University of Nevada, Reno
Biosafety Manual

Introduction to the Manual

The goal of this manual is to provide policies and procedures that when implemented will reduce risks to the UNR community from biological agents, including pathogenic organisms and derived toxins. These policies and procedures reflect current biosafety regulatory expectations, as well as currently accepted biosafety practices, and are designed to safeguard personnel, facilities, and the environment without inhibiting research activities. Principal Investigators and laboratory workers are expected to be familiar with the requirements of this Manual and to implement these requirements in their laboratory operations.

The hazards present in any particular laboratory are rarely limited to biological agents; chemical hazards are almost always of concern, and radiological hazards are also often present. Consequently, biosafety should not be approached separately from other laboratory hazards, but should be viewed as just one component of a total laboratory safety program. Guidance on chemical and radiological hazards can be found in the UNR Chemical Hygiene Plan and UNR Radiation Safety Manual, respectively.

Success of the UNR Biosafety Program requires a team effort involving the Institutional Biosafety Committee, academic departments, Principal Investigators, laboratory workers, and the Environmental Health and Safety Department. **Principal Investigators are responsible for the health and safety of personnel who work under their supervision and occupy their laboratory space**. Consequently, they are in a unique position to positively influence the implementation of the safe work practices contained in this manual. The Institutional Biosafety Committee and the Environmental Health and Safety Department endorse this manual and encourage active participation in maintaining high standards of biosafety at UNR.

UNIVERSITY OF NEVADA, RENO
BIOSAFETY MANUAL

TABLE OF CONTENTS

Chapter 1
Purpose, Scope, and Responsibilities

Purpose
The purpose of the UNR Biosafety Manual is to define policies and procedures that when implemented, will minimize risks to personnel, facilities, and the environment resulting from the use of biological agents during teaching, research, and clinical activities at UNR. The work practices, procedures and policies specified in this manual are based on current regulatory requirements and accepted good biosafety practices. Implementation of these measures will reduce the likelihood that an incident involving a biological agent will occur, and will fulfill regulatory biosafety expectations. Laboratory microbiological work usually involves exposure not only to biological hazards, but to chemical and radiological hazards as well. Consequently, this manual should be used in conjunction with the UNR Chemical Hygiene Plan and the UNR Radiation Safety Manual, as appropriate. These manuals are accessible at http://www.unr.edu/ehs/safety-policies-and-manuals.

Scope
This manual applies to all UNR activities involving biological agents. All UNR faculty, staff, students, visitors, and employees of industry partners when working on UNR sponsored projects or at UNR facilities, are included in the scope of this manual. For purposes of this manual the term biological agents includes the following:

a. microorganisms (bacteria, chlamydiae, rickettsiae, fungi, and parasites)
b. viruses
c. prions and other infectious agents
d. cultured cells
e. human blood, unfixed tissues, and potentially infectious body fluids
f. recombinant and synthetic nucleic acid molecules and toxic products
g. biological toxins
h. animals infected with human pathogens, and animals as sources of zoonotic diseases
i. insects

Responsibilities
The responsibility for biosafety at UNR is a team effort requiring the direct involvement of the UNR Institutional Biosafety Committee, the UNR Biosafety Officer and Environmental Health and Safety Department (EH&S), Principal Investigators (PIs), and laboratory workers.

UNR Institutional Biosafety Committee
The Institutional Biosafety Committee (IBC) approves biological agent use protocols (MOUAs), develops biosafety policies, and provides administrative oversight of the university biosafety program, with the goal of reducing laboratory biosafety risks to the UNR community. The IBC is composed of at least five members that collectively represent experience and expertise in a wide range of biosafety areas applicable to UNR activities. At least two members of the IBC must be from outside the UNR community (community members not otherwise affiliated with

UNR). Non-committee faculty or staff with special expertise may be asked to advise the IBC as appropriate. Additional information on the IBC can be found at the IBC web page

IBC contacts are as follows:

Committee Member	*Affiliation*
William Courchesne, Ph.D., Chair	UNR Microbiology and Immunology Dept.
Ben Owens (BSO)	UNR EH&S Dept.

Responsibilities of the IBC include:

1. Developing biosafety policies applicable to UNR activities, including work practices, biohazardous waste, and medical surveillance of personnel.

2. Reviewing and approving new research proposals in accordance with CDC/NIH guidelines.

3. Setting required containment levels for research projects. Generally, the biosafety levels (BSLs) established by the CDC and NIH will be used as the level of containment; however, the IBC can increase or decrease the level of containment according to the specific circumstances of the project.

4. Developing design specifications and criteria for containment facilities.

5. Investigating significant violations of UNR biosafety procedures or policies, and significant accidents or illnesses involving biological agents. If appropriate, the IBC will recommend disciplinary action to the proper UNR officials.

6. Notifying the NIH Office of Biotechnology Activities of reportable incidents as specified in the latest edition of the *NIH Guidelines*.

UNR Biosafety Officer

The UNR Biosafety Officer (BSO) is responsible for providing guidance on safe handling of biological agents and overall management of the Biosafety program. The BSO is a member of the IBC. Specific responsibilities of the BSO include:

1. Providing technical advice to the IBC and PIs on biosafety protocols.

2. Developing emergency response plans for accidental spills and personnel contamination, and investigating incidents involving biological agents.

3. Making periodic inspections of laboratories to assess biosafety issues.

4. Keeping the IBC informed of pertinent biosafety issues and program status.

5. Providing general biosafety training for UNR personnel on a regular basis.

6. Informing the IBC Chair of biosafety incidents involving personnel exposures, releases outside of containment, non-compliance with local, State, or federal regulations or *NIH Guidelines*, or other biosafety issues.

Principal Investigators

Principal Investigators (PIs) are responsible for the health and safety of all personnel in their laboratory. Specific responsibilities of the PI include:

1. Ensuring that specific laboratory hazards are effectively communicated to laboratory personnel, and that controls are in place to minimize risks associated with these hazards.

    a. Developing laboratory-specific standard operating procedures (SOPs) that cover the hazards and activities (both routine activities and unusual events) relevant to the laboratory.

    b. Ensuring that engineering controls are available, are in good working order, and are used appropriately to minimize exposure to biohazardous agents.

    c. Ensuring that appropriate personal protective equipment is available and used by laboratory personnel.

2. Ensuring that all laboratory personnel receive general biosafety training conducted by EH&S (or equivalent), as well as laboratory-specific training on the hazards, procedures, and practices relevant to the laboratory they are working in. All training must be documented and records maintained.

3. Notifying the IBC and obtaining prior IBC approval for work involving biohazardous material as specified in this manual (see Chapter 2).

4. Ensuring that laboratory workers are provided immunizations and medical surveillance prior to exposure to biohazardous agents as appropriate (based on current recommendations of the Centers for Disease Control and Prevention, and IBC recommendations).

5. Notifying the BSO of any spills or incidents involving biological agents that result in exposure to laboratory personnel or the public, or release to the environment (including laboratory spills).

6. Ensuring that biological agents are disposed of as outlined in this manual.

7. Ensuring that biohazardous materials to be transported are packaged and shipped in accordance with regulations, and that persons performing these duties have appropriate and current training.

8. Ensuring that an accurate inventory of biological agents is maintained.

9. Ensuring that periodic assessments of the laboratory are conducted to self-identify health and safety weaknesses, and that identified weaknesses are remedied in a timely manner.

Laboratory Workers

Laboratory workers are the most important element in developing and maintaining a safe laboratory environment. Laboratory workers are responsible for their own health and safety, as well as that of their coworkers. An incident caused by one laboratory worker can have a widespread effect on others. Specific responsibilities include:

1. Following procedures and practices established by the University and the laboratory.

2. Knowing how to access the Biosafety Manual (this document) and being knowledgeable of requirements and procedures contained in the Manual.

3. Using practices and procedures specified in this manual, presented in training, and other accepted good laboratory practices to minimize exposures to biological agents, and to avoid other incidents (such as fire, explosion, etc.).

4. Attend biosafety and other laboratory safety training as required.

5. Report unsafe laboratory conditions, incidents or near incidents involving personnel exposure, releases outside of containment, or other biosafety issues to the PI, EH&S, or other responsible party.

6. Utilize control measures such as biological safety cabinets and personal protective equipment to prevent exposure to biological agents, and contamination of personnel and facilities.

Chapter 2
Approval of Research Projects

Projects Requiring Approval

All projects involving biological agents must be reviewed and approved by the Institutional Biosafety Committee (IBC) prior to commencement of the work (see Scope on page 1-1 for a listing of biological agents). Principal Investigators must submit a "Memorandum of Understanding and Agreement" (MOUA) to the IBC in order to initiate the approval process. The MOUA form is available at the EH&S homepage (then "Forms," then "Biological Safety").

Biological Agents

All work involving biological agents must be reviewed by the UNR Institutional Biosafety Committee (IBC) for adherence to NIH/CDC biosafety guidance published in the latest edition of *Biosafety in Microbiological and Biomedical Laboratories*, the latest edition of *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*, applicable regulations, as well as UNR policies and current biosafety practice.

Biosafety Level 1 (BSL-1) and Animal Biosafety Level 1 (ABSL-1)

Organisms in this category are not known to cause disease in healthy human adults. IBC approval is required for all BSL-1 work. Principal Investigators must submit a MOUA to the IBC in order to initiate the approval process.

Biosafety Level 2 (BSL-2) or 3 (BSL-3) and Animal Biosafety Level 2 (ABSL-2) or 3 (ABSL-3)

All work involving biological agents classified as BSL-2 or BSL-3 must be reviewed by the IBC. Containment levels, facility requirements, and work practices will generally follow NIH/CDC guidance; however, the IBC can raise or lower these requirements as appropriate. Principal Investigators are required to submit a MOUA to the IBC in order to initiate the approval process.

Biosafety Level 4 (BSL-4) and Animal Biosafety Level 4 (ABS-L4)

Projects involving BSL-4 organisms are currently prohibited at UNR.

Recombinant and Synthetic Nucleic Acid

As a condition of funding from the National Institutes of Health (NIH), all research at UNR involving recombinant or synthetic nucleic acid must be conducted in accordance with the most current version of *NIH Guidelines for Research Involving Recombinant or Synthetic DNA Molecules (NIH Guidelines)*. PIs are required to make an initial determination of the required biological and physical containment required. The approval level required for the proposed research is dependent on the NIH category to which the work corresponds. **Approval by the IBC is required for all proposed experiments involving recombinant or synthetic nucleic acid, including those exempt from NIH Guidelines. Exceptions to the requirement to submit a MOUA are described in the "Transgenic Animals" section later in this chapter).** Principal Investigators must submit a MOUA to the IBC in order to initiate a request for approval. The following paragraphs summarize experiments covered by

the NIH Guidelines and the required level of approval; refer directly to the NIH Guidelines for a more detailed description of experiments and specific requirements.

Experiments requiring IBC approval, RAC review, and NIH approval (NIH Guidelines section III-A)
Experiments involving the deliberate transfer of a drug resistance trait to microorganisms that do not acquire the trait naturally, where such acquisition could compromise the use of the drug to control disease in humans, veterinary medicine, or agriculture are included in this category. These experiments are considered a "Major Action" and require review by the NIH Recombinant DNA Advisory Committee (RAC) and specific approval by NIH Director prior to initiation. The NIH Office of Science Policy web site has additional information on the RAC and Major Actions. Approval by the IBC is required prior to initiation of these experiments.

Experiments requiring IBC and NIH approval (NIH Guidelines section III-B)
Experiments in this category include the deliberate formation of recombinant or synthetic nucleic acid containing genes for the biosynthesis of toxic molecules with an LD_{50} for vertebrates less than 100 ng/kg. This includes microbial toxins such as botulinum toxins, tetanus toxins, and diphtheria toxin. NIH Office of Science Policy (OSP) and IBC approval is required prior to initiation of these experiments.

Experiments requiring IBC and IRB approval, and RAC review (NIH Guidelines section III-C)
These experiments involve the deliberate transfer of recombinant or synthetic nucleic acid, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, into human research participants (human gene transfer). No research participant may be enrolled until the RAC review process has been completed (Appendix M-I-B of the NIH Guidelines).

Experiments requiring IBC approval before initiation of work (NIH Guidelines section III-D)
This category includes the following categories of experiments:

1. The introduction of recombinant or synthetic nucleic acid molecules into Risk Group 2, 3, or 4 agents.
2. Cloning of DNA from Risk Group 2, 3, or 4 agents into nonpathogenic prokaryotic or eukaryotic host-vector systems.
3. The use of infectious DNA or RNA viruses or defective DNA or RNA viruses in the presence of helper virus in tissue culture systems.
4. The use of whole transgenic animals, including creation of transgenic animals, and viable recombinant or synthetic nucleic acid molecule-modified microorganisms tested on whole animals. Certain experiments involving whole transgenic animals are exempt from *NIH Guidelines* and do not require submittal of a MOUA. See the section below titled "Transgenic Animals" for additional information.
5. Genetic engineering of plants using recombinant or synthetic nucleic acid molecule methods, use of such genetically engineering plants for experimental purposes, or use of plants with microorganisms or insects that contain recombinant or synthetic nucleic acid molecules.

6. Culture of more than 10 liters of organisms that contain recombinant or synthetic nucleic acid molecules.
7. The use of influenza viruses generated by recombinant or synthetic methods,

Experiments requiring IBC notice simultaneous with initiation of work (NIH Guidelines section III-E)

Experiments in this category are low risk and can be conducted using BSL-1 containment. Examples include experiments involving the formation of recombinant or synthetic nucleic acid molecules that do not contain more than two-thirds of the genome of any eukaryotic virus and plant and animal work (including the generation of transgenic rodents) involving recombinant or synthetic nucleic acid molecule methods that can be conducted at BSL-1. IBC approval is required and a MOUA must be submitted simultaneous with initiation of the experiments.

NIH exempt experiments (NIH section III-F)

The experiments listed below are exempt from the *NIH Guidelines*; however, UNR biosafety policy requires the PI to submit a MOUA to the IBC simultaneous with initiation of the work, with subsequent approval by the IBC. Exceptions to the requirement to submit a MOUA for NIH exempt experiments involving transgenic animals are described in the section titled "Transgenic Animals."

The following recombinant or synthetic nucleic acid molecules are exempt from the *NIH Guidelines*:

1. Synthetic nucleic acids that cannot replicate or generate nucleic acids that can replicate in any living cell, and not designed to integrate into DNA, and do not produce a toxin with an LD_{50} for vertebrates of less than 100 ng/kg bodyweight.
2. Recombinant or synthetic nucleic acid molecules that are not in organisms, cells, or viruses and that have not been modified or manipulated to make them capable of penetrating cellular membranes.
3. Recombinant or synthetic nucleic acid molecules that consist solely of the exact recombinant or synthetic nucleic acid sequence from a single source that exists contemporaneously in nature.
4. Recombinant or synthetic nucleic acid molecules that consist entirely of nucleic acids from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host or when transferred to another host by well-established physiological means.
5. Recombinant or synthetic nucleic acid molecules consisting entirely of nucleic acids from a eukaryotic host including its chloroplasts, mitochondria, or plasmids (excluding viruses) when propagated only in that host.
6. Recombinant or synthetic nucleic acid molecules consisting entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent.
7. Genomic DNA molecules that have acquired a transposable element provided the transposable element does not contain any recombinant and/or synthetic DNA.

8. Recombinant or synthetic nucleic acid molecules that do not present a significant risk to health or the environment, as listed in the *NIH Guidelines*, Appendix C.

Transgenic Animals

Creation of transgenic animals, including knockout animals, is included in the scope of the *NIH Guidelines*, and therefore, requires submittal of a MOUA for IBC approval prior to initiation of the work, with additional information and exceptions to this requirement provided below.

Experiments Involving Transgenic Rodents

The creation of transgenic rodents that can be housed under BSL-1 containment is covered under Section III-E-3 of the *NIH Guidelines* and requires submittal of a MOUA simultaneous with initiation of the work, with subsequent approval by the IBC. The creation of transgenic rodents that must be housed at BSL-2 or higher containment levels is covered under Section III-D-4 and requires submittal of a MOUA to the IBC, with approval by the IBC prior to initiation of the work.

Breeding Transgenic Rodents

Breeding of two different lines of transgenic rodents, including knockout lines, can potentially generate a new line of transgenic rodent and is therefore covered by the *NIH Guidelines*. **The breeding of two different lines of transgenic rodents may require submittal of a MOUA for IBC approval. The following experiments involving breeding of transgenic rodents do not require submittal of a MOUA.**

The breeding of a transgenic rodent and a non-transgenic rodent with the intent of creating a new strain of transgenic rodent that can be housed at BSL-1 containment will not require submittal of a MOUA if both parental rodents can be housed at BSL-1 containment and:

1. Neither parental transgenic rodent contains more than one-half of the genome of an exogenous eukaryotic virus from a single family of viruses, or a transgene that is under the control of a gammaretorviral long terminal repeat, and
2. The transgenic rodent that results from this breeding is not expected to contain more than one-half of an exogenous viral genome from a single family of viruses.

Breeding within the same line of transgenic rodents, including knockout lines, at BSL-1 is exempt from the *NIH Guidelines* and submittal of a MOUA to the IBC is not required. If the transgenic rodents require housing at BSL-2 or higher containment level, then the breeding is covered by section III-D-4-b and requires submittal of a MOUA and IBC approval before initiation.

Breeding of Transgenic Animals Other Than Rodents

The breeding of all other transgenic animals is covered by the *NIH Guidelines* section III-D-4-a or III-D-4-b, depending on the containment level required, and requires submittal of a MOUA and prior approval by the IBC.

Purchase or Transfer of Transgenic Animals

The purchase or transfer of transgenic rodents, including knockout rodents, which can be housed at BSL-1, is exempt from the *NIH Guidelines* and submittal of a MOUA is not required. The purchase or transfer of transgenic rodents that must be housed at BSL-2 or higher containment is covered by section III-D-4 and requires submittal of a MOUA with IBC approval before purchase or transfer. The purchase or transfer of any other animal other than rodent species, at any biosafety level, is covered by the *NIH Guidelines* and requires submittal of a MOUA and IBC approval prior to purchase or transfer.

MOUA Amendment and Termination

Changes involving new biological agents, significant procedural changes, or any other modifications must be approved by the IBC. PIs wanting to modify a current MOUA are required to submit a MOUA Amendment form to the UNR Biosafety Officer (www.unr.edu/ehs, then "Forms"). Significant amendments will require review by the IBC. The PI is required to notify the UNR Biosafety Officer when a project is no longer active.

MOUA Expiration and Annual Update

MOUAs are approved for three years; however, PIs are required to verify and update approved projects on an annual basis. The purpose of the annual update is to allow the PI to verify continuance of the project, discontinue the project, or amend the MOUA. The UNR Biosafety Officer or other designated person will contact each PI and request verification of the accuracy of the MOUA. The PI is required to update any incorrect information by submitting a MOUA Amendment form. Significant modifications will require IBC approval.

Additional Information

1. NIH *Principal Investigator Responsibilities Under the NIH Guidelines*.
2. NIH *Guidance on Biosafety Considerations for Research with Lentiviral Vectors*.
3. NIH *Genetically Modified (Transgenic) Animals and the Use of Recombinant or Synthetic Nucleic Acid Molecules in Animals*.

Chapter 3
Biosafety Regulations and Guidelines

There are several local, state, and federal agencies that either regulate or provide guidelines covering the use of biological agents. A summary of these regulations and guidelines is provided below. Copies of these documents can be obtained from EH&S.

1. Centers for Disease Controls and Prevention (CDC) and the National Institutes of Health (NIH): *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th Edition. This document contains guidelines for microbiological safe work practices, safety equipment, and facilities that constitute the four established biosafety levels. The BMBL is generally considered the standard for biosafety and is the basis for this manual. **Compliance with the BMBL is a regulatory requirement for work involving select agents and toxins.**

2. National Institutes of Health (NIH): *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (NIH Guidelines). This document provides guidelines for constructing and handling recombinant and synthetic nucleic acid molecules, and organisms containing such nucleic acid. Although these guidelines are not subject to regulatory enforcement (with the exception of work involving select agents and toxins), institutions that receive any NIH funding for research involving recombinant or synthetic nucleic acid molecules are required to comply with these guidelines as a condition of funding. This document requires that each institution establish an Institutional Biosafety Committee with the authority to approve proposed research involving recombinant or synthetic nucleic acid molecules, using the NIH Guidelines as a minimum standard.

3. Occupational Safety and Health Administration (OSHA): *Bloodborne Pathogens*. This regulation covers occupational exposure to human blood and other potentially infectious material, including human tissue and cells. OSHA specifies a combination of engineering controls, work practices, and training to reduce the risk of infection. Personnel potentially exposed to human blood and other potentially infectious material must be offered immunization against the Hepatitis B virus and receive annual training. Personnel who work with HIV or Hepatitis B virus in a research laboratory must receive additional training and demonstrate proficiency in working with human pathogens

4. Department of Health and Human Services (CDC) and Department of Agriculture (APHIS): *Possession, Use, and Transfer of Select Agents and Toxins.* These regulations cover the possession, use, and transfer of biological agents and toxins that affect humans, animals, and plants and which have been determined to be potential bioterrorism agents (known as select agents). Entities and personnel who wish to work with select agents must be registered with the CDC or APHIS before acquiring or having access to select agents. Individuals who require access to select agents require a FBI background check and submittal of fingerprints, and must be approved by the Select Agent Program. These regulations mandate strict requirements for biosafety, emergency planning, and security of

select agents and toxins, and requires that laboratories that possess select agents comply with the BMBL (see above) and the OSHA Laboratory Standard (see UNR Chemical Hygiene Plan) if select agent toxins are used. Each transfer of a Select Agent must have prior approval of the Select Agent Program through completion of APHIS/CDC Form 2, which requires signature by the Select Agent Responsible Official (UNR Biosafety Officer) or designated alternate. Accurate inventory records of Select Agents, including transfers, must be maintained. See Chapter 12 of this manual for additional information.

5. Washoe County: *Regulations of the Washoe County District Board of Health Governing Solid Waste Management* (see Section 080 "Biohazardous Waste").
   These regulations include requirements for biohazardous waste storage, treatment, and disposal, including specific requirements for decontamination of biohazardous wastes by autoclaving or treatment with chemical disinfectants.

Chapter 4
Biosafety Principles

Containment

Laboratory biosafety practices are based on the principle of containment of biological agents to prevent exposure to laboratory workers and the outside environment. Primary containment protects the laboratory workers and the immediate laboratory environment from exposure to biological agents. Primary containment is achieved through good microbiological technique and the use of safety equipment and personal protective equipment. Secondary containment protects the environment outside the laboratory, and is provided by facility design and operational procedures.

Laboratory Practice and Technique

The use of good microbiological technique is the most important element of containment. Personnel working with biological agents must be aware of hazards, and must be trained to safely handle and dispose of these materials. Although we are all responsible for our own safety, the Principal Investigator is responsible for ensuring that persons working in their laboratory are adequately trained.

This Biosafety Manual has been developed to provide general policies and procedures when working with biological agents at UNR. Each individual laboratory must supplement this manual with laboratory specific policies; procedures and training that will minimize the specific risks present in the laboratory.

Safety Equipment

Safety equipment includes biological safety equipment, safety centrifuge cups, and other engineered controls designed to minimize exposure to biological agents. Biological safety cabinets (BSCs) are the most important safety equipment for protection of personnel and the laboratory environment, and most BSCs also provide product protection. Safety equipment is most effective at minimizing exposure when workers are trained on the proper use of such equipment, and the equipment is regularly inspected and maintained.

Personal Protective Equipment

Personal protective equipment includes safety eyewear, lab coats, gloves, and other protective equipment, and is used to supplement the containment provided by laboratory practices and safety equipment. Personal protective equipment is considered the least desirable containment method because its failure results in direct exposure of personnel to the biological agent.

Facility Design

Facility design features include physical separation of laboratories from public access, specially designed ventilation systems (to prevent airborne biological agents from migrating outside the laboratory), and autoclaves. These design features protect personnel working outside the immediate laboratory, as well the outside environment.

Biosafety Levels

The CDC/NIH has developed four biosafety levels that describe laboratory practices and techniques, safety equipment, and facility design features recommended for work with specific infectious organisms. Descriptions of the biosafety levels, as well as assigned biosafety levels for specific organisms, are contained in the CDC/NIH document, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th Edition. The recommended biosafety level for an organism represents conditions under which the agent can normally be handled safely; however, specific circumstances may dictate that the recommended conditions be raised or lowered. As outlined in the BMBL, the four biosafety levels are summarized below:

Biosafety Level	Agents	Practices	Safety Equip.	Facilities
1	Not known to cause disease in healthy adults.	Standard Microbiological Practices	None required	Open bench top, sink required
2	Associated with human disease, *hazard:* auto-inoculation, ingestion, mucous membrane exposure	BSL-1 practice plus: - Limited access - Biohazard warning signs - Sharps precautions - Biosafety manual	*Primary barriers:* Class I or II BSCs or other containment used for manipulations of agents that cause splashes or aerosols of infectious materials; *PPE:* lab coats; gloves; eye/face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: - Controlled access - Decontamination of all waste - Decontamination of lab clothing before laundering - Consider medical surveillance program	*Primary barriers:* Class I or II BSCs or other physical containment devices used for all manipulations of agents; *PPE:* protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: - Physical separation from access corridors - Self-closing, double door access - Exhausted air not recirculated - Directional airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agent with unknown risk of transmission	BSL-3 practices plus: - Clothing change before entering - Shower on exit - All material decontaminated on exit from facility	*Primary barriers:* All procedures conducted in Class III BSCs or Class I or Class II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: - Separate building or isolated zone - Dedicated supply/exhaust, vacuum, and decon systems - Other requirements outlined in BMBL

Consult the BMBL for a more complete description of the four biosafety levels, as well as recommended biosafety levels for specific organisms.

In addition to the four biosafety levels described above, there are also four biosafety levels for work with infectious agents in vertebrate animals and plants. For a complete description of the animal and plant biosafety levels, consult the BMBL.

Routes of Transmission

Skin and Mucous Membrane Contact

Low energy procedures such as decanting of liquids, pipetting, removal of screw caps, vortex mixing, streaking agar plates, and inoculation of animals, can result in the generation of infectious droplets, as well as result in direct contact with infectious material. Eye contact is also a route of exposure and safety eyewear must be worn as needed to prevent sprays or splashes to the eyes.

Ingestion

Mouth pipetting presents the highest risk for ingestion of infectious material. Splashing of material into the mouth, and indirect oral exposure through touching the mouth with contaminated hands, and eating and drinking in the lab can also result in ingestion of infectious material.

Percutaneous Inoculation

Use of syringes and needles are considered the greatest risk of exposure through inoculation. Inoculation can also occur as a result of cuts and scratches from contaminated items, and animal bites.

Inhalation

Many procedures have the potential for generation of respirable aerosols, including: sonication, centrifugation, "blowing out" of pipettes, heating inoculating loops, and changing litter in animal cages.

Chapter 5
Laboratory Biosafety Practices

Basic Laboratory Practices

The following prudent biosafety practices are recommended by the National Academy of Sciences in the publication *Biosafety in the Laboratory* and in part constitute basic good biosafety practices. Although these practices may be considered "common sense" and overly simplistic by experienced laboratorians, strict adherence to these basic principles will greatly reduce the likelihood of laboratory acquired infections.

Biosafety Practice	Routes of Exposure Blocked
1. Do not mouth pipette	Inhalation, ingestion, skin and mucous membrane contact
2. Manipulate infectious fluids carefully to avoid spills and the production of aerosols	Inhalation, skin and mucous membrane contact
3. Restrict use of needles, syringes, and other sharps to those procedures for which there are no alternatives; dispose of sharps in leak- and puncture-proof containers	Percutaneous, inhalation
4. Use lab coats, gloves, safety eye wear, and other personal protective equipment	Inhalation, skin and mucous membrane contact
5. Wash hands after all laboratory activities, following the removal of gloves, and immediately following contact with infectious agents	Ingestion, skin and mucous membrane contact
6. Decontaminate work surfaces before and after use, and immediately after spills	Ingestion, skin and mucous membrane contact
7. Do not eat, drink, store foods, or smoke in the laboratory	Ingestion, skin and mucous membrane contact

Biological Hazard Information

Laboratory workers must be knowledgeable of the hazards associated with the biological agents present in the laboratory, and have hazard information available to them. The following are sources of hazard information for biological agents.

Microbial Agents

1. The CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th Edition has descriptions of biosafety levels and recommended biosafety practices for specific biological agents.

2. The Public Health Agency of Canada publishes Pathogen Safety Data Sheets that describe hazardous properties of human pathogens and recommendations for laboratory work.

3. The American Biological Safety Association (ABSA) maintains a database for Risk Group Classification for Infectious Agents.

Toxins

Purified biological toxins are chemical hazards, although many such toxins produce adverse effects at doses significantly below that of "traditional" laboratory chemicals. Laboratory use of purified toxins falls under the UNR Chemical Hygiene Plan, and Safety Data Sheets (SDSs) must be maintained and available.

1. SDSs for the specific toxin should be received from the vendor upon receipt of the toxin.

2. Toxicology textbooks such as *Casarett's and Doull's Toxicology* are also good sources of hazard information for some toxins.

3. The BMBL also contains information on biological toxins and associated safe work practices.

Written Standard Operating Procedures

This manual, in combination with the referenced CDC/NIH publications *Biosafety in Microbiological and Biomedical Laboratories* and *Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules,* provides general standard operating procedures (SOPs) for working with biological agents. However, because these SOPs cover relatively general topics, individual laboratories are required to develop laboratory specific SOPs that cover the biosafety concerns and laboratory procedures for that particular laboratory. For example, laboratory-specific SOPs should address safe manipulation of specific organisms, specific exposure control methods, and specific decontamination and waste handling requirements. There is no standard format required for SOPs and laboratories are encouraged to use any format that effectively conveys the biosafety information (including use of pictures and illustrations). The laboratory-specific SOPs do not need to duplicate the more general SOPs contained in this manual but should supplement this document.

Security and Inventory of Biological Agents

Each PI is responsible for ensuring that his or her laboratory implements sufficient security measures and procedures to prevent unauthorized access to biological agents. Select Agents (see Chapter 12) and other higher risk microorganisms and toxins must be stored in a locked container, and an inventory must be maintained with sufficient detail to enable identification of missing materials.

Prevention and Containment of Aerosols and Droplets

Handling of liquids or dry powders is likely to generate aerosols or droplets. Procedures such as centrifuging, mixing, and pipetting that involve high energy tend to produce respirable aerosols that stay airborne for extended periods and are small enough to be inhaled. Low energy procedures including opening containers and streaking plates produce droplets that settle quickly on surfaces, skin, and mucous membranes.

Biological Safety Cabinets

Procedures involving infectious material should be performed inside a biological safety cabinet (BSC) whenever possible. A properly operating, properly used BSC (see Chapter 8) will contain aerosols and droplets generated during handling of infectious agents.

Pipetting

Do not mouth pipette! Always use a mechanical pipetting device. Pipettes should be drained gently with the tip against the inner wall of the receiving vessel and liquid should not be forcibly expelled from the pipette.

Blending

Use a safety blender that has leak proof bearings and a tight fitting lid with a sealable gasket. Use the blender inside a BSC when blending material containing infectious agents.

Centrifugation

The potential for contamination and infection is high if liquid and aerosol is released during centrifugation. Sealed centrifuge buckets, or safety cups should be used to prevent release of liquid and aerosol. Ultracentrifuges operate under vacuum and should contain an in-line HEPA filter between the chamber and the vacuum pump. Small bench top centrifuges can be placed inside a BSC to contain infectious aerosols and prevent personnel exposures.

When centrifuging BSL-3 agents outside of a BSC, approved respiratory protection should be worn when opening the centrifuge as a precaution against possible release of the agent during centrifugation. Rotors containing infectious agents must be loaded and unloaded (or otherwise opened) in a BSC.

Inoculating Loops

Flaming inoculating loops can result in spatter and release of aerosols and droplets. Use of an electric microincinerator will effectively control spatter resulting from sterilization of inoculating loops.

Use of Absorbent Materials

Work surfaces should be covered with absorbent paper or "diaper" sheets to collect splashes and drips, and minimize the spread of contamination. The absorbent paper should be changed at the end of the laboratory procedure as part of the final cleanup, or at least daily during use.

Personal Protective Equipment

Although not a substitute for use of BSCs and good laboratory practices, personal protective equipment (PPE) is considered a primary barrier to infectious agents and proper use will reduce the likelihood of infection. PPE is the least desirable exposure control method because its failure results in direct exposure to the agent. PPE is most effective when used to supplement primary control methods such as biological safety cabinets, safety centrifuge cups, and other containment devices. For example, manipulation of infectious agents that have a very low infectious dose and which are readily transmitted by inhalation of aerosols, such as *Mycobacterium tuberculosis,* may necessitate the use of respiratory protection in addition to containment within a BSC.

Laboratory Coats and Gowns

Laboratory coats protect street clothes against chemical and biological spills, and provide additional body protection. Generally, a 100% cotton lab coat is recommended over polyester-cotton blends for general microbiological work. The wearing of lab coats is considered to be standard microbiological practice for BSL-1 and BSL-2 laboratories. For BSL-3 laboratories, CDC/NIH guidelines recommend solid-front or wrap-around gowns or suits, rather than front-buttoning lab coats. It is good laboratory practice (and a requirement for BSL-3 labs) to remove lab coats or gowns before leaving the laboratory to minimize the spread of contamination outside the laboratory. Lab coats should be left in the laboratory and must not be taken home for washing.

Gloves

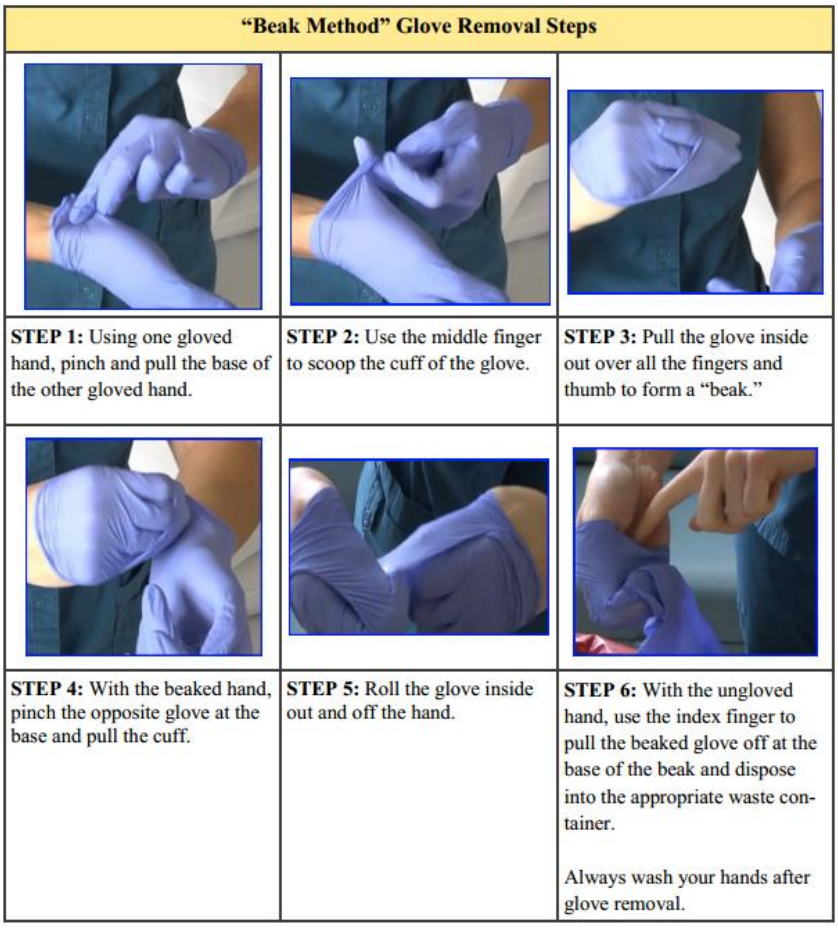
Gloves are available that provide protection against a variety of hazards, including infectious agents, chemicals, and radioactive material. Unfortunately, there is no single glove type that provides adequate protection for all hazards (or even all chemicals!). Always check gloves for pinholes prior to use.

Standard latex examination type gloves provide protection against microbiological hazards, including human blood and body fluids. Latex gloves do not generally provide adequate protection against liquid chemicals; additionally, many people develop latex allergies as a result of wearing latex gloves. Disposable nitrile gloves are an alternative to latex examination gloves that provide similar dexterity and increased chemical resistance. Nitrile gloves still provide protection against microbiological hazards, but without the latex allergy hazard. Although disposable nitrile gloves generally provide better chemical protection than latex gloves (for

example, nitrile gloves are recommended when handling tamoxifen), they are not considered to be chemical resistant gloves. For instances where chemical contact is likely or cannot be tolerated due to high toxicity, consult the UNR Chemical Hygiene Plan or contact the UNR Chemical Hygiene/Biosafety Officer (Ben Owens) at 327-5196 for recommendations concerning chemical resistant gloves.

Glove selection for protection against radioactive materials should be based on the resistance of the glove to the liquid solvent that the radioactive material is contained in. Essentially any rubber-type glove material will provide protection against dry chemicals or radioactive material. For more information about radiation safety and control of radioactive contamination, contact the UNR Radiation Safety Officer (Myung Chul Jo) at 784-4540.

Contamination control requires that gloves be removed prior to exiting a BSC or touching non-contaminated laboratory areas and equipment (such as clean areas, phones, computers, door knobs, etc.). Be careful to remove gloves from the inside out so as to minimize contact between outside of gloves and bare skin during removal (to prevent self-contamination). The proper procedure for removing gloves is shown in photos below.



Proper removal of potentially contaminated gloves

Eye and Face Protection

Safety glasses, goggles, and face shields provide protection against chemical reagents and disinfectants. Additionally, they also provide protection against infection that can result from the splashing of pathogenic organisms into the eyes. Normal prescription eyeglasses are not safety glasses and do not provide adequate eye protection for laboratory operations. Further guidance on the use of protective eye and face wear for chemical hazards can be found in the UNR Chemical Hygiene Plan.

Goggles with indirect venting provide a good barrier against splashes to the eyes. A face shield can be worn in addition to goggles (face shields may not provide adequate eye protection by themselves) to provide protection against splashes to the face and mouth.

Respiratory Protection

Required Use of Respiratory Protection

Prior approval from EH&S is required whenever personnel are required to wear any respirator (including N-95 filtering facepiece respirators, which are also known as dust masks) to prevent exposure to infectious agents, or when required by regulation or administrative procedure. **When use of respiratory protection is required, personnel must be medically evaluated, fit-tested, and trained prior to using respiratory protection.** An exception to the fit test requirement is use of a powered air-purifying respirator (PAPR) with loose fitting hood; however, use of these respirators still requires a medical evaluation and training prior to initial use.

Voluntary Use of Respirators

Some personnel may want to voluntarily wear a N95 filtering facepiece respirator or other respirator in the absence of a recognized inhalation risk, regulatory requirement, or administrative requirement. In these circumstances, personnel are permitted to voluntarily wear a respirator; however, certain requirements must still be met so **EH&S must be contacted prior to voluntary use of respirators**.

Voluntary use of N-95 filtering facepiece respirators requires only that specific information contained in the applicable OSHA standard (29 CFR 1910.134, Appendix D) be provided to each person (in place or more detailed training); a medical evaluation and fit-test are not required. Filtering facepiece respirators are particulate filters and therefore are only effective against particles; they do not provide protection from inhalation of gases or vapors. Voluntary use of respirators other than a N95 filtering facepiece respirator requires a medical evaluation and training.

Respirator Fit-Tests

Contact Crista Hartman in EH&S (327-5055) to schedule respirator fit test or if there are questions about the respiratory protection program.

Storage and Labeling of Biological Agents

BSL-3 infectious agents must be stored using double containment and it is recommended that BSL-2 agents also be stored using double containment. Both the primary and secondary

containers must be durable and leak proof so as to prevent accidental exposure. Primary containers must be clearly labeled to indicate the identity of the agent and include the universal biohazard symbol (see below) as physical space on the container permits. At a minimum, secondary (or outside) containers must include the universal biohazard symbol (identity of contents is also desirable). Freezers, refrigerators, and other storage areas must also be labeled with the biohazard symbol; exceptions to this policy will be considered on an individual basis by the IBC. Waste, and contaminated equipment or other objects to be decontaminated must also be labeled with the biohazard symbol.

Universal Biohazard Symbol

The OSHA Bloodborne Pathogen Standard specifically requires that containers of human blood or other potentially infectious material (OPIM), contaminated waste, and refrigerators, freezers, and other storage containers used to store or transport blood or OPIM, be labeled with the universal biohazard symbol (fluorescent orange or orange-red). See the UNR Exposure Control Plan for additional information on handling and labeling of blood and OPIM.

Biohazard Labels and Signs

Signs must be posted at entrances to laboratories and other rooms where human, animal, or plant pathogens, or biological toxins that are listed as select agents or that have a LD_{50} for a mammalian species below 100 ng/kg of bodyweight, are present. A biohazard sign template is available that can be used to communicate room-specific information.

EH&S posts signs at laboratory entrances that include the universal biohazard symbol, the biosafety level of the agents present in the room, and the name and contact information for the primary and secondary emergency contacts. Laboratory occupants should notify EH&S when any information on the door card needs to be updated. If there are any specific entry requirements (such as personal protective equipment or immunization) the laboratory occupants should post that information in a professional looking manner, or contact EH&S for assistance.

Additionally, individual cages used to house animals infected with BSL-2 or BSL-3 agents, or dosed with biological toxins listed as select agents or that have a LD_{50} for a mammalian species below 100 ng/kg of bodyweight, should be labeled with the biohazard symbol, biosafety level, and biological agent or toxin.

Chapter 6
Laboratory Training

Laboratory Training

Training is required for all laboratory workers (faculty, staff, students, and visiting scientists) at UNR. The exact training required for a particular person will depend on the hazards to which he or she is exposed. It is the responsibility of the Laboratory Supervisor to ensure that all personnel receive training that is appropriate for their job duties and exposure potential. A schedule of upcoming training classes provided by EH&S is available at the online EH&S training registration site.

Laboratory Safety Training

EH&S conducts laboratory safety training in a modular format that includes the following topics: chemical hygiene, hazardous waste, chemical spill response, laboratory ventilation, and biosafety. Because virtually every laboratory worker utilizes chemicals, the chemical hygiene, hazardous waste, chemical spill response, and laboratory ventilation topics are required for all laboratory workers. The biosafety training is designed for laboratory workers who work with biological agents.

General Biosafety Training

EH&S offers a general biosafety training class as part of laboratory safety training. The biosafety training lasts approximately one hour and covers regulatory requirements, the UNR Biosafety Program, general biosafety work procedures, and biohazardous waste disposal. UNR laboratory workers who work with biological agents are expected to attend this course. Workers must receive training prior to beginning laboratory work with biological agents. Currently, each individual is required to attend this general biosafety training once; however, completion of an online refresher course is required annually. Additionally, ongoing training is required as part of laboratory specific training requirements (see section below).

Bloodborne Pathogens Training

Laboratory workers who are exposed to human blood and body fluids, unfixed human tissue, or human cells are within the scope of the OSHA Bloodborne Pathogens Standard. It is the position of the Centers for Disease Control and Prevention (CDC) and OSHA that all cell lines of human origin are considered potentially infected with bloodborne pathogens, and that these materials be handled using a minimum of BSL-2 containment and procedures (see Chapter 9). Consequently, all people who work with human cell lines are required to be in the Bloodborne Pathogens Program (see UNR Bloodborne Pathogens Exposure Control Plan) and complete annual Bloodborne Pathogens training.

HIV/HBV Laboratory Training

Personnel who work in research laboratories that culture, produce, or otherwise perform microbiological manipulation of human immunodeficiency virus (HIV) or hepatitis B virus (HBV) must receive additional training beyond the standard bloodborne pathogens training. Prior to working with HIV or HBV, laboratory workers must demonstrate proficiency in standard

microbiological techniques, and in the practices and techniques specific to the laboratory. Additionally, workers must have prior experience in handling human pathogens before working with HIV or HBV. Personnel who do not have experience working with human pathogens must be trained in the laboratory before working with HIV or HBV. Initial training must not involve the use of infectious agents. Training and work activities should be progressive as proper techniques are demonstrated and workers are permitted to handle infectious agents only after demonstrating proficiency to the satisfaction of the Laboratory Supervisor. Although this specialized laboratory-specific training is the responsibility of the Laboratory Supervisor, the training should be coordinated with the Biosafety Officer to ensure proper documentation and recordkeeping.

Packaging and Shipping of Infectious Agents Training

Personnel who package and ship infectious agents such as microorganisms, blood samples, and clinical samples for pathological testing, are required by federal and international regulations to receive training every two years (see Chapter 15). EH&S offers this training periodically and upon request.

Laboratory Specific Training

Individual laboratories are required to develop specific training for the particular agents and procedures that will be used in that laboratory. This training should be specific to the hazards in the laboratory and to each person's laboratory duties. Each person in the laboratory must understand the hazards associated with laboratory operations, how to prevent exposures to biological and chemical agents, and exposure evaluation procedures. This laboratory specific training should not duplicate the general biosafety training, but should supplement it. Training records must be maintained by each laboratory. The names and signatures of the instructor(s) and laboratory personnel, signature of the PI (if not the instructor), topic of training, and date that training was conducted, should be recorded on a documentation form and maintained by the laboratory. Ongoing training is required as new hazards and procedures are introduced into the laboratory. The occurrence of spills, spread of contamination, near misses, etc. also indicate the need for refresher training.

BSL-3 Training

All personnel who work in BSL-3 laboratories must receive training on BSL-3 practices and procedures that is specific for the BSL-3 laboratory in which they will work. This training must be documented as described above, with records maintained by the laboratory. Training topics should include:

1. BSL-3 biosafety principles and procedures (as specified by the most current versions of the BMBL and the NIH Guidelines).
2. Proper use of biological safety cabinets.
3. Personal protective equipment use (selection, donning, and doffing).
4. Decontamination and biohazardous waste management.
5. Agent inventory and facility security.
6. Incident response (spill response, personnel contamination, evacuation procedures, etc.).

Chemical Hygiene Training

Virtually every laboratory utilizes hazardous chemicals to some extent so all laboratory workers must attend laboratory safety training, which is described above

Radiation Safety and Laser Safety Training

Personnel that utilize radioisotopes or x-ray generating devices must attend radiation safety training. Likewise, personnel that operate Class 3B or 4 lasers must receive laser safety training. Contact Myung Chul Jo (784-4540), the UNR Radiation Safety Officer (EH&S), for information and scheduling.

Chapter 7
Decontamination

Decontamination

Decontamination of cultures and objects contaminated by biological agents is routinely performed in microbiological laboratories. Decontamination is a vital component of microbiological safety practice and serves to protect laboratory personnel (as well as others) from infection, as well as the release of infectious organisms to the outside environment. Decontamination of media, work surfaces, and equipment is also necessary to prevent contamination of cultured organisms.

Chemical Disinfection

Decontamination of work surfaces, equipment, biological safety cabinets, and other inanimate objects using antimicrobial agents is referred to as disinfection. Several chemical agents are used as disinfectants. Laboratory workers should remember that there are hazards associated with all of these chemical disinfectants. Inhalation and skin contact should be minimized, and eye contact avoided. Appropriate gloves and safety eyewear should always be worn when handling these chemicals.

The susceptibility of microbes to chemical disinfects varies on their physical structure and physical state (e.g., more resistant spore state). For example, the waxy outer cell wall of mycobacteria provides increased resistance. Viruses that lack lipids in the outer capsid make them more hydrophilic and generally more resistant to chemical disinfectants than lipophilic viruses. The order of resistance of microbes to chemical disinfectants, from most resistant to least resistant is presented on the next page.

Descending order of resistance to chemical disinfectants.*

MOST RESISTANT

LEAST RESISTANT

Bacterial Spores
Bacillus anthracis
Clostridium sporogenes

Mycobacteria
Mycobacterium tuberculosis
Nontuberculosis mycobacteria

Nonlipid or Small Viruses
Coxsackievirus
Rhinovirus

Fungi
Cryptococcus spp.
Candida spp.

Vegetative Bacteria
Pseudomonas aeruginosa
Staphylococcus aureus

Lipid or medium-Size Viruses
Cytomegalovirus
Hepatitis B and C virus

* Adapted from *Biological Safety: Principles and Practices*, Fleming, D. O. and Hunt, D. L., eds, 4th Edition (2006), ASM Press, Washington, D. C.

Pertinent information for some of the common chemical disinfectants is summarized in table format at the end of this chapter.

Autoclaving

Autoclaving uses saturated steam under pressure (approximately 15 psi) to achieve a temperature in the autoclave of at least 121 °C (250 °F). Autoclaving can be used to destroy vegetative bacteria, bacterial spores, and viruses. When decontaminating biohazardous waste, it is recommended that the temperature in the waste reach a minimum of 115 °C for a minimum of 20 minutes. The total processing time required to meet these conditions depends on several loading factors (see below); however, a minimum autoclave cycle of one hour must be used when decontaminating biohazardous waste.

There are three factors that in combination determine the effectiveness of autoclaving:

Temperature - autoclave uses steam under a pressure of approximately 15 psi to achieve a chamber temperature of at least 121 °C. Although the autoclave chamber may reach 121 °C,

this does not necessarily mean that the interior of the load will reach this temperature.

Time - an minimum autoclave cycle time of twenty minutes at a chamber temperature of 121 °C (time does not begin as soon as the autoclave cycle is initiated) is commonly recommended for sterilization of clean items. However, the total processing time required to achieve decontamination depends on several loading factors, including the load container (heat transfer properties), the amount of water contained in the load, and the weight of the load. For increased loads, a longer cycle time is required to ensure effective decontamination. **For treatment of biohazardous waste, a minimum autoclave time of 60 minutes is required.** Exemptions to the 60 minute minimum autoclave time will be considered on a case-by-case basis and must be approved by the Biosafety Officer or Institutional Biosafety Committee. Requests for exemption must be submitted to the Biosafety Officer with a written SOP that describes the biohazardous waste, autoclave conditions, autoclave time, and autoclave efficacy testing procedure and frequency of testing. In no case will an autoclave time of less than 30 minutes be approved.

Contact - steam saturation is essential for maximum heat transfer. Steam must contact all areas of the load. Autoclave bags and other containers should be left partially open (or otherwise permit entry of steam) to ensure adequate contact. Studies have shown that adding water to the interior of the bag improves the time-temperature profile of the autoclave cycle, increasing the sterilization efficiency of the autoclave.

There are specific requirements for decontaminating biohazardous waste prior to disposal. See Chapter 12 for autoclave procedures relating to biohazardous waste.

Autoclave Maintenance and Inspection

Autoclaves must be regularly maintained and repaired by qualified technicians. Additionally, **Nevada State law requires that autoclaves with an internal volume greater than 5 cubic feet be inspected annually by a certified inspector**. This service is currently performed at no cost by the University insurance carrier. Call EH&S at 327-5040 to schedule an autoclave for inspection. Additional autoclave use information is available in the UNR Autoclave Safety Manual.

Dry Heat

Dry heat is less effective than moist heat (autoclaving); requiring higher temperature and longer contact time. Nevertheless, dry heat is preferable to moist heat for decontamination of anhydrous materials and closed containers. This is due to the fact that the moisture component of the steam used in an autoclave will not effectively penetrate anhydrous materials and closed containers. The highest dry heat equivalent temperature that these materials will reach in an autoclave is 121 °C. The highest temperature that material will reach in a dry-heat oven will be the actual temperature inside the oven. A temperature of 160-180 °C for 3-4 hours is recommended for decontamination of waste using a dry heat oven.

Summary of Chemical Disinfectants

Disinfectant	Use Parameters	Effective Against[a]					Important Characteristics	Potential Application
		Vege-tative cells	Lipo-philic viruses	Tubercle bacilli	Hydro-phillic viruses	Bacterial spores		
Alcohol (ethyl, isopropyl)	*conc.*: 70-85% *contact time*: 10-30 min.	+	+	+	±		eye irritant, toxic, flammable, inactivated by organic matter	surfaces - work & equipment
Chlorine Compounds	*conc.*: 0.05-0.5% (commercial bleach ≈ 5%) *contact time*: 10-30 min.	+	+	+	+	±	may leave residue; corrosive; skin, eye & respiratory irritant; inactivated by organic matter; makeup at least weekly	spills, equipment surfaces, instruments, glassware, water baths
Quaternary Ammonium Compounds	*conc.*: 0.1-2% *contact time*: 10-30 min.	+	+				toxic, inactivated by organic matter	surfaces (work & equip.), BSCs, floor maintenance, glassware, instruments
Phenolic Compounds	*conc.*: 0.2-3% *contact time*: 10-30 min.	+	+	+	±		leaves residue; corrosive, skin, eye & respiratory irritant; toxic; inactivated by organic matter	surfaces (work & equip.), BSCs, floors, spills, glassware, instruments, water baths
Iodophor Compounds	*conc.*: 0.47% *contact time*: 10-30 min.	+	+	+	±		leaves residue; corrosive, skin & eye irritant; toxic; inactivated by organic matter	surfaces (work & equip.), BSCs, glassware, water baths
Formaldehyde[b] (Formalin)	*conc.*: 4-8% *contact time*: 10-30 min.	+	+	+	+	±	leaves residue; skin, eye & respiratory irritant; toxic (carcinogen)	less effective than other disinfectants but can be used for equipment surfaces, glassware, instruments
Glutaraldehyde	*conc.*: 2% *contact time*: 10-600 min.	+	+	+	+	+	leaves residue; skin, eye & respiratory irritant; toxic	equipment surfaces, glassware, instruments

From: *Laboratory Safety: Principles and Practices*, second edition (1995), Diane O. Fleming, John H. Richardson, Jerry J. Tulis, and Donald Vesley, eds., American Society for Microbiology, Washington, D. C.

a: + = very positive response, ± = less positive response. A blank denotes a negative response or not applicable.
b: due to its irritating characteristics and status as a carcinogen, formaldehyde should not be used without good local exhaust ventilation.

Chapter 8
Laboratory Ventilation for Biosafety

Laboratory Chemical ("Fume") Hoods

Traditional laboratory chemical (or fume) hoods are designed to capture and control chemical vapors and pull them away from the worker. Although the inward flow of air provides protection to the user, chemical hoods do not provide protection for the product (the desired organism being manipulated). Unless a High Efficiency Particulate Air (HEPA) filter is added, chemical hoods do not provide protection against release of viable organisms to the environment. The airflow within a chemical hood is often somewhat turbulent, which can potentially result in exposure of the user to the organisms being used. In short, **a chemical hood is <u>not</u> a biological safety cabinet and should not be used for handling and manipulation of pathogenic agents**.

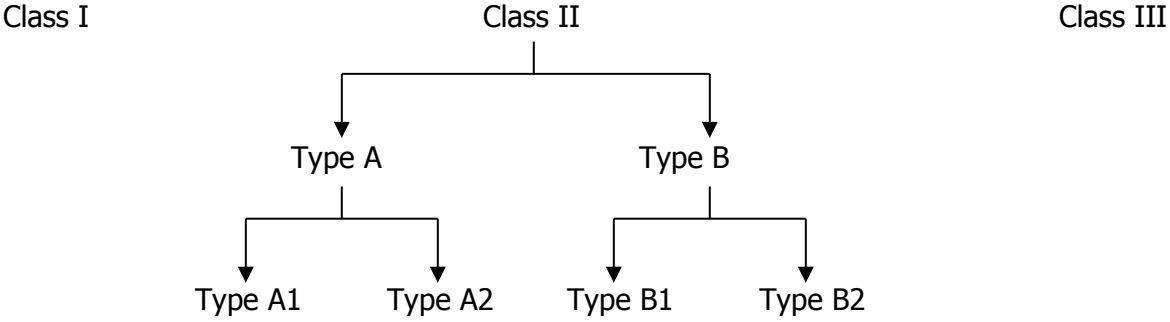
Horizontal Laminar Flow Clean Bench

With horizontal laminar flow clean benches, HEPA filtered air flows horizontally across the workspace directly toward the user (see diagram at end of this chapter). These clean benches provide product protection and were originally designed to provide a particulate free environment for manufacture of semiconductor components. Clean benches do provide product protection against microbial contamination, but they <u>do not</u> provide personal protection or environmental protection. In fact, the horizontal flow of air will blow biological agents directly toward the user and into the laboratory. **Clean benches are not a biological safety cabinet, and they should not be used with any materials (biological, chemical, or radiological) requiring containment for protection of personnel or the environment**. Clean benches are acceptable for tissue culture work only with cell lines considered to represent low risk (BSL-1 agents) to laboratory workers (including immunocompromised individuals who may frequent the lab). Human cell lines and nonhuman primate cell lines are generally considered to be BSL-2 agents and would not be suitable for use in a clean bench.

Biological Safety Cabinets

There are three classes of biological safety cabinets (BSCs), class I, II, and III (see schematic below). All BSCs provide personnel and environmental protection, with Class II and Class III BSCs also providing product protection. Personnel protection is achieved by inward airflow through the front of the cabinet; product protection is achieved by downward HEPA filtered airflow from the top of the cabinet; and environmental protection is achieved by HEPA filtration of exhaust air. Class II BSCs are by far the most common cabinet used in biomedical research laboratories and there are currently no class I or III BSCs in use at UNR.

Classes and Types of Biosafety Cabinets



Class I BSCs

Class I BSCs are similar to chemical hoods in that inflow air enters the front of that cabinet, flows across the work area, exits at the rear of the cabinet, and is exhausted outdoors. The primary difference is that chemical hoods usually do not have any filtration mechanism to prevent contaminants from being released to the outside (unless a filter or scrubber is added), whereas all air exhausted from a Class I BSC must pass through a HEPA filter before being exhausted outdoors. The inflow of air into a Class I BSC provides personnel protection, and HEPA filtration of the exhaust air provides environmental protection; however, Class I BSCs do not provide product protection. Class I BSCs are suitable for work involving BSL-1, -2, or -3 agents when product protection is not required. Class I BSCs are generally only used to house equipment when release of infectious aerosols is possible.

Class II BSCs

Class II cabinets are designed for personnel, product and environmental protection. All class II BSCs are designed for work involving BSL-1, -2, and -3 organisms. Class II BSCs are divided into type A and B cabinets based on construction, airflow, and exhaust systems (see diagrams at the end of this chapter).

Type A1 BSCs (formerly known as Type A)

Type A1 cabinets have an inward airflow of 75 feet per minute (fpm), and recirculate approximately 70% of discharge air through the supply HEPA filter back to the work zone. Some Type A1 cabinets have potentially contaminated air plenums that are positively pressured. Any breach of the positively pressured plenum or ducting would result in loss of containment and possible release of material. All discharge air is HEPA filtered before it is exhausted, either to the room or through ducting to the outside via a canopy connection that serves to minimize the effect of fluctuations in room airflow on cabinet performance. Recirculation of air within the cabinet and discharge of exhaust air directly to the room preclude the use of Type A1 cabinets for volatile chemicals or volatile radionuclides. Minute quantities of volatile toxic chemicals or radionuclides can be used in the Type A2 BSC if it is exhausted to the outside via a canopy connection.

Type A2 BSCs (formerly known as Type A/B3 or Type B3)
Type A2 cabinets are similar to Type A1 cabinets but have two notable differences. Type A2 BSCs maintain an average face velocity of 100 feet per minute and all exhaust ducts and plenums are maintained under negative pressure. Air from the BSC is exhausted through a HEPA filter and either into the room or through ducting to the outside via a canopy connection. Only when the BSC is ducted to the outside does it meet the requirements of the former Class II, Type B3 BSC. Minute quantities of volatile toxic chemicals or radionuclides can be used in the Type A2 BSC only if it exhausts to the outside via a canopy connection.

Type B1 BSCs
Type B1 cabinets maintain an average face velocity of 100 feet per minute and are designed so that small quantities of carcinogens and volatile radionuclides required for microbiological work can be handled safely. To prevent buildup of these chemicals within the cabinet, downflow air is "split", with a portion directed to the front of the cabinet and a portion directed to the back of the cabinet where it is exhausted directly through a HEPA filter and to the outside via hard ducting without recirculation within the cabinet. Volatile chemicals should be handled in the direct exhaust (rear) portion of the cabinet to prevent recirculation. Approximately 30% of outgoing air is recirculated as HEPA filtered downflow air. Type B1 cabinets are suitable for BSL-1, -2, or -3 agents treated with volatile toxic chemicals and volatile radionuclides used in microbiological studies if the work is performed in the direct exhaust (rear) portion of the BSC.

Type B2 BSCs
Type B2 cabinets maintain an average face velocity of 100 feet per minute. These cabinets are referred to as "total exhaust cabinets" because all inflow and downflow air passes through the cabinet only once (without any recirculation), and then is directly exhausted through a HEPA filter and to the outside via hard ducting. Because there is no recirculation of air within the cabinet, downflow air must be drawn in from the room (at the top of the cabinet) and then HEPA filtered prior to entering the cabinet. Type B2 cabinets are suitable for BSL-1, -2, or -3 agents treated with volatile toxic chemicals and volatile radionuclides used in microbiological studies. Because there is no recirculation of air within the cabinet, Type B2 cabinets are expensive to operate and should be specified only when required for use of volatile toxic chemicals and volatile radionuclides. Type B2 cabinets do not provide additional biosafety protection over other Class II BSCs.

Class III BSCs
Class III BSCs are of a glove-box design (gas-tight containment) that provides the highest level of personnel protection, as well as product and environmental protection. Both supply and exhaust air are HEPA filtered. These cabinets should be maintained under a minimum negative pressure of 0.5" w.g. Exhaust air is discharged to the outdoors through double HEPA filters (or HEPA and air incineration). Passage of materials into and out of Class III BSCs requires passage through a dunk tank or double door pass-through box that can be decontaminated (e.g., an autoclave). Class III cabinets provide the highest level of containment and can be

used for work involving any infectious agent; however, they are most appropriate for work involving BSL- 4 agents.

Certification of BSCs

Commercial BSCs are tested by the cabinet manufacturer in accordance with National Sanitation Foundation (NSF) criteria. Cabinets that meet the NSF criteria for performance characteristics including biological containment, ventilation, cabinet leakage, and HEPA filter leakage are NSF certified. Field certification of BSCs is also required to ensure that the cabinet still performs as it did when it obtained NSF certification at the factory. Field certification is required by the CDC and NIH under the following circumstances: 1) upon installation of a new BSC, 2) annually thereafter, 3) after repair or maintenance is performed, and 4) after the BSC is relocated.

NSF standard 49 provides criteria for construction of BSCs, testing by manufacturers (including biological containment testing), and field certification. NSF has also established a certification program for field certifiers to ensure a minimum level of competency and professionalism. It is recommended that NSF field certifiers be used for field certification of BSCs. Field certification tests include:

1. Primary Tests (BSC performance):
    a. Inflow test
    b. Downflow test
    c. Smoke pattern test
    d. HEPA filter leakage
    e. Cabinet leakage (when BSC is newly installed, relocated, or maintenance has been performed that involved removal of access panels)

2. Additional tests (worker comfort and safety): performed at discretion of certifier
    a. Noise
    b. Vibration
    c. Lighting
    d. Electrical leakage, polarity, and ground circuit resistance

The university has a sole BSC certification agreement with C-Scan Technologies to ensure the most competitive pricing for certification of BSCs on the UNR campus. Through this agreement, annual certification of BSCs is conducted by C-Scan and paid for by the Research and Innovation Office and the EH&S Department. Payment for repair and maintenance of BSCs is the responsibility of the individual laboratory or department. This program is coordinated by the Biosafety Officer and questions or issues regarding certification of BSCs should be directed to the BSO.

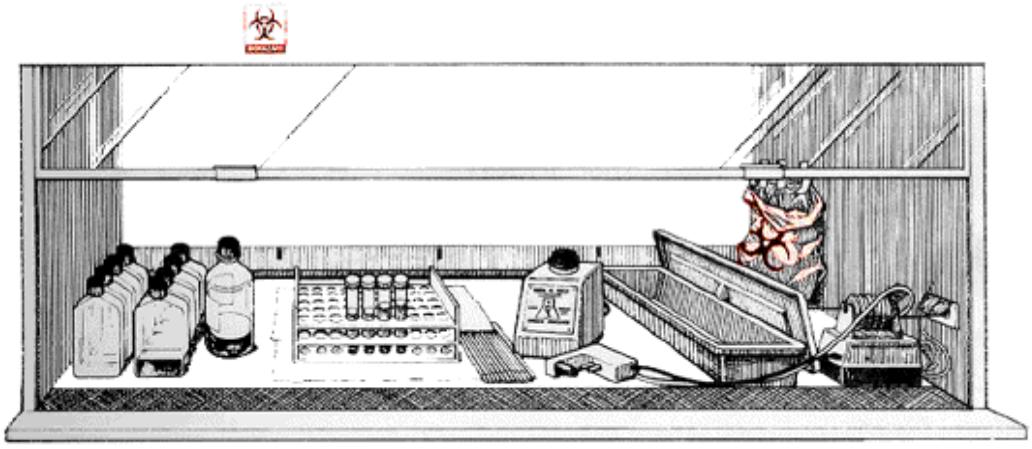
Guidelines for Use of Biological Safety Cabinets

The installation and use of a BSC is an indication that safe work practices are needed to prevent contamination and infection. Modern BSCs are extensively engineered and provide excellent containment of microorganisms; however, they are not substitutes for good work practices and can only serve to complement a safe worker. The following are general recommendations for BSC use.

1. Ready Work Area
   a. Turn off UV lamp (if equipped); turn on fluorescent light. UV light can burn eyes and skin so always minimize exposure by turning off the UV light when near the BSC.
   b. Check air grilles for obstructions; turn on fan (blower).
   c. Allow air to purge workspace for at least 3 minutes.

2. Pre-disinfect
   a. Spray or swab all interior surfaces with an appropriate disinfectant.
   b. Allow the surfaces to air dry.

3. Assemble Materials
   a. Only introduce materials that are required to perform the procedure.
   b. Position materials left to right (or right to left) in the BSC so that clean and contaminated items do not touch. For example, position clean supplies on the left side of the BSC, conduct work with biological agents in the center, and collect contaminated items and waste on the right side of the BSC (see diagram below).
   c. Ensure the view screen is properly located and secured.



4. Pre-Purge Cabinet
   a. Allow the BSC fan to run for at least three minutes with no activity inside (leave fan on!).

5. Prepare Self
   a. Don protective clothing, gloves, respirator, etc., as appropriate.

6. Perform Procedures
   a. Minimize movement of arms during procedure; move arms straight in or out of the BSC when entering or exiting.

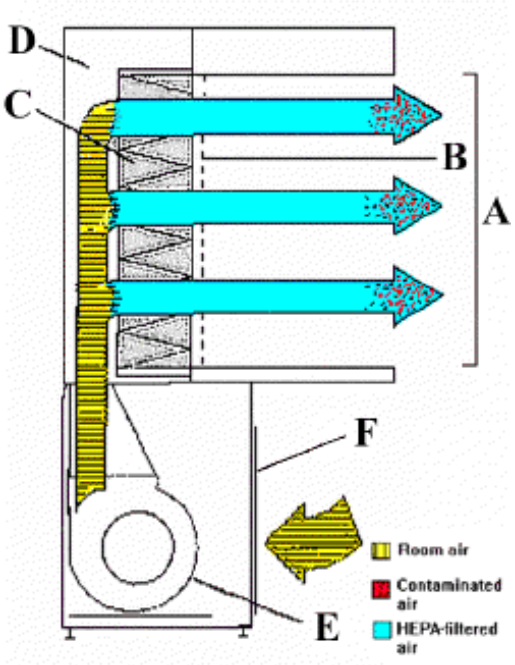
b. Work from a clean area to more contaminated work areas (see diagram above). Do not routinely pass items out of the BSC when working in the cabinet.
c. Remove gloves into contaminated material container and collect as biohazardous waste. Collect all waste in a biohazardous waste container inside the BSC; do not directly discard waste items outside of the BSC.

7. Cleanup and Post-disinfection
   a. Place potentially contaminated materials in a biohazard bag or other appropriate container.
   b. Wipe surfaces of all items in the BSC with an appropriate disinfectant.
   c. After surface decontamination, remove all items from the BSC and autoclave (or otherwise disinfect) waste and other contaminated materials as appropriate.
   d. Disinfect all interior surfaces of the BSC.

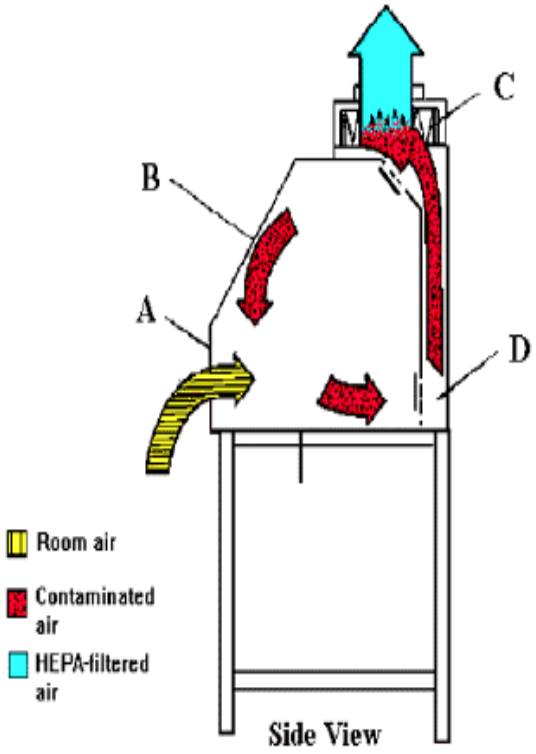
8. Personal Hygiene
   a. Remove protective clothing, respirator, etc., and dispose of as appropriate.
   b. Wash hands.

9. Post-Purge Cabinet
   a. Allow air purge period (minimum of three minutes) with no activity inside (leave fan on!).

10. Shutdown cabinet
    a. Turn off blower and fluorescent lamp.
    b. Turn on UV lamp (if equipped).

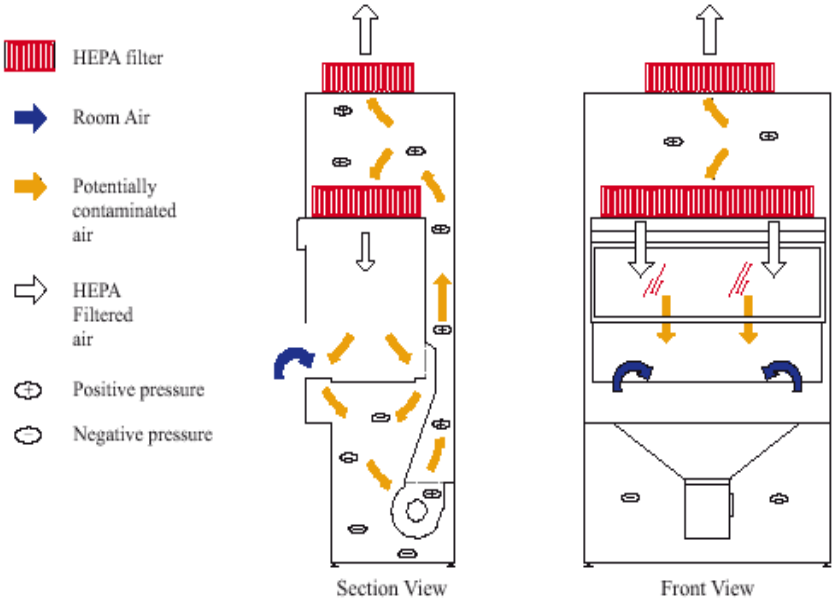


Horizontal Flow "Clean Bench"
A. front opening
B. supply grille
C. supply HEPA filter
D. supply plenum
E. blower
F. grille



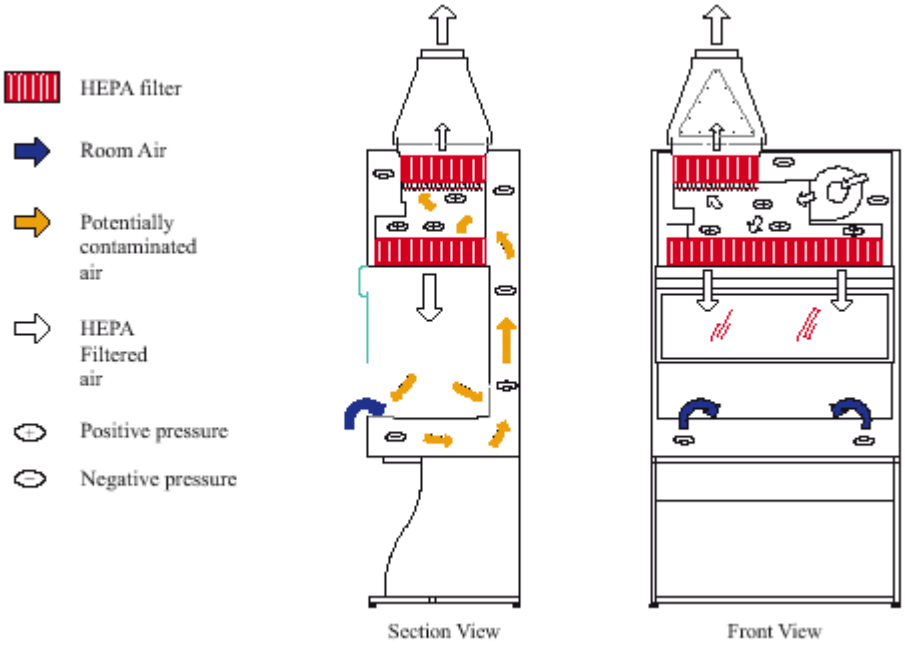
Class I BSC
A. front opening
B. sash
C. exhaust HEPA
D. exhaust plenum

Clean bench and Class I BSC diagrams taken from BMBL, 4th edition 1999, Centers for Disease Control

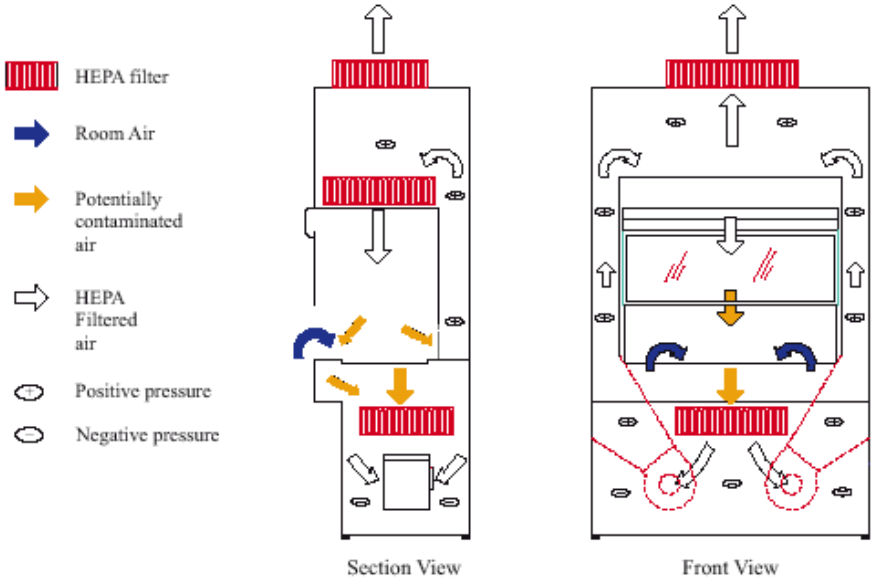


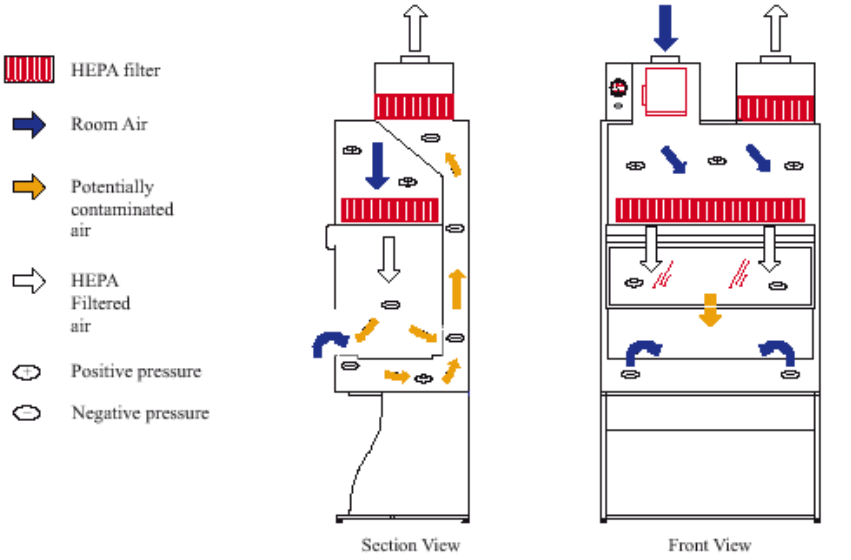
Class II Type A1 BSC
Can be exhausted to room or to outside via canopy connection

Class II Type A2 BSC
Can be exhausted to room or to outside via canopy connection



Class II Type B1 BSC
Hard ducted to outside





Class II Type B2 BSC
Hard ducted to outside

All Class 2 BSC diagrams taken from *Laboratory Biosafety Guidelines*, Chapter 9, Third Edition (2004), Public Health Agency of Canada

Chapter 9
Human Tissue and Cell Culture

Working with Human Tissues and Cells

All unfixed human tissue and cells are to be assumed to be infectious (the concept of "Universal Precautions") and must be handled using Biosafety Level 2 (BSL-2) practices and procedures. Persons who are exposed to these materials in the laboratory are considered to have potential exposure to bloodborne pathogens such as human immunodeficiency virus (HIV) and hepatitis B virus (HBV), and must be included in the UNR Bloodborne Pathogens Program. These persons must be offered the hepatitis B vaccination (they do not have to accept) and receive annual Bloodborne Pathogens Training (see Chapter 6). Cell lines, which have been characterized to be free of recognized bloodborne pathogens, are exempt from the OSHA Bloodborne Pathogens regulations. The Institutional Biosafety Committee will make the final determination on exemption of cell lines based on evidence provided in the MOUA form submitted to the IBC.

Implementation of OSHA Bloodborne Pathogens regulations associated with exposure to human cell and tissue culture is described in the UNR Institutional Biosafety Committee's Position Paper, *Human Cell Lines: Management of Bloodborne Pathogens*.

Transmissible Spongiform Encephalopathies

Spongiform encephalopathies (Creutzfeldt-Jakob, Kuru, and related agents) are fatal prion diseases that have been demonstrated to be present in the brain and spinal cord of infected persons. Infectious prions have also been found in blood and other body fluids, and many other tissues in infected persons; however, neurological tissue contains the highest concentration and represents the highest risk of infection. Prions are resistant to conventional inactivation procedures including chemicals (e.g., formalin, alcohol), boiling, dry heat, and irradiation, and they can be present in fixed tissue from infected persons. Although neurological tissue (brain, spinal cord) represents the highest risk of infection, all tissues from humans and animals infected with these agents should be considered potentially infectious. Laboratory-associated infections have not been demonstrated; however, it is prudent to consider nerve tissue (even fixed tissue) potentially infectious. BSL-2 containment and practices are recommended for all activities utilizing known or potentially infectious tissues and fluids from infected humans and animals.

Cell Culture

Human or animal pathogens might be associated with cell or tissue cultures. Cell cultures known (or suspected) to contain an etiologic agent or an oncogenic virus are classified at the same biosafety level as that recommended for the agent. The following cell cultures and tissues require BSL-2 or higher containment and procedures:

1. All cultured cells derived from human sources, including immortalized and "well established" cell lines.
2. All cultured cells derived from nonhuman primate tissue.

3. All cultured cells exposed to, or transformed by, a primate oncogenic virus.
4. All human clinical materials, such as samples of human tissue obtained from surgery, biopsy, or autopsy.
5. All primate tissue.
6. All virus-containing primate cultured cells.
7. All mycoplasma containing cultured cells.

Notes:

1. Using cells of human origin invokes the Bloodborne Pathogens Standard and its specific training and work requirements (see the above information on working with human tissues and cells).
2. Any exemption from the BSL-2 requirement must be approved by the IBC. Requests for exemption from the BSL-2 requirement must be submitted to the IBC with supporting information.

Chapter 10
Incident Response

Personnel Exposures

Exposure to an infectious agent, which includes recombinant or synthetic nucleic acid molecules, generally does not constitute an emergency in the same sense that a severe physical injury does. In the absence of physical injury, the emphasis when responding to an exposure incident involving a biological agent is on decontamination of affected persons to minimize the likelihood of infection and the spread of contamination. In some cases, the effectiveness of post-exposure prophylactic measures decreases significantly with time; therefore, it is recommended that a medical evaluation be obtained as soon as possible following all exposure incidents.

First Aid for Personnel Exposures

Percutaneous Inoculation:
Wash affected area thoroughly using soap and water. Use of chemical disinfectants to treat the affected area is generally not recommended and can produce skin irritation that could increase the likelihood of infection.

Skin Exposure:
Wash affected area using soap and water. Use of chemical disinfectants or abrasives to treat or clean the affected area is generally not recommended and can increase the likelihood of infection.

Eye and Mucous Membrane (Nose and Mouth) Exposure: Rinse affected areas thoroughly with water using an eye wash, sink faucet, or other means. It is recommended that eyes be rinsed for a minimum of 10 minutes. If ingestion or contact with the mouth or oral cavity occurs, the mouth should be rinsed thoroughly several times using water or antiseptic mouthwash if available.

Inhalation: If inhalation of infectious aerosols is suspected, decontaminate potentially exposed areas as described above.

Personnel Exposure Response Procedure

1. Alert others in the immediate area that an exposure incident has occurred and ask for assistance as needed.

2. Perform first aid as described above. Remove contaminated clothing as required to allow thorough removal of potential contamination and to prevent spread of contamination.

    a. If a large area of skin was exposed, shower and wash hair if a personnel shower is available in the immediate area. If a shower is not available, don "cover-up" clothing to cover all affected areas (including hair) as required to prevent spread of contamination.

3. Seek medical evaluation as described below.

Seeking Medical Evaluation

Personnel who are employed by UNR (for example, faculty, staff, graduate research or teaching assistants) or are formally designated volunteers are covered by Workers' Compensation. Employees who experience an occupational injury or exposure to a biological agent or chemical should go to one of the below facilities for medical evaluation and treatment.

Students with no employee status who are exposed to a biological agent or chemical, or injured, can seek medical attention at the Student Health Clinic or the medical facility of their choice. The medical facility should be called for guidance on decontamination, entrance to the facility, etc., prior to transport of the affected person. Students with no employee status are responsible for payment of any healthcare costs.

Reno

Specialty Health Clinic
330 E. Liberty, Suite 100
Reno, NV 89501
(775) 398-3630
Monday – Friday, 8:00 am – 5:00 pm

For urgent care treatment outside of the Specialty Health Clinic business hours, go to:

St. Mary's Urgent Care
1595 Robb Drive, Suite #2
Reno, NV 89523
(775) 284-5556
Monday – Friday 8:00 am – 6:00 pm; Saturday and Sunday 9:00 am – 5:00 pm

For treatment outside of Specialty Health Clinic or St. Mary's Urgent Care business hours, or medical emergencies, go to:

St. Mary's Hospital
235 W. 6th St.
Reno, NV 89503
(775) 770-3800

Las Vegas

Monday – Friday, 7:00 am – 6:00 pm
Concentra Medical Center
3900 Paradise Rd., Suite V
Las Vegas, NV 89169
(702) 369-0560

Open 24 hours – 7 days a week
Concentra Medical Center
5850 S. Polaris Rd., #100
Las Vegas, NV 89118
(702) 739-9957

Note: Other medical facilities can also be used as response time or available medical expertise dictates. Information for all authorized occupational health clinics is available at: http://www.bcn-nshe.org/downloads/workerscomp/AuthorizedOccupationalHealthClinics.pdf.

Upon arrival at the medical facility, report the incident as work-related. The affected employee will be asked to complete a C-4 form (Employee's Claim for Compensation/Report of Initial Treatment). After returning to work, the affected employee must complete a C-1 form (Notice of Injury or Occupational Disease Incident Report). Contact the Workers' Compensation Office (784-4394) if there are any questions regarding reporting or filing of a Workers' Compensation claim.

Transport of Exposed Personnel

In the event of a personnel exposure or injury, UNR employees that are aware of the incident are expected to take action to arrange the transport of affected personnel to a medical facility; however, UNR employees are not required to actually transport injured or exposed personnel. Due to potential for spread of infectious contamination and worsening of the affected person's medical condition during transport, it is generally recommended that exposed or injured persons be transported by ambulance. Other transport options include transport by a UNR employee when requested by the affected person and agreed to by the transporting employee (preferably using a university-owned vehicle), and self-transport when the injured person is judged to be able to do so safely (however, self-transport is not recommended if other options are available).

Biohazardous Spill Cleanup

Spills of biohazardous agents, which include recombinant and synthetic nucleic acid molecules, do not all represent the same risk to personnel or the environment, making each spill somewhat unique. Nevertheless, preplanning of spill response will lower the risk of cleaning up a spill and will increase the likelihood that the spill is handled appropriately. Laboratory supervisors should prepare their laboratory for typical spill scenarios expected in the laboratory. Laboratory workers should be informed of the hazards of the biological agents used in the laboratory, the risk associated with the agents during spill scenarios, how to safely cleanup spills involving the agents, and proper disposal of cleanup materials.

Each laboratory area should have spill cleanup materials available to respond to the largest spill anticipated for that area. It is recommended that as a minimum, the following spill cleanup materials be available in the laboratory:

- Gloves - thick chemical resistant gloves or double pair of thin, nitrile gloves recommended
- Safety Goggles - face shield is strongly recommended to avoid splashes to the nose and mouth
- Lab coat or smock to protect clothing and body
- Absorbent pads
- Disinfectant appropriate for the agents used in the laboratory
- Forceps or other devices to pick up contaminated material (especially sharps)

- Sharps disposal container
- Autoclavable biohazard bags

The chemical spill kits distributed by EH&S to laboratories contain thick nitrile gloves, absorbent pads, and forceps. Additional items needed for cleanup of biological agents can be added to your chemical spill kit in order to customize it for your laboratory.

Biological Agent Spill Cleanup Procedures
There are several factors that must be considered when assessing the risk that a spill represents. These factors include:

- Volume and concentration of the spilled material
- The infectious dose of the spilled material and routes of transmission
- Location of the spill
- Degree of aerosolization of the agent resulting from the spill
- Susceptibility of the spilled material to disinfection
- Nature of the affected surface(s) and its ability to "hide" organisms from disinfection
- Immune status of immediate personnel

As with any spill scenario (biological, chemical, or radiological) **the safety of personnel is the most important consideration**. Cleanup is to begin only after it is determined that the personnel who will clean up the spill have appropriate knowledge, training, and equipment.

The following are general spill cleanup procedures that are appropriate for most spill scenarios involving biological agents, including recombinant and synthetic nucleic acid molecules; however, the appropriate response to any spill is based on an assessment of the risk associated with that particular situation.

The following guidance is intended for spills of BSL-1 and BSL-2 agents. Spill response for BSL-3 agents is provided by the facility-specific procedures developed for each BSL-3 laboratory.

<u>Spills Inside Biological Safety Cabinets</u>

1. Wear laboratory coat (disposable recommended), safety glasses, and gloves (appropriate for the biological agent and the chemical disinfectant) during cleanup.

2. Allow the biological safety cabinet to run continually during cleanup.

3. Surround the affected spill area with absorbent material to prevent spread of the spill.

4. Cover the spill with absorbent material and gently apply disinfectant appropriate for the biological agent in sufficient quantity to saturate the absorbent material. Alcohol or other flammable liquids are not recommended. Allow a minimum of 20 minutes contact time (or as directed by manufacturer's instructions) and then place absorbent material into a biohazardous waste bag. Allow 30 minutes of contact time if the spill involves a pathogenic

spore-forming agent. If practical, use tongs to handle absorbent material to avoid contact with gloved hands.

5. Thoroughly apply disinfectant to the area that was under the absorbent material and allow contact time as practical (20 minutes or as directed by disinfectant manufacturer). Wipe the walls and work surface of the BSC, and any equipment in the cabinet with disinfectant-soaked towels.

6. Place contaminated items in an appropriate container (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items) for autoclaving.

7. Thoroughly wipe non-autoclavable items with an appropriate disinfectant before removing from the BSC. When possible, allow items to have a minimum of 20 minutes contact time with disinfectant (or as directed by manufacturer's instructions) to ensure disinfection.

9. Remove protective clothing and place in a biohazard waste bag for autoclaving.

10. Thoroughly wash hands, forearms, and face with soap and water.

11. Allow BSC to run for a minimum of 5 minutes before resuming work in the cabinet or shutting the cabinet off.

<u>Spills In the Laboratory, Outside the Biological Safety Cabinet</u>

1. If a BSL-1 agent (or less than 100 ml of a BSL-2 agent) is spilled, proceed to step 4.
   a. The specification of 100 ml is a guideline. In some cases, a smaller spill volume of a BSL-2 agent may justify a more conservative response, including evacuation of the lab as described in step 2. Each laboratory should conduct their own risk assessment and develop a laboratory-specific SOP as appropriate.

2. If the spill involves greater than 100 ml of a BSL-2 agent, immediately evacuate all personnel from the affected area. Wait for aerosol to settle (usually a minimum of 30 minutes) before entering the spill area. **Exception:** If the laboratory is not under negative pressure, cleanup should begin as soon as possible to minimize the spread of aerosols.

3. Notify EH&S at 327-5040 (24 hour contact number) as soon as possible for assistance with the cleanup. Consult with the laboratory supervisor (as available) and EH&S to determine the most appropriate spill response. If it is determined that laboratory personnel can safely clean up the spill, proceed as indicated below.

4. Remove any contaminated clothing and place in a biohazard waste bag for autoclaving, and wash all areas affected by skin contact with soap and water. Notify the BSO of all personnel contamination incidents.

5. Wear a long-sleeved gown or lab coat (disposable recommended), safety glasses (face shield also recommended), and gloves (appropriate for biological agent and disinfectant).

Shoe covers can be worn if available to help prevent contamination of shoes and to facilitate contamination control.

6. Place absorbent pads over the spill (to absorb liquid), then gently add disinfectant in sufficient quantity to saturate the absorbent pads.

7. Allow a minimum of 20 minutes contact time (or as directed by manufacturer's directions) with the disinfectant and then place absorbent material into a biohazardous waste bag. Allow a minimum of 30 minutes contact time if the spill involves a pathogenic spore-forming agent. If practical, use tongs to handle absorbent material to avoid contact with gloved hands..

8. Thoroughly apply disinfectant to the area that was under the absorbent material and allow contact time as practical (20 minutes or as directed by disinfectant manufacturer). Collect absorbent material in a biohazardous waste bag for autoclaving.

9. Wipe down all equipment, tools, etc. that might be contaminated with disinfectant and allow contact time with disinfectant as practical. If the agent was spilled on the floor, it is generally recommended that the entire floor be mopped with disinfectant.

10. Place contaminated items in an appropriate container (biohazard waste bag, sharps container, or autoclavable pan with lid for reusable items) for autoclaving.

11. Remove protective clothing and place in a biohazard waste bag for autoclaving.

12. Thoroughly wash hands, forearms, and face with soap and water. It is recommended that cleanup personnel shower as soon as possible.

<u>Spills Inside a Centrifuge</u>

1. Clear the area of all personnel and allow aerosol to settle (usually a minimum of 30 minutes) before re-entering the area.

2. Wear a laboratory coat (disposable recommended), safety glasses, and gloves during cleanup. If it is felt that the rotor or safety cups were breached, a N95 respirator, Powered Air-Purifying Respirator (PAPR), or other HEPA filtered respirator may be needed. Contact laboratory supervisor or EH&S for specific guidance.

3. Transfer the centrifuge rotor to a biological safety cabinet for cleanup.

4. Using an appropriate disinfectant, thoroughly disinfect the inside of the centrifuge, and the rotor.

5. Discard cleanup materials and protective clothing as biohazardous waste.

6. Thoroughly wash hands, forearms, and face with soap and water.

Spills Outside the Laboratory During Transport

1. If infectious agent is release outside of the transport containment, immediately clear the area of all personnel and secure the area.

2. Cleanup should be initiated as soon as possible to prevent spread of aerosol. Attempt cleanup **only** if appropriate cleanup materials and protective clothing are available.

3. Notify EH&S at 327-5040 (24 hour contact number) as soon as possible for assistance with the cleanup.

Because it is impossible to prevent aerosolization when a spill occurs outside of the laboratory, the primary emphasis when transporting biological agents is on spill prevention. All biological agents are to be transported from the laboratory inside an unbreakable, well-sealed, primary container containing absorbent material that is contained inside of a second unbreakable, well-sealed, secondary container. Both the primary and secondary containers must be labeled with the universal biohazard symbol and the identity or biosafety level of the agent. BSL-3 or risk group 3 agents must be transported using tertiary containment.

Chapter 11
Incident Reporting

Internal Reporting of Incidents Involving Biological Agents

The below spill scenarios must be reported to the Biosafety Officer or other appropriate EH&S staff member if the Biosafety Officer is not immediately available. The Biosafety Officer can be contacted at 327-5196 (office) and the EH&S 24-hour number is 327-5040. Incidents or safety concerns can also be reported online from the EH&S home page (www.unr.edu/ehs, then click on the "Report an Incident" quick tab.)

Report the following incidents as soon as possible:

- All spills or personnel exposure incidents involving BSL-3 agents or select agents and toxins (reporting of minor spills contained in a biological safety cabinet can wait until business hours if no personnel exposure is suspected)
- All personnel exposure incidents involving BSL-2 agents or biological toxins
- The following spills or personnel exposure incidents involving recombinant or synthetic nucleic acid:
  - Overt exposures of personnel to BSL-2 recombinant or synthetic nucleic acid
  - Overt or potential exposures of personnel to BSL-3 recombinant or synthetic nucleic acid
- High risk spills of any BSL-2 agent or biological toxin, even when released within a biological safety cabinet or other containment (high risk due to large volume or high potential for aerosolization)
- Any spill or release of a biological agent outside of a laboratory room or release to the outside environment by any route (e.g., ventilation system, sewer, or spill during transport)

Report the following incidents the next business day:

- Spills or other incidents that involve exposure of personnel to BSL-1 agents which are not expected to result in adverse health effects.
- Spills of BSL-2 agents not included above (e. g., low risk spills)
- Incidents or situations that had potential to expose personnel or result in release outside of containment ("near misses")

Notification of the Institutional Biosafety Committee

The Biosafety Officer will notify the Institutional Biosafety Committee (IBC) Chair of reported incidents no later than the next business day. The Biosafety Officer will investigate reported incidents and provide a written critique of the incident to the IBC Chair, the responsible Principal Investigator, and university administrators as appropriate. The IBC Chair will decide if the incident will be reviewed by the IBC.

External Reporting of Incidents Involving Biological Agents

In some cases, incidents involving biological agents must be reported to external agencies within specified time limits. Guidance on external reporting of biological agent incidents is

provided below. Contact the UNR Biosafety Officer for more specific guidance on incidents not specifically addressed.

Incidents Requiring Reporting to an External Agency

Select Agents

The Biosafety Officer (Ben Owens) is also the UNR Select Agent Responsible Official (RO) and he or one of the designated Alternate Responsible Officials (AROs) is responsible for reporting incidents involving select agents to the CDC or APHIS in accordance with the current select agent regulations (see Chapter 13 for more information). The RO or ARO is also responsible for submitting reporting forms required by the select agent regulations.

Recombinant or Synthetic Nucleic Acid

The IBC Chair is responsible for reporting incidents involving recombinant or synthetic nucleic acid to the NIH Office of Biotechnology Activities (OBA) as specified in the latest edition of the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*. Alternatively, the IBC Chair can ask the BSO to make any required notifications to the NIH OBA.

Biohazardous Waste

Incidents involving spills or releases of biohazardous waste that result in personnel exposure, a health and safety hazard to the public or agricultural or domestic animals, or discharge to the environment, must be reported to the Washoe County District Health Department Environmental Health Services as soon as possible. Significant changes to the storage, transport, or treatment of biohazardous waste requires revision of the UNR Biohazardous Waste Operations Plan and must be approved by the Washoe County District Health Department. The Biosafety Officer or alternate EH&S staff member is responsible for reporting incidents. The Biosafety Officer is responsible for revising the Biohazardous Waste Operations Plan.

Release of Biological Agents

Release of infectious agents outside of the laboratory, whether to the larger building or to the environment must be reported to Washoe County Health District Environmental Health Services as soon as possible. Infectious agents that are classified as select agents or recombinant or synthetic nucleic have additional reporting requirements as specified above. The Biosafety Officer or alternate EH&S staff member is responsible for reporting such incidents to the Washoe County Health District, and the Biosafety Officer is responsible for reporting incidents involving select agents and recombinant or synthetic nucleic acid as described above.

Exposure to Personnel

Personnel exposure incidents involving a causative agent of a communicable disease must be reported to Washoe County Health District Environmental Health Services as soon as possible. Reportable incidents are those that involve specific contact of an infectious agent with eyes, mouth, or other mucous membranes; parenteral contact; contact with non-intact skin; exposure to aerosols; and diagnosed illnesses known or suspected of being laboratory acquired. Biological agents that are classified as select agents or recombinant or synthetic nucleic acid have additional reporting requirements as specified above. The Biosafety Officer or alternate EH&S staff member is responsible for reporting such incidents to the Washoe County Health

Department, and the Biosafety Officer is responsible for reporting incidents involving select agents and recombinant or synthetic nucleic acid as described above.

Fatalities or Hospitalizations

Any occupational incident that results in the fatality of an employee within 8 hours. Additionally, work-related in-patient hospitalization of one or more employees, amputation, or loss of an eye, must be reported to the Nevada State OSHA within 24 hours. The Biosafety Officer or alternate EH&S staff member is responsible for making this report.

Chapter 12
Biohazardous Waste

Biohazardous Waste

Biohazardous waste includes waste materials derived from cultures and stocks of infectious agents, human pathological wastes, contaminated animal carcasses and body parts, all sharps, human blood and body fluids, and chemotherapeutic waste.

Biohazardous Waste Regulations

Proper handling and disposal of biohazardous waste is necessary to prevent infection of personnel (laboratory workers, custodians, laboratory visitors, etc.) and release to the environment. OSHA and Washoe County regulations require that biohazardous waste be properly labeled, stored, and disposed. In some instances improper disposal of biohazardous waste has resulted in regulatory action and negative media attention. Thus, the handling and disposal of biohazardous waste has important implications with regard to environmental health and safety, regulatory compliance, legal liability, and public opinion.

The Washoe County Health Department (WCHD) is responsible for promulgation and enforcement of local biohazardous waste regulations. Biohazardous waste regulations are available on the WCHD web site (go to "Solid Waste Management", then Section 080).

UNR Biohazardous Waste Operational Plan

The WCHD biohazardous waste regulations require the university to maintain a current operational plan that describes all aspects of biohazardous waste management at UNR. For full details on biohazardous waste management, consult the UNR Biohazardous Waste Operational Plan. The following information is a partial summary of the operational plan and is provided as a quick reference for laboratory personnel.

Labeling and Storage of Biohazardous Waste

Labeling

Waste Containers

At a minimum, all biohazardous waste must be labeled with the universal biohazard symbol (easily visible). Additional information such as the type of waste (such as "sharps", or "liquid waste") and origin of the waste is recommended as is appropriate.

Storage Rooms

Rooms or areas used to store biohazardous waste must be labeled with the following warning:

"CAUTION - INFECTIOUS WASTE STORAGE AREA - UNAUTHORIZED PERSONS KEEP OUT"; and in Spanish, *"CUIDADO - ZONA DE RESIDUOS INFECTADOS - PROHIBIDA LA ENTRADA A PERSONAS NO AUTHORIZADAS"*.

Laboratories where waste is accumulated during experimentation, and where biohazardous waste is not stored, are not considered storage areas and do not have to be labeled as above.

Storage

Biohazardous waste must be stored separated from other wastes. Biohazardous waste cannot be stored for more than seven days above a temperature of 0 °C. Waste can be stored up to 30 days if it is kept at or below 0 °C. Sharps can be stored for up to 30 days regardless of the storage temperature. The date that waste is first placed in storage must be clearly marked on the outside of each biohazardous waste storage container or packaging.

These storage timelines apply to any area where biohazardous waste is stored. Storage times do not apply during active accumulation of biohazardous waste; however, waste containers that are full or obviously not being actively used to receive biohazardous waste are considered to be in storage.

Laboratories must decontaminate and dispose of laboratory biohazardous waste on a regular basis to avoid accumulation times that result in unsafe conditions or odor. Matching the biohazardous waste container size to the waste generation rate will avoid an excessively long container fill time and will minimize the need to dispose of partially filled biohazardous waste containers.

Handling and Disposal of Biohazardous Waste

Sharps

Sharps include **all** syringes, lancets, scalpels and other similar medical instruments (whether contaminated or not), as well as contaminated Pasteur pipettes, broken glass and other instruments or materials that can cut or puncture personnel.

Sharps must be collected in rigid containers that are leak proof and resistant to puncture from the sharps. Sharps containers must be designed so that sharps can be safely introduced into the container but not easily retrieved (old coffee cans are not acceptable). Containers must be red in color and labeled with the universal biohazard symbol. When the sharps container is approximately 3/4 full, autoclave the container (if possible), secure the lid (tape securely), and submit a request for pickup by EH&S. Approximately once per month sharps are picked by a local waste contractor for treatment and disposal. Sharps containers are available from EH&S by request through the EH&S Waste Management web site.

Contaminated laboratory glassware can be decontaminated by autoclaving or chemical disinfectant and then disposed of as uncontaminated laboratory glassware or broken glass (see below). Small pieces of broken glassware can be disposed of as sharps, or broken glassware can be decontaminated and disposed of as is other contaminated glassware.

Uncontaminated Laboratory Glassware and Broken Glass

Collect uncontaminated laboratory glassware and broken glass in rigid containers (separate from other waste) that will prevent cuts and punctures to personnel. Containers should be

labeled "broken glass." Broken glass is to be disposed of as ordinary trash (to go to sanitary landfill).

Solid Biohazardous Waste

Solid biohazardous waste includes microbial agents, tissue culture, and contaminated material (such as petri dishes, pipettes, gloves, towels, etc.). These materials must be collected in autoclavable biohazard bags that are labeled with the universal biohazard symbol (the bag or the symbol must be red or orange in color). Biohazardous waste **must** be autoclaved prior to disposal and a visual steam sterilization indicator (such as autoclave strips or tape) must be included on **every** biohazard bag. After autoclaving, the waste is considered non-infectious and can be disposed of as ordinary trash; however, it is recommended that the autoclaved bag be placed inside an opaque bag prior to disposal. Autoclave bags are available from EH&S and can be requested through the EH&S Waste Management web site.

Solid biohazardous waste generators who do not have an autoclave available for decontamination can coordinate disposal of such waste directly through Waste Management (329-8822). Biohazardous waste generated on the main contiguous UNR campus can be transported to another campus building that has an autoclave suitable for treating the waste; however, autoclaving must be coordinated in advance with the person responsible for the autoclave used (see the UNR Biohazardous Waste Operations Plan for responsible persons).

Solid biohazardous waste that is stored, or that will be transported to another university building or off-campus location for autoclaving, must be double contained and labeled with the biohazard symbol.

Liquid Biohazardous Waste

Liquid biohazardous waste includes all blood and liquid waste from humans or animals, and all other liquid biohazardous waste (such as microbial cultures). Collect liquid waste in closeable, rigid plastic, leak proof containers labeled with the universal biohazard symbol.

Human and animal blood and body fluids can be disposed of by flushing directly to the sanitary sewer (wear laboratory coat, safety glasses and face shield, and gloves, and be careful to minimize splashing). All other liquid waste must be autoclaved or treated with a 1/10 dilution of commercial bleach (an approximate final concentration of 0.5% sodium hypochlorite) with a minimum of 30 minutes residence time prior to disposal. Treatment of spore-forming pathogenic organisms requires that the 1/10 diluted bleach solution be adjusted to pH 7 prior to treatment. **These specific conditions are the only chemical treatments currently allowed for treatment of liquid biohazardous waste.**

Animal Carcasses, Body Parts, and Tissue

Treatment and disposal of animal carcasses, body parts, and tissues is coordinated by the Office of Animal Resources (OAR).

Non-infectious carcasses, body parts, and tissues are to be placed in an opaque plastic bag and the bag taped shut with duct tape. Store the bagged materials in a freezer or cold storage area until transport to the OAR facility.

Infectious carcasses are to be collected in an autoclavable plastic bag labeled with the biohazard symbol and taped shut. Secure limbs and sharp protrusions so they will not puncture the bag. Twist the open end of the bag, fold the end over and tape securely. These materials must be stored at -20 °C until transport to the OAR facility.

All animal carcasses are picked up by a local contractor and transported to their facility for incineration.

Human Pathological Waste

Pathological waste includes **all** recognizable human anatomical remains. Collect human pathological waste in a red, biohazard bag (or other appropriate, labeled container) and place inside a rigid container that can be sealed and which is labeled with the universal biohazard symbol. All human pathological waste will be sent off campus for cremation or interment. Coordinate disposal of human pathological waste through the Curator of the Gross Anatomy Laboratory, Department of Physiology and Cell Biology (784-6169).

Autoclaving Biohazardous Waste

Autoclaving is an accepted procedure for decontamination of certain biohazardous waste. Biological cultures and stocks, contaminated solid waste, liquid waste, and small animal carcass waste can be sterilized through autoclaving. After sterilization in a steam autoclave, these materials are considered non-infectious. Except for animal carcasses, this bagged waste can then be disposed of as ordinary trash (to go to sanitary landfill); however, it is recommended that autoclave bags containing sterilized waste be placed in an opaque trash bag prior to disposal. Materials that contain hazardous chemicals or radioisotopes are not to be autoclaved (contact EH&S at 327-5040 for assistance).

The Washoe County Health Department has promulgated regulations that cover the treatment and disposal of biohazardous waste. Specific UNR procedures for management of biohazardous waste are contained in the UNR Biohazardous Waste Operational Plan. The following information summarizes the procedures related to autoclaving biohazardous waste; see the Operational Plan for more detailed information.

To ensure that biohazardous waste is properly decontaminated during autoclaving, Washoe County regulations require that the following procedures be followed.

1. Treatment of infectious waste must be conducted as specified in the UNR Biohazardous Waste Operational Plan. For treatment of microbial cultures and contaminated solid waste (e.g., gloves, absorbent pads, and pipette tips) a **minimum time of 60 minutes** at 121 °C (250 °F) and 15 psi is required. The total processing time required to decontaminate infectious waste depends on the specific loading factors (container type, water content, quantity, etc.) and in some cases a longer autoclave time may be required.

   Note: Autoclave times of less than 60 minutes must be approved by the IBC. Such proposals must be submitted to the IBC in writing with justification for the shorter time

period, and a written SOP that describes the material to be autoclaved, typical waste volume, waste container, autoclave time, and procedure and frequency of autoclave efficacy testing.

- Sterilization by autoclaving is accomplished through exposure and penetration of the contaminated material by superheated steam for an adequate amount of time. Because steam may not effectively penetrate a sealed plastic autoclave bag, bags containing dry loads must not be tightly sealed (rubber band closures will allow bags to "breath") or adequate amounts of water must be added to the load. Consult the manufacturer's instructions for sterilizing materials inside sealed plastic autoclave bags.

2. All autoclaved waste must include a visual steam sterilization indicator. If the visual indicator fails then biohazardous waste is not considered to be sterilized and must be re-autoclaved.

Autoclave Efficacy Testing

Autoclaves used to treat biohazardous waste must be tested for sterilization efficacy at least once every three months using commercially available *Geobacillus stearothermophilus* spore ampoules, which contain a suspension of viable spores and are designed for this purpose.

1. Place ampoule of *G. stearothermophilus* spores and in the center of an autoclave load (a representative non-biohazardous waste load can be used as a surrogate).

2. Process the load under normal operating procedures.

3. After the autoclave cycle is completed retrieve the spore ampoule and incubate it with a non-autoclaved control ampoule at the recommended growth temperature (as prescribed by the spore ampoule manufacturer's directions).

4. After completion of the incubation period, inspect the ampoules for growth as described in the manufacturer's instructions. Lack of growth in the test ampoule indicates that the autoclave conditions were sufficient to inactivate the heat-resistant spores, and thus provides good assurance that the autoclave conditions are sufficient to kill more heat sensitive microbial agents.

5. If an autoclave fails an efficacy test, any waste that was autoclaved during the failed test is not considered to be sterilized and must be re-autoclaved. The failed autoclave cannot be used to treat biohazardous waste until efficacy testing demonstrates adequate autoclave conditions are achieved.

In addition to quarterly efficacy testing requirements, the performance of autoclaves used to treat biohazardous waste must be verified through efficacy testing at the following times:

a. Prior to initial use to treat biohazardous waste (whether unit is new, reconditioned, or pre-existing)

b. Upon relocation of pre-existing, previously tested autoclaves

c. After maintenance, repair, or calibration that has potential to negatively affect autoclave performance

Autoclave Records

The following records must be maintained for each autoclave used to treat biohazardous waste for a minimum of three years:

a. Each autoclave cycle used to treat biohazardous waste, to include:
   i. date and operator name
   ii. autoclave temperature and pressure
   iii. general description of waste load, including waste containers (e.g., 2 small bags of solid waste)
   iv. quantity of biohazardous waste* (record number of containers by type or volume of liquid waste)
   v. autoclave run time
   vi. results of visual indicators (e.g., heat sensitive tape) used to verify adequate autoclave conditions

* Quantity of solid waste in pounds is estimated during end or year reporting by assigning nominal weights to container types or converting liquid volume to mass.

b. All autoclave maintenance, calibration, and repair

c. All autoclave efficacy tests and results

Contact the BSO for a template of an example autoclave log sheet that includes fields for all required information.

Chapter 13
CDC/USDA Select Agents

Introduction

The Centers for Disease Control and Prevention (CDC) and the United States Department of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS) have identified specific biological agents and toxins that are considered to be a severe threat to public health and safety as bioterrorism agents. These materials are referred to as select agents and toxins by the CDC and high consequence livestock pathogens and toxins, and listed plant pathogens, by APHIS. Transfer, possession, use, and disposal of all of these agents and toxins are strictly regulated. **Tier 1 select agents and toxins** is a subset that represents the greatest risk of deliberate misuse with significant potential for mass casualties or severe effects to the economy, infrastructure, or public confidence. **There are additional regulations that apply to Tier 1 select agents and toxins.** Because the list of select agents may be revised, it is recommended that the most current list of select agents and toxins be checked before acquiring pathogenic agents and biological toxins by accessing the Federal Select Agent Program web site (click on "Select Agents & Toxins" to find the current list).

The regulations associated with select agents and toxins are very complex and strict, and there are significant monetary fines and criminal penalties associated with non-compliance. The information in this Chapter is a summary of the select agent regulations; it is not a complete description of the regulatory requirements associated with select agents and toxins. **Investigators must review and understand the select agent regulations and their responsibilities prior to acquiring or working with any select agent or toxin (see above link to regulatory information).**

Responsible Official

Select agent regulations require that a Responsible Official (RO) be designated for each institution that possesses and uses select agents or toxins. The UNR Biosafety Officer, Ben Owens, is the RO for UNR. Additionally, an alternate Responsible Official has been designated to act in the RO's absence. **The RO has institutional responsibility for the biosafety, security, and regulatory compliance of select agents, and as such, must be contacted prior to obtaining any select agents.**

UNR Select Agent Responsible Official and Alternates				
Name	**Position Title**	**Department**	**Phone**	**E-Mail**
Ben Owens	Responsible Official	EH&S	327-5196	bowens@unr.edu
Cheston Carpenter	Alternate Official	EH&S	784-4342	chestonc@unr.edu

Authorization to Possess and Use Select Agents
Prior to obtaining any select agent or toxin, a MOUA (see Chapter 2) covering the proposed work must be submitted to, and approved by, the UNR Institutional Biosafety Committee.

PIs who want to acquire, possess, or use any select agent or toxin **must be registered and approved with the appropriate agency (CDC or USDA) prior to obtaining the agent(s) or toxins(s)**. Both the institution (UNR) and individual personnel must be approved by the appropriate agency. Investigators who want to possess and use select agents or toxins must contact the RO (Ben Owens) for assistance with the registration application process. Approval by the CDC or USDA will take a few months (plan a minimum of three months) and will require a site inspection by Select Agent Program inspectors. PIs should plan research projects accordingly.

The select agent regulations contain very strict requirements regarding biosafety, training, incident response, security and accountability, as well as other requirements. Investigators wanting to acquire select agents or toxins should review the applicable select agent regulations thoroughly before initiating the registration application process.

Exemptions and Exclusions
Diagnostic labs that do not maintain select agents or toxins are largely excluded from the select agent regulations; however, there are notification and possession time limits and other requirements that do apply. Additionally, the CDC and APHIS can grant exclusions for temporary public health emergency situations, and other special circumstances. Consequently, any laboratory that conducts diagnostic or verification testing for any select agent or toxin must self-identify and contact the UNR RO as soon as possible. **Identification of any select agent or toxin in a specimen or isolate must be reported to the RO as soon as possible.**

Several specific select agent microbial strains and toxin forms have been determined to not present a severe threat to public health and safety, and are therefore excluded from the CDC/APHIS select agent regulations. The list of excluded biological agents and toxins is dynamic and the most current list is available on the Select Agent Program web site. Only the specific strains and toxins listed are excluded. Altered strains or toxin forms derived (even directly) from excluded agents and toxins are not themselves excluded unless specifically approved by the CDC or APHIS. Toxins are also excluded based on threshold quantities. Laboratories maintaining exempt quantities of select agent toxins must keep an accurate inventory of toxin amounts to verify that total quantities are below the threshold, and must keep toxins stored in locked containers (e.g., freezer).

Safety Plan
Each select agent laboratory must develop and implement a written safety plan that addresses the biological and chemical safety issues associated with the specific select agents and toxins maintained by the laboratory. In particular, the plan must address the hazards associated with the select agents and toxins, methods to be used to prevent exposures, including use of laboratory ventilation (biological safety cabinets and lab hoods) and personal protective

equipment, disinfection and decontamination methods, waste handling and disposal procedures, and the proper response to spills, personal contamination, and other incidents.

Biosafety plans for select agents and toxins must meet the agent-specific and biosafety level criteria listed in the most current version of the BMBL. Use of recombinant or synthetic nucleic acid molecules that are considered to be a select agent must meet the applicable criteria listed in the most current version of the NIH guidelines. Chemical safety plans for select toxins must meet the criteria listed in the OSHA Standard *Occupational Exposure to Hazardous Chemicals in Laboratories*.

Security Requirements

All persons who will have access to any select agent or toxin must be approved by the Department of Justice. Approval requires that each individual successfully pass a background security check (conducted by the FBI in accordance with the USA PATRIOT Act) and submit fingerprints to the FBI, with approval by the Select Agent Program and the RO. Additionally, access to Tier 1 select agents and toxins requires successful screening through the UNR Select Agent Personnel Suitability Assessment Program and approval by the RO. Contact the RO for more information on obtaining approval for access to select agents and toxins.

Anyone who has not been approved for access to select agents and toxins must be denied access unless they are escorted by an approved person (including Tier 1 select agent and toxin approval as applicable). Everyone who enters a laboratory where select agents are stored or present must have security approval or be accompanied by an approved person. This includes visiting scientists (UNR or off campus), maintenance workers, custodians, and vendors. Persons without a security approval cannot have direct (hands on) access to select agents and toxins, even if under direct escort of an approved person.

Each laboratory that possesses or uses select agent or toxins must have a written security plan. This plan must be based on a site-specific security risk assessment and must addresses the following topics:

- Physical security
- Cyber security
- Inventory of select agents
- Select agent transfers
- Training
- Reporting of unauthorized persons and missing materials
- Provisions for cleaning, maintenance, and repairs

Laboratories that possess Tier 1 select agents and toxins require additional security measures over and above those required for other select agents and toxins.

Any theft or loss of select agents must be immediately reported to the RO and the UNR Police (334-COPS). The RO will make the required notification to the Select Agent Program.

Incident Response

Each laboratory that possesses or uses select agents or toxins must develop a written incident response plan that is laboratory specific and based on a site-specific risk assessment. This plan must be coordinated with the department, building, and university incident response plans. The plan must address the hazards of the select agents, planning and coordination with emergency responders, building evacuation, site security and control, decontamination and emergency medical treatment, and other incident response issues.

Training

All persons approved for access to select agents and toxins must receive documented training covering safety of select agents and toxins and their safe handling, use, and disposal; security requirements and procedures; inventory and accounting procedures; and emergency response procedures. Personnel who require access to Tier 1 select agents and toxins must receive additional training on Tier 1 select agents and toxins, including the Personnel Suitability Assessment Program. **Training is required before beginning work with select agents and annually thereafter**.

Transfers

Select agents and toxins can only be transferred between entities that are currently approved by the CDC or USDA to possess and use the specific select agents and toxins to be transferred. All transfers of select agents and toxins require prior approval of the Select Agent Program. Both the sender and recipient must complete a transfer form (APHIS/CDC Form 2), and the recipient submits the completed form to the Select Agent Program. The Form 2 requires the signature of the RO from both the sender and recipient facilities. **Contact the UNR RO, Ben Owens, at 327-5196 or bowens@unr.edu prior to any transfer of select agents**.

Inventory and Disposal of Select Agents

An accurate record of all select agents and toxins, from receipt to destruction or disposal, must be maintained. The inventory must include specific information on individual containers and vials, as well as a record of each use and disposal. The select agent and toxin inventory must be verified at least monthly to account for all quantities and containers of select agents and toxins. Any discrepancies between the inventory record and the actual inventory must be reported as soon as possible to the UNR RO (Ben Owens).

Records

The select agent regulations require that several records be maintained, including the following:

- Detailed inventory of each select agent and associated containers
- Access to select agents and toxins
- Access to areas where select agents and toxins are used or stored
- Safety, security, and incident response plans
- Training records
- Transfer documents (From 2) and other APHIS/CDC select agent/toxin forms
- Safety and security incident reports

Each individual laboratory that possesses or uses select agents or toxins is responsible for maintaining these records. EH&S will keep records of any training that it conducts, including select agent training presented by the RO. Laboratories must maintain records of training conducted by laboratory personnel or any other applicable training. EH&S will maintain APHIS/CDC forms; however, laboratories must also maintain copies of these forms. The recordkeeping requirements are complex, and therefore the select agent regulations should be reviewed for a complete description of the recordkeeping requirements.

Chapter 14
Animal Care and Use in University Research, Testing, and Education Programs

University Policy

All vertebrate animal use inclusive of research, teaching, and testing activities at UNR must be conducted in accordance with all applicable laws, regulations, and guidelines regardless of the source of funding or location of activity. The program of animal care and use at UNR is bound by the animal welfare standards, oversight mechanisms, and reporting requirements delineated by the USDA Animal Welfare Regulations, the Public Health Service Policy on Humane Care and Use of Laboratory Animals (last revised in March 2015), and the standards and guidelines of AAALAC International. Accordingly, compliance with these regulations requires that assurance is provided that all personnel at risk are appropriately encompassed within the program of safety, inclusive of specific safeguards for animal experimentation with hazardous agents.

Proper attention is expected for the development and implementation of proper procedures for animal care and housing, storage and distribution of hazardous agents, dose preparation and administration to animals, body fluid and tissue handling, waste and carcass disposal, items which might be used temporarily and then removed from the work location (e.g., written records, experimental devices, sample vials), and appropriately selected methods for personnel protection.

The UNR Biosafety Manual provides the campus-wide guidance upon which project-specific standard operating procedures involving the use of hazardous agents in animals are developed, which then must be approved prior to implementation by the Institutional Animal Care and Use Committee (IACUC). Thus, vertebrate animal studies involving the use of etiological agents or recombinant or synthetic nucleic acid molecules must be pre-approved by both the IACUC and the IBC.

Animal Resources and the IACUC

Animal Resources is a service core with campus-wide animal welfare compliance authority which reports to the UNR Vice President for Research and Innovation and the Assistant Vice-President for Research Administration. For professional guidance on technical and species-appropriate methods for the care and use of vertebrate animals in teaching and research activities, please contact the Office of Animal Resources for a copy of their "Investigator Handbook" and the IACUC approved policies and procedures for using hazardous agents in animals.

The staff of Animal Resources provide animal husbandry, technical services, veterinary care, and welfare compliance oversight for all projects at UNR, and is overseen by Benjamin Weigler, DVM, MPH, Ph.D., DACLAM, DACVPM. Dr. Weigler is the campus Attending Veterinarian and also serves on the IBC to help the BSO foster communication and coordination between the work of the IACUC and the IBC.

Details regarding the expectations and services of Animal Resources and the IACUC can be found on their website (www.unr.edu/animal-resources). Requests to the IACUC and the IBC for the use of hazardous agents in animal studies must detail the specific methods to be used for housing and caring for animals exposed experimentally, with emphasis on the management and safety practices for containment of each class of agent as well as the protection of personnel. The guiding documents for development of experimental plans are this biosafety manual, Animal Resources policies and procedures, and applicable sections of the BMBL and NIH Guidelines. In serious cases of non-compliance, progressive discipline according to University personnel policies will result, including the potential loss of privileges for the use of research and teaching animals and access to the UNR campus animal facilities.

Chapter 15
Packaging and Shipping Infectious Agents

Introduction
The International Civil Aviation Organization (ICAO) is a specialized agency within the United Nations that develops and maintains principles that ensure the safety of international civil aviation. The ICAO *Technical Instructions for the Safe Transport of Dangerous Goods by Air* are the regulations that govern the shipping of dangerous goods. These technical instructions have been incorporated into US law and are an acceptable method of transport in the US (49 CFR 171.11).

Packaging and shipping biological materials involves certain risks with numerous potential liabilities. The International Air Transport Association (IATA), *Dangerous Goods Regulations* (DGR), latest edition, is the worldwide gold standard for shipping. The IATA regulations apply to all air transport, both domestic and international. By following the IATA DGR you ensure that your package will also meet U.S Department of Transportation (DOT) requirements for ground transport. **All responsibility** for packaging and shipment of these agents have been assigned to the shipper. These include classification of shipment, proper packaging of shipment, proper marking and labeling of package, and proper documentation of shipment.

Training Requirements
Those involved in the packaging and shipping of Dangerous Goods must undergo training every two (2) years or when activities change. It is the responsibility of the department to ensure that training is completed; however, the Environmental Health and Safety Department (EH&S) can provide this training. The shipper is under obligation to receive further qualification when shipping hazardous materials of a class or division where current training is insufficient.

Classification of Shipment Type
A shipment of biological material will fall into one of the five following categories:

- Unregulated biological material
- Category A Infectious Substances
- Category B Infectious Substance
- Patient specimens
- Genetically Modified Organisms (GMOs)

Shipment Classifications
Unregulated Biological Materials
Unregulated biological materials are not subject to IATA or DOT infectious substance shipping regulations; however, these materials may require a permit for shipment abroad. Please check with EH&S if you have any question as to whether your shipment is unregulated. Some examples of unregulated biological materials include:

- Substances which do not contain infectious agents and which should not cause disease in humans or animals. These include non-infectious cells or tissue cultures; blood, plasma, or sera from humans or animals not suspected of having an infectious disease; DNA, RNA, or any other genetic elements that are not themselves infectious.
- Patient specimens that are not thought to be infectious
- Microorganisms that are not pathogenic to humans, animals, or plants.
- Substances that have been neutralized or inactivated such that they are no longer infectious.
- Environmental samples that are not known, or thought to be, infectious.
- A biological product such as an antibody or drug.

Category A Infectious Substances

Category A infectious substances are capable of causing disease in humans or animals. The proper shipping name for Category A Infectious Substances is *Infectious Substance Affecting Humans* (UN2814), or *Infectious Substance Affecting Animals* (UN2900). These substances are classified as DOT hazard class 6.2 (infectious). Some examples of Category A infectious substances are:

UN2814 Infectious Substances Affecting Humans

Bacillus anthracis (cultures only)
Brucella abortus (cultures only)
Brucella melitensis (cultures only)
Brucella suis (cultures only)
Burkholderia mallei – Pseudomonas mallei – Glanders (cultures only)
Burkholderia pseudomallei – Pseudomonas pseudomallei (cultures only)
Chlamydia psittaci – avian strains (cultures only)
Clostridium botulinum (cultures only)
Coccidioides immitis (cultures only)
Coxiella burnetii (cultures only)
Crimean-Congo hemorrhagic fever virus
Dengue virus (cultures only)
Eastern equine encephalitis virus (cultures only)
Escherichia coli, verotoxigenic (cultures only)
Ebola virus
Flexal virus
Francisella tularensis (cultures only)
Guanarito virus
Hantaan virus
Hantavirus causing hemorrhagic fever with renal syndrome
Hendra virus
Hepatitis B virus (cultures only)
Herpes B virus (cultures only)
Human immunodeficiency virus (cultures only)

Highly pathogenic avian influenza virus (cultures only)
Japanese Encephalitis virus (cultures only)
Junin virus
Kyasanur Forest disease virus
Lassa virus
Machupo virus
Marburg virus
Monkeypox virus
Mycobacterium tuberculosis (cultures only)
Nipah virus
Omsk hemorrhagic fever virus
Poliovirus (cultures only)
Rabies virus (cultures only)
Rickettsia prowazekii (cultures only)
Rickettsia rickettsii (cultures only)
Rift Valley fever virus (cultures only)
Russian spring-summer encephalitis virus (cultures only)
Sabia virus
Shigella dysenteriae type 1 (cultures only)
Tick-borne encephalitis virus (cultures only)
Variola virus
Venezuelan equine encephalitis virus (cultures only)
West Nile virus (cultures only)
Yellow fever virus (cultures only)
Yersinia pestis (cultures only)

UN2900 Infectious Substances Affecting Animals
African swine fever virus (cultures only)
Avian paramyxovirus Type 1 – Velogenic Newcastle disease virus (cultures only)
Classical swine fever virus (cultures only)
Foot and mouth disease virus (cultures only)
Goatpox virus (cultures only)
Lumpy skin disease virus (cultures only)
Mycoplasma mycoides – Contagious bovine pleuropneumonia (cultures only)
Peste des petits ruminants virus (cultures only)
Rinderpest virus (cultures only)
Sheep-pox virus (cultures only)
Swine vesicular disease virus (cultures only)
Vesicular stomatitis virus (cultures only)

Category B Infectious Substances
Category B infectious substances are materials that are infectious, but do not meet the definition of Category A infectious substances. These include patient samples, tissue cultures,

and cells that are presumed to contain, or have a reasonable probability of containing, a pathogenic organism (e.g., blood known to contain HIV). The proper shipping name for these substances is *Biological Substance, Category B* (UN3373).

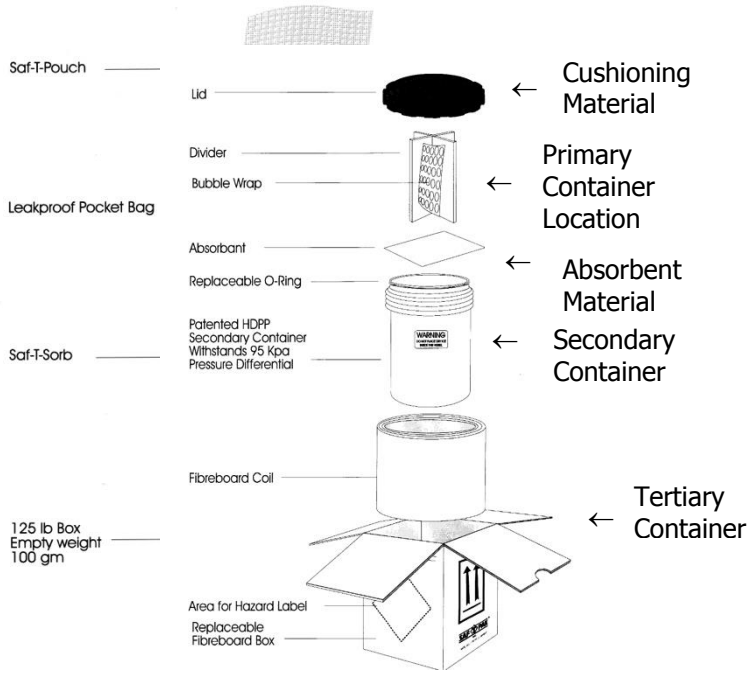
Genetically Modified Organisms (GMOs)

Genetically modified organisms (GMOs) are organisms in which their genetic material has been altered through recombinant DNA techniques. If a GMO is non-infectious it is classified as a DOT class 9 (miscellaneous) hazard and is assigned to UN3245. If a GMO is potentially infectious, it must be assigned as a Category A infectious agent (UN2814 or UN2900), or Category B infectious agent (UN3373).

Packaging Biological Materials

Category A infectious substances must be packaged per IATA packaging instructions 602 as shown below. UN approved packaging such as that from Saf-T-Pak must be used.

Specimen containers are to be placed in the cushioning material, which is then placed into the secondary leak-proof container. Absorbent material, sufficient to absorb the quantity of the shipment, must be placed next to the primary container. The specimens and an inventory of the contents are then placed into the outer container.



Containers:

For Category A Infectious Substances IATA Packaging Instructions 602 apply. Three containment levels are used to package Infectious Agents for shipment. The three levels ensure containment of the agent and include:

- Primary – plastic, metal or glass leak-proof container, containing the product.
- Secondary - plastic, metal or glass leak-proof container, containing the primary container and sufficient absorbent material for the entire quantity of the product.
- Tertiary – contains secondary container, description of contents, dry ice or other preserving material, and any packaging material required to prevent shifting of contents.

Preservatives
When preservatives are shipped with a specimen or agent, the preservative must also be declared on the Shipper's Declaration (see page 15-8). The hazard class label that applies to the preservative, and the quantity of the preservative, must also be on the outer package. Additional information may be found in the IATA *Dangerous Goods Regulations*, latest edition.

Preservative	Hazard Class	UN Code	Packing Instruction
Dry Ice*	9	1845	904
Formalin, Formaldehyde Solution	8	2209	820
Nitrogen, Refrigerated Liquid	2.2	1977	202

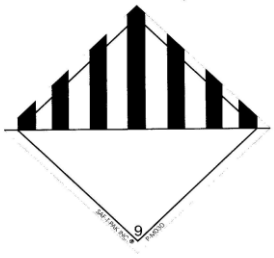
*The container must be designed to allow the release of carbon dioxide gas.

Labeling and Marking
The outer container, usually a fiberboard box, must be marked with the following information:

- Hazard Label, Class 6.2 (for all category A infectious agents)
- UN Number (see table below)
- Proper shipping name of agent, with technical name in brackets, and quantity.
- Name, address and phone number of shipper
- Name, address and phone number of receiver
- 24-hour contact phone number of responsible party (shipper)
- Two pairs of orientation arrows

UN Number	Infectious to
UN 2814	Humans
UN 2900	Animals
UN 2814	Humans & Animals

Hazard Label Class 6



Hazard Label Class 9

Note: Labels may be obtained from the supplier of the packaging material or from other label manufacturers.

Preservative:
- Class Label (determined by the preservative used, such as Class 9 for dry ice)
- Quantity of preserving agent
- UN Shipping Code of the preservative

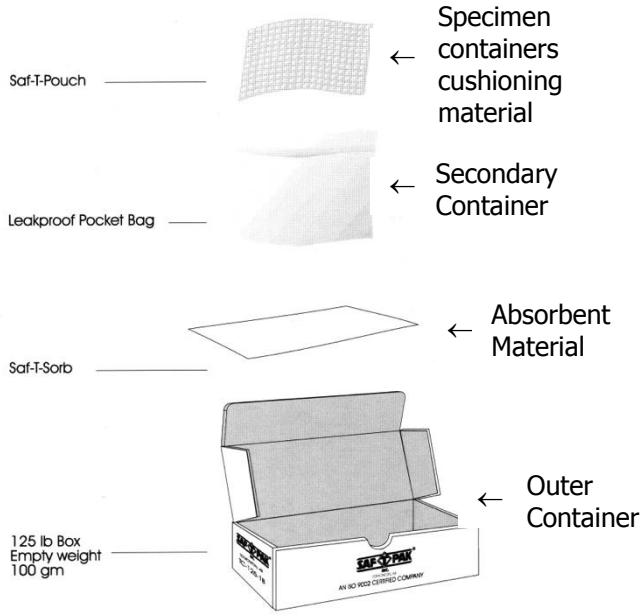
Example: Identification on tertiary container

5g of culture containing hepatitis B virus are to be shipped in 3 kg dry ice. The tertiary container would be labeled with the following information:
- Infectious Substance Affecting Humans (Hepatitis B virus), 5g, UN2814
- Dry Ice UN1845 3 kg
- Hazard Class Label 6, "Infectious Substance", and Hazard Class Label 9, "Miscellaneous", would be affixed to the container.

Note: The above information must also be located **inside** the tertiary (or outer) container.

Category B infectious substances are shipped utilizing IATA packaging instructions 650. The manufacturer will identify compliance with package specifications on the outer shipping container. Orientation labels may be used.

Specimen containers are to be placed in the cushioning material, which is then placed into the secondary leak-proof container. Absorbent material, sufficient to absorb the quantity of the shipment, must be placed next to the primary container. The specimens and an inventory of the contents are then placed into the outer container. The package must then be labeled with the UN3373 Label.



The full and accurate completion of the Shipper's Declaration is essential to the safe transportation of the infectious agent or diagnostic specimens. These are legal documents signed by the shipper, which creates a contract between the shipper and the carrier. The document must be accurate, legible, and neat. There must be no spelling errors. The carrier will provide you with a Shippers' Declaration and detailed instructions (see example on page 15-8). This document must be completed electronically.

- The declaration form must be completed in English.
- Three copies of the declaration must be completed. One copy will remain with the shipper (investigator). Two copies will be sent with the shipment. The following instructions refer to the Shipper's Declaration on page 15-8.

SPACE	**INSTRUCTIONS**
1	**Shipper's:** ♦ Name, ♦ Address, ♦ Phone number.
2	**Receiver's:** ♦ Name, ♦ Address, ♦ Phone number.
3	**Line out the item that does <u>not</u> apply.** Passenger aircraft can only be used to ship quantities less than 50 ml. Cargo aircraft must be used to ship quantities between 50 ml and 4 L.
4	**Line out the item that does <u>not</u> apply.** Radioactive or Non-Radioactive
5	♦ Identify shipment as Category A Infectious Substance. ♦ Identify infectious to human, animals or both ♦ Identify the specimen by name in parenthesis. ♦ Identify preserving agent*
6	**Class or Division ***
7	**UN Code ***
8	**Packaging Group *** There is no packaging group for biological agents. Dry Ice is Packing Group III
9	♦ Identify the quantity ♦ Identify type of outer container for the shipment
10	**Packaging Instructions *** Dry Ice is PI 904
11	<u>Must</u> contain the following information: ♦ 24 hour emergency contact number for the shipper (Primary Investigator, Lab Supervisor)
12	**Name, Place, Date, and Signature of the shipper.**

***** As described in the latest edition of the IATA Dangerous Goods Regulations

- Example Shipper's Declaration for Dangerous Goods

SHIPPER'S DECLARATION FOR DANGEROUS GOODS

Shipper Ben Thompson
1 College Road
Durham, NH 03824
602-862-1234
(1)

Air Waybill No.
Page 1 of 1 Pages
Shipper's Reference Number (optional)

Consignee Sam Research
2 Langley Road
Boston, MA 11111
(2)

Person Responsible: Chem-Tel 1-800-255-3924

Two completed and signed copies of this Declaration must be handed to the operator.

WARNING

Failure to comply in all respects with the applicable Dangerous Goods Regulations may be in breach of the applicable law, subject to legal penalties.

TRANSPORT DETAILS

This shipment is within the limitations prescribed for: *(delete non-applicable)*

PASSENGER AND CARGO AIRCRAFT	CARGO AIRCRAFT ONLY
	XXXX

(3)

Airport of Departure

Airport of Destination

(4)

Shipment Type *(delete non-applicable)*

NON-RADIOACTIVE	RADIOACTIVE
XXXXX	

NATURE AND QUANTITY OF DANGEROUS GOODS

Dangerous Goods Identification				Quantity and Type of Packing	Packing Instructions	Authorization
UN or ID No. (5)	Proper Shipping Name (5)	Class or Division (Subsidiary Risk)	Packing Group			
UN2814	Infectious substance, affecting humans (Mycobacterium tuberculosis)	6.2 (6)		25 mg (9)	602	
UN1845 (7)	Dry ice	9	III (8)	5 kg All packed in one fibreboard box	904 (10)	

Additional Handling Information (11)

Emergency Telephone Number Chem-Tel 1-800-255-3924

I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name, and are classified, packaged, marked and labelled/placarded, and are in all respects in proper condition for transport according to the applicable international and national governmental regulations. I declare that all of the applicable air transport requirements have been met.

Name/Title of Signatory Ben Thompson/professor

Place and Date Durham, NH/November 21, 2006

Signature *(see warning above)* Ben Thompson (12)

University of New Hampshire Office of Environmental Health and Safety