and equipped to use. An Ohio EPA guidance document is available for generators of infectious waste.
Contract Waste Service
The university maintains a contract with a state licensed infectious waste service company to pick-up, transport, treat, dispose of and document infectious waste. Contact the Biosafety Officer (740-593-1662) to be set-up with this service. It is the responsibility of the laboratory, department or center/institute to pay all costs incurred for infectious waste disposal. Users of the contract service must use the following procedures.

1) The contractor provides cardboard boxes with special plastic liners to the waste generator (laboratory). These boxes and bags must meet specific performance requirements, so only items specifically designated for infectious waste may be used.

2) The generator sets up the box, taping the bottom, and lines the box with the plastic liner.

3) The waste collection boxes must be stored in a secure location that is labeled with the biohazard symbol. The collection area must have a spill clean-up procedure posted (General Spill Procedure or Appendix E) and must have spill cleanup supplies available.
   a. The clean up supplies must include – absorbent, materials to make a 10% bleach solution or an EPA registered disinfectant, red/orange biohazard bags, gloves, other needed personal protective equipment, a first aid kit, and boundary tape or other method of demarcating a spill area.

4) Generated infectious waste must be segregated at the source of generation. For example, sharps waste must be placed into a sharps container when generated and other infectious waste could be placed into small biohazard bags to segregate it from regular waste. For disposal the wastes must be placed into the lined box.
   a. All sharps must be placed into a puncture proof sharps container, before being placed into the collection box. When it is reaches the full line, the entire sharps container should be placed into the collection box.
   b. Any liquid or semi-liquid materials must be double bagged before being placed into the collection box.

5) Laboratories may choose to perform an initial decontamination of the waste prior to placement in the box (autoclaving, bleach treatment, etc.) This initial decontamination is not required, but often suggested based on the type of waste and frequency of waste pick-up by the contractor.

6) The large collection boxes must be stored in a method to maintain the integrity of the boxes. The boxes must be stored in a non-putrescent state, and refrigerated if necessary.
7) Once the collection box is full, the generator must tie off the plastic liner and seal the box shut with tape.

8) Generators may either establish a regular pick-up schedule with the contractor or the generator must contact the contractor to schedule each pick-up of a full box.

9) When the box is picked up, the waste contractor will have a manifest shipping document that someone from the generator area will sign. The contractor will leave a yellow copy of this form with the signer. These yellow forms must be sent to EHS (EHS, Hudson Health Center), where EHS will maintain the records according to EPA regulations.

Treatment of Cultures with Sodium Hypochlorite Solution
1) This treatment option may only be used on cultures at BSL 1 or BSL 2 that are either surface colonies or colonies in suspension, which will allow the sodium hypochlorite solution to come into direct contact with the colonies.

2) The sodium hypochlorite solution must be mixed immediately prior to each use and contain 15% by volume household bleach in water. This solution results in a hypochlorite concentration of 0.45-0.79% or 4500-7900ppm.

3) The cultures must be submerged in the hypochlorite solution for at least 20 minutes.

4) The hypochlorite solution must be decanted from any culture that is put into the solid non-hazardous waste. The hypochlorite solution must be discarded after use either by pouring into the sanitary sewer or placing it (double-bagged) into the infectious waste boxes for commercial pick-up.

5) The laboratory must ensure that all cultures and hypochlorite solutions are appropriate labeled and handled. Household bleach is corrosive, irritating and toxic.

6) Records must be kept of all infectious waste cultures treatments. These records must include the date of treatment and the number of cultures treated. A tracking sheet designed by EHS may be used or laboratories may develop their own sheet, as long as that sheet includes the same information. These records must be maintained for three years after the latest treatment date recorded on the sheet.

Disposal of Non-Regulated Biological Wastes
Non-regulated biological wastes should be inactivated using methods appropriate to the agents in use. These methods may include: chemical treatment, heat treatment, autoclaving, etc. The laboratory must have a defined inactivation procedure for each category of waste, but is not required to keep documentation of each waste inactivation event.
**Multihazard Wastes**
Multihazard wastes are any waste materials that present more than one of the following hazards: biohazard, radioactive, chemical hazard. Contact EHS prior to generation of multihazard waste to determine the proper disposal method.

**11.0 BIOLOGICAL SAFETY CABINETS**

Biological safety cabinets are required for any work that generates aerosols at BSL 2 or with RG 2 agents. Activities that generate aerosols include pipetting, blending, centrifuging, etc. Biological safety cabinets (or biosafety cabinets, or BSCs) provide protection to the user, the environment and to the work inside the cabinet by filtering the air through HEPA filters. There are also other laminar flow hoods available, such as clean benches, that provide protection for the work being performed, but do not protect the user or the environment; these are not BSCs. For a full description of BSC operation, selection and use, see the CDC publication “Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets”. In general, a class II cabinet is required for BSL 2 or RG 2 work at Ohio University. See below for an overview of BSC use procedures.

**OPERATION OF CLASS II BSCs**

1) Turn on cabinet fan 15 minutes before beginning work. (If the optional UV light is on, turn it off before proceeding to step 2.)

2) Disinfect the cabinet work surface with an appropriate disinfectant.

3) Place all work supplies inside the cabinet, including any containers needed for disposal. Movement of hands in and out of the cabinet can spread contamination or create turbulence that affects the BSC performance. Do not block the BSC’s air grills.

4) Wait 2-3 minutes to purge any contaminates.

5) Keep all work items at least 4 inches back from the edge of the cabinet. Set-up and conduct work from the clean to the contaminated areas of the cabinet in order to protect the work being conducted.

6) Open flames should not be used in the biosafety cabinet because they disrupt the air flow and pose a fire hazard that could damage the cabinet’s HEPA filter. Alternate methods such as disposable inoculating loops are recommended. If a flame is necessary, a touch-plate micro burner with a pilot light or electric incinerator must be used.
7) When work is completed, wipe all surfaces both of the cabinet and of any equipment, with appropriate disinfectant.

Most BSCs at Ohio University vent filtered air into the laboratory. The filters remove particles, such as viruses and bacteria. The filters do not remove vapors or gases. Therefore, laboratory-venting BSCs must not be used for hazardous chemical work. In some cases, BSC can be vented outside of the building to allow for work with hazardous chemicals.

BSCs require regular certification and maintenance by certified technicians. Certification is required upon installation, after major repairs, when moved and at least annually. The certification must be conducted in accordance with the National Sanitation Foundation, Standard 49. The laboratory or department is responsible for scheduling and funding certification, repairs and filter changes. Contact EHS for information on certified technicians. The BSC will occasionally need to be decontaminated by a certified technician, such instances may include: prior to major repairs, prior to moving, or in cases of large spill or gross contamination. Contact EHS for more details about BSC decontamination.

12.0 TRANSPORTATION OF BIOLOGICAL MATERIALS (including import/export permits)

Personal Transport
Personal transportation of RG 2 agents or any agent from a BSL 2 laboratory must follow these procedures. It is recommended that RG 1 and BSL 1 work also follow these procedures. For personal walking transportation between laboratories or between buildings, the following steps must be taken.

1) The agent must be in a leak-proof primary container (for example: screw top vial, vacutainer tube, etc.)

2) The primary container must be placed in a leak resistant secondary container (for example: a cooler, a plastic box, etc.)

3) The secondary container must be labeled with the biohazard symbol.

4) It is recommended that the name of the biohazardous agent be available outside of the secondary container. The name could be written on the outside of the container or written on paperwork that is carried by the person performing the transport.

For personal transport in vehicles, consult with the BSO for current Department of Transportation regulations.
Shipping and Receiving
Shipping and receiving of biological materials is regulated by several different agencies, depending on the shipment. These regulations may include training, packaging, and labeling requirements, in addition to obtaining required permits. All shipping and receiving of biological materials must be conducted in accordance with current regulations. When shipping or receiving biological materials, the recommended steps are as follows:

1) Determine what you want to ship/receive and to/from where.

2) Determine if any permits are necessary, and start the application process.

3) Choose a shipper; determine their shipping procedure; and determine any applicable shipping regulations.

4) Once permits are obtained, package and ship according to the correct procedures.

5) At any point contact EHS for assistance. EHS also offers some hazardous materials shipping training.

Permitting and shipping regulations are continually modified, so links to current information are provided. Contact EHS for assistance. Material Transfer Agreements are typically handled through the Technology Transfer Office (593-1818). The Office of Research Compliance can assist with some import/export permits (593-0664).

Permits
- U.S. Department of Commerce export permits are required for a wide variety of biological materials [http://www.bis.doc.gov/licensing/exportingbasics.htm](http://www.bis.doc.gov/licensing/exportingbasics.htm)

Shipping Regulations
13.0 BIOLOGICAL EMERGENCIES AND SPILLS

Emergency Incidents

If an emergency is immediately dangerous to you or others in the building or if the emergency cannot be handled by university personnel (for example: fire):

1) Evacuate the lab if your life is in danger or if you can evacuate without spreading contamination.
2) Pull the fire alarm as you evacuate the building.
3) Call 911 to reach fire/ambulance/police.
4) If you can do so safely, also call Ohio University Police Department (OUPD) 740-593-1911 and then EHS 740-593-1666.
5) Call the laboratory supervisor if they are not aware of the emergency.

For medical emergencies:

1) Call 911 to reach the ambulance.
2) Stay with the injured party until medical assistance arrives.
3) Inform the 911 operator of any potential contamination before the medical staff arrives.
4) After the 911 operator tells you to hang-up, call the laboratory supervisor, if they are not aware of the emergency. Then call EHS 740-593-1666.

For smaller incidents that are not emergencies, but require assistance from other university staff:

- Minor medical incidents: call OUPD 740-593-1911 to transport students or staff to Hudson Health Center, and then inform the laboratory supervisor if necessary.
- Spills for which you require assistance to clean-up: inform the laboratory supervisor if necessary and then call EHS 740-593-1666.

Reporting

1) For minor incidents that are handled in the laboratory inform the laboratory supervisor.

2) Laboratory supervisors are responsible for reporting all incidents to the appropriate university offices, possibly including: Department Chair, Center/Institute Director, Workers Compensation Office, OUPD, or EHS.

3) Some incidents involving biohazardous material require reporting to agencies outside of the university (EPA, NIH, etc.). Report all biohazardous incidents to EHS BSO, so that when necessary, the proper reporting will take place. Route all reporting to such agencies through EHS.
4) Following all injuries:
   a. Complete an OU Incident Report Form
      i. This form may also be used to document incidents that do not result in injuries.
   b. If the incident included a sharps injury, also complete the Sharps Injury Report Form

Biohazardous Agent Spills

Each laboratory must have a plan and equipment available to deal with spills that occur in their laboratory. Each event may require variations of procedures and equipment, but basic outlines are provided below. See Appendix E for a printable overview of spill procedures, and Appendix F for information on chemical disinfectants.

Spill Kit – These supplies must be available in the laboratory, either as a specific kit or as part of the standard lab equipment. All workers in the lab must know the location of this equipment.

- Concentrated Disinfectant
- Disposable Absorbent Material (paper towels, commercial absorbents, etc.)
- Forceps and/or Dust Pan & Brush for picking up broken glass (make sure they can be disinfected)
- Gloves and Splash Goggles – both utility gloves and lab/exam style gloves should be available
- Biohazard bags
- Instructions on how use the specific equipment (ex: how to dilute the disinfectant) and general clean up procedures.

For all spills
1) Notify others and restrict access to the area, if necessary.

2) Remove sharps, broken glass, etc., using a tool, before absorbing up the spill. Place the sharps directly into a biohazard sharps container for disposal.

Spill inside a biological safety cabinet
1) Leave cabinet turned on.

2) Disinfectant and gloves should already be available at cabinet, but if they aren’t available, get them now.

3) Put on PPE

4) Spray or wipe the walls, work surfaces and equipment with disinfectant. If necessary, flood the work surface, drain pans and catch basin.

5) Wait for the contact time of the disinfectant (usually 15 minutes).
6) Absorb the disinfectant and spill with disposable absorbent. Make sure to get all surfaces, including under the exhaust grill and tray. Make sure no absorbent debris is left in the cabinet.

7) Place all absorbent material into the biohazardous waste. If the absorbent is ‘dripping’, double bag it before placing into the infectious waste box.

8) Wash hands and any other exposed area.

9) Notify EHS if the spill overflows into the inner workings of the cabinet, as a more thorough decontamination may be necessary.

**Small spill outside of a biosafety cabinet**
(approximately <500 mL and able to be absorbed by the spill kit supplies)

1) Don the appropriate personal protective equipment (at least splash goggles, gloves, and lab coat).

2) Cover the spill with absorbent material.

3) Pour disinfectant over the spill and absorbent material.

4) Allow contact time appropriate to the disinfectant, usually 15 minutes.

5) Pick or scoop up absorbent material, from the outside to the inside of the spill, and put directly into a biohazardous waste container. If the absorbent is ‘dripping' double bag it before placing into the infectious waste box.

6) Rewipe the spill area with disinfectant.

7) Wash hands and any other exposed area.

**Large spill outside of a biosafety cabinet**
(approximately >500 mL or any spill that cannot be handled with the lab spill kit supplies)

1) Get Help!

2) Notify the lab manager and EHS 740-593-1666.

3) Prevent spreading contamination by restricting access the area (put up a sign, lock the door, etc.)
Personnel Exposure to Biohazardous Agents

Exposure to Intact Skin

1) Remove contaminated clothing. Clothing should be cut-off rather than pulling the contaminated clothing over the face where contact with the eyes, nose or mouth may occur.

2) Wash the contaminated skin with soap and water for 1 minute.

3) If necessary, seek medical attention at the Occupational Health Clinic or by calling 911.

4) Inform your supervisor, complete an incident report form. If necessary, inform EHS.

Exposure to Broken Skin, Damaged Skin, a Puncture Wound

1) Remove contaminated clothing. Clothing should be cut-off rather than pulling the contaminated clothing over the face where contact with the eyes, nose or mouth may occur.

2) Wash the contaminated skin with soap and water for 5 minutes.

3) If necessary, seek medical attention at the Occupational Health Clinic or by calling 911.

4) Inform your supervisor, complete an incident report form. If necessary, inform EHS.

Exposure to the Eyes

1) Flush your eyes with water, using an eyewash, for 15 minutes. Hold your eyelids open and rotate your eyes to flush all surfaces.

2) Remove contaminated clothing. Clothing should be cut-off rather than pulling the contaminated clothing over the face where contact with the eyes, nose or mouth may occur.

3) If necessary, seek medical attention at the Occupational Health Clinic or by calling 911.

4) Inform your supervisor, complete an incident report form. If necessary, inform EHS.
Exposure to other Mucous Membranes (Nose, Mouth)

1) Flush the exposed area with water for 5 minutes.

2) Remove contaminated clothing. Clothing should be cut-off rather than pulling the contaminated clothing over the face where contact with the eyes, nose or mouth may occur.

3) If necessary, seek medical attention at the Occupational Health Clinic or by calling 911.

4) Inform your supervisor, complete an incident report form. If necessary, inform EHS.

Ingestion or Inhalation

1) Move to fresh air.

2) If necessary, seek medical attention at the Occupational Health Clinic or by calling 911. The Poison Control Center can be reached at 1-800-222-1222.

3) Do not induce vomiting, unless instructed to by a medical professional.

4) Inform your supervisor, complete an incident report form. If necessary, inform EHS.

14.0 TRAINING AND INFORMATION

Training and assistance for researchers is available in the form of:

1) Consultation assistance from the Biosafety Officer, IBC members, EHS Staff, Laboratory Animal Resources Staff, and the Occupational Health Clinic. Contact them directly.

2) Computerized and written information from this manual, EHS web site, governmental agencies, and many other sources. See Appendix C.

3) Training programs are available from EHS; see the website for current offerings. Ask EHS about additional training, if your desired training is not already listed on the EHS website. As needed, the Biosafety Officer is available for assistance when designing and presenting training programs for individual needs or audiences.
15.0 PERSONAL PROTECTIVE EQUIPMENT (PPE)

1) Appropriate personal protective equipment (PPE) is required to be worn during all work with biohazardous agents.

2) Typical PPE for BSL 1 work minimally requires gloves and may also require lab coats and safety glasses or safety goggles. BSL 2 work requires gloves, lab coats and safety glasses or safety goggles. In rare cases, respiratory protection may be required or desired. All use of respiratory protection, must follow the university’s Respiratory Protection Program.

3) The investigator is responsible for determining the specific PPE requirements in their laboratory, informing laboratory workers and enforcing the use of that PPE. PPE requirements must be documented; this may be done using the university’s PPE Checklist or on the laboratory specific information sheet in Appendix G.

4) Gloves:
   a. Gloves should be worn when working with an etiologic agent that may cause infection by entry through skin abrasions. Gloves must be worn when one anticipates hand contact with blood, potentially infectious materials, mucous membranes, or non-intact skin.

   b. Generally latex or nitrile ‘exam’ style gloves are used for laboratory work. Heavy duty utility gloves may be used for housekeeping duties.

   c. Disposable gloves should be replaced as soon as possible if contaminated, torn, punctured or damaged in any way. The contaminated gloves must be placed into the infectious or biological waste stream. Reusable gloves are not recommended for work with biohazardous agents; if used, they must be decontaminated after each use.

5) Signs indicating "Eye Protection Required" should be prominently displayed in all areas where a hazardous eye exposure may exist. Safety glasses provide protection from impacts, while safety goggles provide protection from impacts and from splashes.
APPENDIX A: Emergency Phone Numbers

(A sticker with emergency contact phone numbers is available from EHS.)
Emergency Phone Numbers

Athens County Emergency Number 911
(fire, police, medical or other major emergencies)
Ohio University Police Department 593-1911
Ohio University Facilities Management 593-2911
National Poison Control Centers Hotline 1-800-222-1222
Ohio University Environmental Health & Safety 593-1666
Ohio University Radiation Safety Program 593-4176 or 592-2950
Ohio University Biosafety/Infectious Waste/ Lab Safety Program 593-1662
Ohio University Hazardous Materials Management Program 593-1663
Ohio University Fire Staff 593-1665 or 597-1748
Ohio University Laboratory Animal Resources 593-2997

My Department Chair/Director ___________________________ ______
My Supervisor ___________________________ ______
My Lab/Area/Office ___________________________ ______
Other ___________________________ ______
Appendix B: Select Agents & Toxins List

The most current list is available online at the Select Agent Homepage
http://www.selectagents.gov/
HHS Select Agents and Toxins

Abrin
Botulinum neurotoxins
Botulinum neurotoxin producing species of Clostridium
Cercopithecine herpesvirus 1 (Herpes B virus)
Clostridium perfringens epsilon toxin
Coccidioides posadasii/Coccidioides immitis
Conotoxins
Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus
Ebola viruses
Francisella tularensis
Lassa fever 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
Rickettsia rickettsii
Saxitoxin
Shiga-like ribosome inactivating proteins
Shigatoxin
South American Haemorrhagic Fever viruses
Flexal
Guanarito
Junin
Machup
Sabia
Staphylococcal enterotoxins
T-2 toxin
Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses
Central European Tick-borne encephalitis
Far Eastern Tick-borne encephalitis
Kysanur Forest disease
Omsk Hemorrhagic Fever
Russian Spring and Summer encephalitis
Varioia major virus (Smallpox virus)
Varioia minor virus (Alastrim)
Yersinia pestis

Overlap Select Agents and Toxins

Bacillus anthracis
Brucella abortus
Brucella melitensis
Brucella suis
Burkholderia mallei (formerly Pseudomonas mallei)
Burkholderia pseudomallei (formerly Pseudomonas pseudomallei)
Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan Equine Encephalitis virus

African horse sickness virus;
African swine fever virus;
Akabane virus;
Avian influenza virus (highly pathogenic);
Bluetongue virus (exotic);
Bovine spongiform encephalopathy agent;
Camel pox virus;
Classical swine fever virus;
Ehrlichia ruminantium (Heartwater)
Foot-and-mouth disease virus;
Goat pox virus;
Japanese encephalitis virus;
Lumpy skin disease virus;
Malignant catarrhal fever virus (Alcelaphine herpesvirus type 1);
Menangle virus;
Mycoplasma capricolum subspecies capripneumoniae (contagious caprine pleuropneumonia)
Mycoplasma mycoides subspecies mycoides small colony (MmmSC) (contagious bovine pleuropneumonia)
Peste des petits ruminants virus;
Rinderpest virus;
Sheep pox virus;
Swine vesicular disease virus;
Vesicular stomatitis virus (exotic): Indiana subtypes VSV-IN2, VSV-IN3
Virulent Newcastle disease virus

USDA Select Agents & Toxins

African horse sickness virus;
African swine fever virus;
Akabane virus;
Avian influenza virus (highly pathogenic);
Bluetongue virus (exotic);
Bovine spongiform encephalopathy agent;
Camel pox virus;
Classical swine fever virus;
Ehrlichia ruminantium (Heartwater)
Foot-and-mouth disease virus;
Goat pox virus;
Japanese encephalitis virus;
Lumpy skin disease virus;
Malignant catarrhal fever virus (Alcelaphine herpesvirus type 1);
Menangle virus;
Mycoplasma capricolum subspecies capripneumoniae
Mycoplasma mycoides subspecies mycoides small colony (MmmSC) (contagious bovine pleuropneumonia)
Peste des petits ruminants virus;
Rinderpest virus;
Sheep pox virus;
Swine vesicular disease virus;
Vesicular stomatitis virus (exotic): Indiana subtypes VSV-IN2, VSV-IN3
Virulent Newcastle disease virus

USDA Plant Protection and Quarantine (PPQ)
Select Agents and Toxins

Peronosclerospora philippinensis (Peronosclerospora sacchari)
Phoma glycinicola (formerly Pyrenochaeta glycines)
Ralstonia solanacearum race 3, biovar 2
Rattayibacter toxicus
Sclerophthora rayssiae var varzea
Synchytrium endobioticum
Xanthomonas oryzae
Xylella fastidiosa (citrus variegated chlorosis strain)

11/17/2008

1 A virulent Newcastle disease virus (avian paramyxovirus serotype 1) has an intracerebral pathogenicity index in day-old chicks (Gallus gallus) of 0.7 or greater or has an amino acid sequence at the fusion (F) protein cleavage site that is consistent with virulent strains of Newcastle disease virus. A failure to detect a cleavage site that is consistent with virulent strains does not confirm the absence of a virulent virus.
Appendix C: Reference Websites
Ohio University Sites

Environmental Health & Safety http://www.ohio.edu/ehs/


Office of Research and Sponsored Programs Vice President for Research http://www.research.ohiou.edu/orsp/index.php

Office of Research Compliance Vice President for Research and Department of Laboratory Animal Resources (LAR) http://www.research.ohiou.edu/compliance/index.php?section=236

Federal Regulations & Guidelines

OSHA: Occupational Safety and Health Administration/DOL http://www.osha.gov


CDC: Centers for Disease Control and Prevention/HHS http://www.cdc.gov/
Office of Safety and Health http://www.cdc.gov/od/ohs/
“Biosafety in Microbiological and Biomedical Laboratories” (CDC/NIH Manual) http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm

Etiologic Agent Import Permit Program http://www.cdc.gov/od/eaipp/


Packaging of Infectious Materials http://www.cdc.gov/od/ohs/biosfty/shipdir.htm


Workplace Health & Safety http://www.cdc.gov/workplace/

USEPA: U.S. Environmental Protection Agency http://www.epa.gov


NIH, Office of Recombinant DNA Activities http://oba.od.nih.gov/rdna/rdna.html


National Cancer Institute (NCI) http://cancernet.nci.nih.gov/


USDA Plant Health Inspection Service (APHIS) http://www.aphis.usda.gov

USDA, APHIS, Permits http://www.aphis.usda.gov/permits/
USDA, APHIS, Forms http://www.aphis.usda.gov/forms/

OEPA: Ohio Environmental Protection Agency http://www.epa.ohio.gov


ODH: Ohio Department of Health http://www.odh.ohio.gov/

Professional Biosafety Sources

American Biological Safety Association (ABSA) http://www.absa.org

Risk Group and Biosafety Level Definitions and Lists http://www.absa.org/riskgroups/index.html


International Air Transport (IATA) (packaging an air transport for biologicals) http://www.iata.org


Commercial Sources

Saf-T-Pak -- Packaging for Infectious Materials http://www.saftpak.com

Lab Safety Supply (Safety Equipment and Supplies) http://www.labsafety.com

Fisher Safety Products (Safety Equipment and Supplies) http://www.fishersci.com

Baker Company (Biosafety Cabinets) http://www.bakerco.com

NuAire (Biosafety Cabinets) http://www.nuaire.com

Thermo Scientific (Biosafety Cabinets) http://www.thermo.com/com/cda/landingpage/1,10255,640,00.html

Laboratory Animal Resources

Animal Care and Use Committee American Society of Mammalogist http://www.mammalsociety.org/committees/index.asp


Animal Welfare Information Center (AWIC) http://www.nal.usda.gov/awic

Guide for the Care and Use of Laboratory Animals http://www.nap.edu/readingroom/books/labrats/

Recommendations for the Care of Amphibians and Reptiles in Academic Institutions http://netvet.wustl.edu/species/reptiles/pough.txt

American Association for Laboratory Animal Science http://www.aalas.org


Human Subjects Research

Office for Human Resource Protections http://www.hhs.gov/ohrp/

Office of Research Compliance, Human Subjects http://www.ohio.edu/research/compliance/
APPENDIX D: RISK GROUPS AND BIOSAFETY GUIDELINES
Risk Group and Biosafety Level Definitions:

**European Economic Community (DIRECTIVE 93188/EEC, Oct, 1993)**

1. **Group 1** biological agent means one that is unlikely to cause human disease;

2. **Group 2** biological agent means one that can cause human disease and might be a hazard to workers; it is unlikely to spread to the community; there is usually effective prophylaxis or treatment available;

3. **Group 3** biological agent means one that can cause severe human disease and present a serious hazard to workers; it may present a risk of spreading to the community, but there is usually effective prophylaxis or treatment available;

4. **Group 4** biological agent means one that causes severe human disease and is a serious hazard to workers; it may present a high risk of spreading to the community; there is usually no effective prophylaxis or treatment available.

**NIH Guidelines on Recombinant DNA (April 2002)**

1. **Risk Group 1** (RG 1) agents are not associated with disease in healthy adult humans.

2. **Risk Group 2** (RG 2) agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.

3. **Risk Group 3** (RG 3) agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.

4. **Risk Group 4** (RG 4) agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

**Canadian Laboratory Biosafety Guidelines (2nd ed. 1996)**

1. **Risk Group 1** (low individual and community risk) This group includes those microorganisms, bacteria, fungi, viruses and parasites, which are unlikely to cause disease in healthy workers or animals.

2. **Risk Group 2** (moderate individual risk, limited community risk) A pathogen that can cause human or animal disease but under normal circumstances, is unlikely to be a serious hazard to healthy laboratory workers, the community, livestock, or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventive measures are available and the risk of spread is limited.

3. **Risk Group 3** (high individual risk, low community risk) A pathogen that usually causes serious human or animal disease, or which can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that can be treated by antimicrobial or antiparasitic agents.

4. **Risk Group 4** (high individual risk, high community risk) A pathogen that usually produces very serious human animal disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice-versa directly or indirectly or casual contact.

**CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (4th Edition 1999)**

1. **BIOSAFETY 1** is suitable for work involving well-characterized agents not known to cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment.

2. **BIOSAFETY LEVEL 2** is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment.

3. **BIOSAFETY LEVEL 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.

4. **BIOSAFETY LEVEL 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.
Appendix E: Overview of Biological Spill Procedures and the Infectious Waste Spill Procedure
## Biological Spill Procedures Overview

<table>
<thead>
<tr>
<th>Where</th>
<th>Hazard Type</th>
<th>Initial Steps</th>
<th>Needed Supplies</th>
<th>Steps</th>
<th>Follow-Up</th>
</tr>
</thead>
</table>
| Inside Cabinet| BSL 1 or BSL 2       | Leave cabinet running. Inform others of the spill.                             | Disinfectant Absorbent Gloves               | 1. When present, use a tool to remove sharps and place them into the appropriate waste container.  
2. Spray all surfaces with disinfectant.  
3. If necessary, flood surfaces with disinfectant.  
4. Wait appropriate contact time, usually 15 minutes.  
5. Absorb up all spill material and disinfectant; then place absorbent materials into the appropriate waste container. | Inform laboratory supervisor.  
If necessary, inform EHS. |
| Outside Cabinet| Any Size or BSL 2 Small Spill (~<500 mL) | Inform others of the spill. Get help if needed.                              | Disinfectant Absorbent Gloves Goggles Lab Coat | 1. When present, use a tool to remove sharps and place them into the appropriate waste container.  
2. Cover the spill with absorbent material.  
3. Pour disinfectant over the spill, and allow the appropriate contact time, usually 15 minutes.  
4. Pick-up all the absorbent materials and put them into the appropriate waste container.  
5. Rewipe the spill with disinfectant. | Inform laboratory supervisor.  
If necessary, inform EHS |
| Outside Cabinet| BSL 2 Large Spill (~>500 mL) | Get Help! Contact Laboratory Supervisor and EHS                              | Prevent spreading contamination by restricting access to the area (put up a sign, lock the door, etc.) | - | - |
1. Restrict access to the spill area and inform others of the spill. Get help, if needed.
2. Prepare the disinfecting solution.
   For bleach solutions use: 10% household bleach in water (10% by volume)
   For other disinfectants include the instructions here:
3. Get personal protective equipment (PPE) and spill clean-up supplies. Put on the PPE.
   Gloves and other PPE are located:
   Spill clean-up kit or supplies are located:
   Infectious waste boxes & bags are located:
   First aid kit is located:
4. Use tongs or other tools to remove any sharps, broken glass, etc. from the spill. Place the sharps into a sharps container.
5. Absorb the spill using the clean-up supplies, and place the used absorbent into an infectious waste container. Clean the spill of any gross organic materials, putting those materials into an infectious waste container.
6. Lay absorbent, paper towels, or similar material on top of the spill. Pour the disinfecting solution onto the towels. Allow the disinfecting solution to remain on the spill area for the appropriate contact time. Add more solution if the spill area starts to dry.
   10% Bleach Solution Contact Time: 30 minutes
   __________ Solution Contact Time:
7. Put all the disinfectant and absorbent material into the infectious waste container.
8. Clean and disinfect any non-disposable items.
9. Clean the spill area with water.
10. Remove protective equipment, and wash hands.
Appendix F: Disinfectant Information

General information is provided in this appendix. For detailed information or to determine the best disinfectant for the specific agents in use, see current literature or the following references.


### Chlorine Compounds (ex. Clorox)

<table>
<thead>
<tr>
<th>Use Dilution (in water)</th>
<th>10% (see variation for EPA waste treatment)</th>
<th>5%</th>
<th>1%</th>
<th>70%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact Time</td>
<td>15 minutes (see variation for EPA waste treatment)</td>
<td>10 minutes (or as listed on bottle)</td>
<td>10 minutes (or as listed on bottle)</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Inactivates Bacteria?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Inactivates Viruses?</td>
<td>Most</td>
<td>Most (not effective on coxsackie virus)</td>
<td>Most</td>
<td>Most</td>
</tr>
<tr>
<td>Inactivates Spores?</td>
<td>Some</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Preparation</td>
<td>Prepare once/week (see variation for EPA waste treatment)</td>
<td>shelf-life &gt;1 month, if protected from light &amp; air (see label)</td>
<td>shelf-life &gt;1 month, if protected from light &amp; air (see label)</td>
<td>shelf-life &gt;1 month, if protected from light &amp; air</td>
</tr>
<tr>
<td>Recommended Uses</td>
<td>Surfaces and some liquids</td>
<td>Surfaces</td>
<td>Surfaces</td>
<td>Surfaces</td>
</tr>
<tr>
<td>Hazards</td>
<td>Corrosive; Skin, Eye &amp; Respiratory Irritant; Toxic</td>
<td>Corrosive; Skin &amp; Eye Irritant; Toxic</td>
<td>Corrosive; Skin &amp; Eye Irritant; Toxic</td>
<td>Flammable; Eye Irritant; Toxic</td>
</tr>
<tr>
<td>Notes</td>
<td>Leaves a residue which must be cleaned with water. Is inactivated by organic matter.</td>
<td>Leaves a residue that must be cleaned with water.</td>
<td>Leaves a residue that must be cleaned. Is not appropriate for liquid treatment. May stain surfaces or clothing. Use EPA registered disinfectants, not skin antiseptics.</td>
<td>100% is a preservative. Not active when large amounts of organic matter are present. Evaporates quickly, so contact may not be sufficient for killing.</td>
</tr>
</tbody>
</table>
Appendix G Laboratory Specific Information

The following laboratory specific information sheet is intended to be completed by the Principal Investigator and either posted in the laboratory or a copy given to all new lab workers. The information sheet is intended to be a one stop location to get information or to learn where detailed information is located. Feel free to add additional information relevant to the laboratory. Not all questions apply to every lab; complete the questions applicable to the specific laboratory. A word copy of the information sheet is available on the EHS website http://www.ohio.edu/ehs/biosafety/index.htm.
Biosafety Information Provided to Lab Workers

's Laboratory in room .
(PI's name) (Building) (Number)

This is a Biosafety Level ☐ 1 (BSL 1) or ☐ 2 (BSL 2) Laboratory

Biohazardous Agents in Use

<table>
<thead>
<tr>
<th>Group (ex. human cells) or Specific Agent (ex. E. coli)</th>
<th>Risk Group (1 or 2)</th>
<th>Special Containment or Handling Practices beyond Standard Microbiological Practices? Yes or No – If yes list details or where to find them.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Routine Procedures (If desired specific procedures can be listed here.)

<table>
<thead>
<tr>
<th>Where are sharps containers located?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What chemical is used for routine decontamination of work surfaces?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Where can the procedure for routine decontamination of work surfaces be found?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Are any vaccinations, medical exams or routine medical surveillance suggested or required?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Where can lab specific procedures that differ from the Biosafety Manual be found?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Where is the secondary container for transporting materials between labs?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What training is required to work in this lab?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Where can information about personal protective equipment for this lab be found?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>
Waste Procedures
What types of biological waste are generated in this lab?

☐ EPA Regulated Infectious Waste  ☐ Non-Regulated Biological Waste

**EPA Regulated Waste Procedures**
Which method(s) is used?

☐ Disposal by Contract Service  ☐Culture Treatment

What is the location of the following spill supplies?

<table>
<thead>
<tr>
<th>Item</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbent Material</td>
<td></td>
</tr>
<tr>
<td>Disinfecting Agent (Bleach or EPA</td>
<td></td>
</tr>
<tr>
<td>Registered Disinfectant)</td>
<td></td>
</tr>
<tr>
<td>Biohazard Bags</td>
<td></td>
</tr>
<tr>
<td>Gloves</td>
<td></td>
</tr>
<tr>
<td>Other necessary PPE?</td>
<td></td>
</tr>
<tr>
<td>First Aid Kit</td>
<td></td>
</tr>
<tr>
<td>Boundary Tape or other method to</td>
<td></td>
</tr>
<tr>
<td>demarcate a spill</td>
<td></td>
</tr>
<tr>
<td>Written Spill Procedures</td>
<td></td>
</tr>
</tbody>
</table>

**Non-Regulated Biological Waste**
Where can the waste treatment procedure for each kind of waste be found? Or insert the procedure here.

**Emergency Procedures**
**Contact Phone Numbers**

<table>
<thead>
<tr>
<th>Police/Fire/Medical</th>
<th>911</th>
<th>Lab Supervisor</th>
</tr>
</thead>
<tbody>
<tr>
<td>OUPD</td>
<td>593-1911</td>
<td>Lab Supervisor (alt #)</td>
</tr>
<tr>
<td>Poison Control</td>
<td>800-222-1222</td>
<td>Alternate Lab Sup.</td>
</tr>
<tr>
<td>EHS</td>
<td>593-1666</td>
<td>Alt. Lab Sup. (alt #)</td>
</tr>
</tbody>
</table>

**Location of:**

<table>
<thead>
<tr>
<th>Eyewash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety Shower</td>
</tr>
<tr>
<td>Exits</td>
</tr>
<tr>
<td>Lab/dept. meeting location in case of evacuation</td>
</tr>
</tbody>
</table>

**Spill Supplies**

<table>
<thead>
<tr>
<th>Item</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbent Material</td>
<td></td>
</tr>
<tr>
<td>Disinfecting Agent</td>
<td></td>
</tr>
<tr>
<td>Tongs or Dust Pan</td>
<td></td>
</tr>
<tr>
<td>Gloves</td>
<td></td>
</tr>
<tr>
<td>Goggles</td>
<td></td>
</tr>
<tr>
<td>Other necessary PPE?</td>
<td></td>
</tr>
<tr>
<td>Biohazard bags</td>
<td></td>
</tr>
<tr>
<td>Written Spill Procedures</td>
<td></td>
</tr>
</tbody>
</table>