

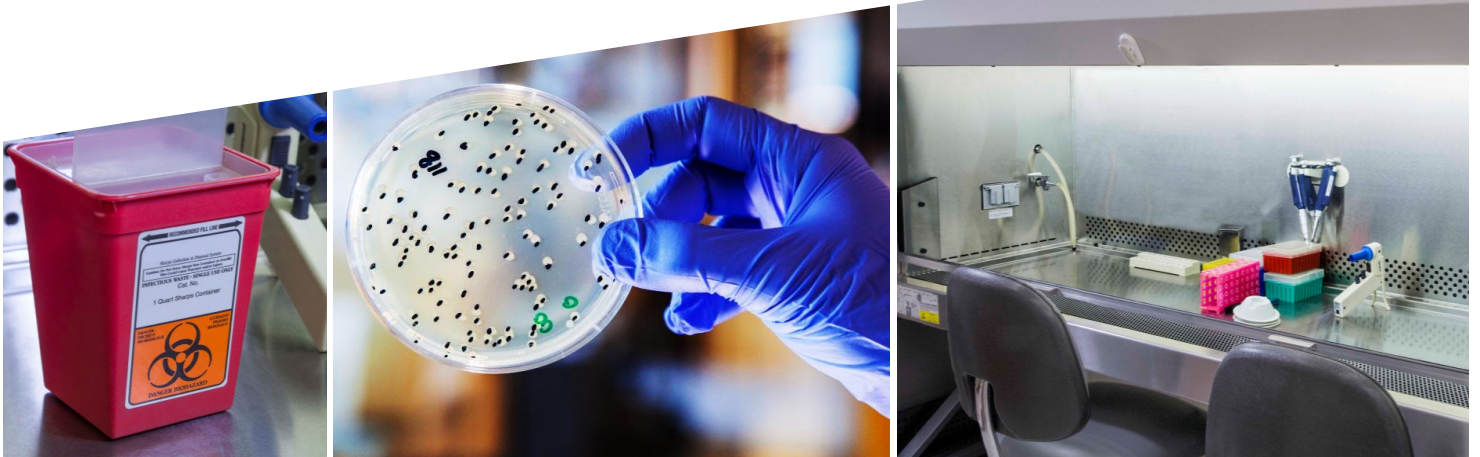


UNIVERSITY OF OREGON

# Biological Safety Manual

ENVIRONMENTAL HEALTH & SAFETY

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## I. Biological Safety Program

### 1.1 Purpose

The University of Oregon's Biological Safety Program facilitates safe research involving recombinant or synthetic nucleic acid molecules (rsNA) and biohazardous materials. The Program seeks to fulfill this goal by providing support to the Institutional Biosafety Committee (IBC), managing the Bloodborne Pathogen Exposure Control Plan (ECP), and consulting on exposure assessments for the Occupational Health Program. This manual outlines appropriate practices, university policies and regulatory requirements for working safely with biological materials.

The University of Oregon is actively committed to preserving the health and safety of its students, staff, and faculty, and to protecting the environment and the community. It is recognized that use of potentially pathogenic microorganisms and organisms containing rsNA is necessary in many university research and teaching laboratories. To ensure the safe handling of these organisms, the University requires compliance with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (“NIH Guidelines”) and other applicable federal, state, and local regulations, and incorporates best practices as outlined in CDC's *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

### 1.2 Scope

The University of Oregon Biological Safety Manual is applicable to all laboratory, research, teaching, service, and support activities that may involve exposure to biohazards. Biohazards are microorganisms, microbial toxins, or other biological agents that can infect and/or cause disease in humans, animals, or plants. Often referred to as “infectious agents,” examples include bacteria, bacterial toxins, viruses, fungi, rickettsia, prions, protozoans, parasites, genetically-modified organisms, or rsNA molecules. In addition, biohazards include human blood, body fluid, tissues, and cell lines of human origin. The Biological Safety Program applies to all clinical, laboratory, research, service, and support activities the University sponsors or participates in.

### 1.3 Organization and Responsibilities

The Principal Investigator (PI) is directly and primarily responsible for the safe operation of the laboratory. His/her knowledge and judgment are critical in assessing risks and appropriately applying the recommendations in this manual. However, safety is a shared responsibility among all of the laboratory staff. Many resources exist to assist the PI with these responsibilities, including the Institutional Biosafety Committee (IBC) and the EHS department.

#### ***The University of Oregon***

The president of the University of Oregon is ultimately responsible for all environmental health and safety issues at the institution. This responsibility is exercised through the normal chain of authority within the university by delegating the charge for ensuring safe work practices and adherence to established policies and guidelines to the provost, vice-



presidents, deans, directors, department chairs, PIs, supervisors and, ultimately, each employee (UO Safety Policy).

***Environmental Health & Safety/ Biological Safety Officer***

- Assist departments in providing training and guidance for implementation of this policy.
- Develop, implement, and maintain a comprehensive biosafety program at the University of Oregon, including policies and procedures regarding biosafety principles and practices;
- Consult with UO Principal Investigators regarding mitigation of biological hazards, methods for compliance with applicable regulations, and biological waste disposal;
- Administer the Institutional Biosafety Committee;
- Track and coordinate annual inspections of biological laboratories and certification of biosafety cabinets on campus;
- Investigate accidents involving infectious agents and assist with corrective actions;
- Develop and provide training programs related to biosafety.

***The Institutional Biosafety Committee***

- Review recombinant and synthetic nucleic acid research conducted at or sponsored by the University for compliance with the NIH Guidelines, and approve those research projects that are found to conform with the NIH Guidelines;
- Review non-rsNA projects as defined in the IBC charter;
- Notify the PI of the results of the IBC's review and approval;
- Report any significant problems with or violations of the NIH Guidelines and any significant research-related accidents or illness to the appropriate Institutional official and to the NIH Office of Science Policy (OSP) as required;
- Follow the guidelines for membership and meetings as defined by NIH.

***Principal Investigators***

- Conduct the primary risk assessment of their experiments;
- Ensure the safe operation of their laboratory;
- Train laboratory personnel in safe work practices;
- Comply with all applicable state and federal regulations and guidelines;
- Register the following experiments with the IBC or EHS, as required:
  - recombinant and synthetic nucleic acid activities;
  - work with infectious agents;



- experiments involving the use of human blood or other potentially infectious materials, such as unfixed human tissues, human cell lines, and certain body fluids;
- animal and plant pathogens
- Report all spills, accidents, injuries, and potential exposures to the BSO

### **Laboratory Personnel**

- Comply with safety recommendations for the work being performed;
- Report to their physician any concerns about risks and hazards of their laboratory work, particularly if they are pregnant or immunocompromised;
- Report all accidents, spills, or injuries to the PI.

## **II. Registration of Research**

### 2.1 Review and Approval of Experiments

Principal investigators are responsible for registering with EHS any experiments involving human materials, non-human primate materials, Risk Group 1, 2 or 3 organisms, select agents and toxins, and recombinant or synthetic nucleic acids (rsNA), including those exempt from the *NIH Guidelines*. The biosafety office audits all laboratories where BSL2 containment is required, and all BSL1 laboratories that are subject to the *NIH Guidelines*. The IBC, which oversees rsNA research at the University of Oregon, or the BSO will review and approve the registration.

### 2.2 Experiments Covered by the *NIH Guidelines*

Many experiments involving rsNA require registration and approval by the IBC before work may be initiated. Experiments that require IBC approval before initiation include those that involve:

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

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

- the formation of rsNA molecules containing no more than 2/3 of the genome of any eukaryotic virus propagated in tissue culture



- rsNA-modified whole plants, and/or rsNA-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-C, or III-D of the *NIH Guidelines*
- generation of transgenic rodents that require BSL1 containment.

### 2.3 Experiments Exempt from the *NIH Guidelines*

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

- Use synthetic nucleic acids that can neither replicate nor generate nucleic acids capable of replicating in any living cell; are not designed to integrate into DNA, and do not produce a toxin that is lethal for vertebrates at an LD50 of <100 ng/kg body weight.
- use rsNA molecules that are not in organisms or viruses
- consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent
- consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well-established physiological means
- consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species)
- consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent
- do not present a significant risk to health or the environment as determined by the NIH Director, with the advice of the Recombinant DNA Advisory Committee (RAC), and following appropriate notice and opportunity for public comment
- contain less than one-half of any eukaryotic viral genome propagated in cell culture.
- use *E. coli* K12, *Saccharomyces cerevisiae*, or *Bacillus subtilis* host-vector systems, unless genes from Risk Group 3 or 4 pathogens or restricted animal pathogens are cloned into these hosts
- involve the purchase or transfer of transgenic rodents for experiments that require BSL1 containment.

### 2.4 Human Blood, Unfixed Tissue, and Cell Culture

Please refer to the *Bloodborne Pathogens Exposure Control Plan* for detailed information on handling human-source material. Work with human-source material is regulated by the



Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard (29 CFR, Part 1910.1030) and comparable Oregon State regulations (OAR 437, Division 2, Subdivision Z). Human blood, unfixed tissue, cell culture, and certain other body fluids are considered potentially infectious for bloodborne pathogens such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). All human clinical material should be presumed infectious and handled using BSL2 work practices. This concept is called Universal Precautions. Investigators are responsible for notifying EHS of their use of human materials so training and immunization can be provided as required by OSHA.

## 2.5 Biological Select Agents and Toxins

Biological Select Agents and Toxins (BSAT) are microorganisms and toxins that have potential for misuse by terrorists. The Federal Select Agent Program regulates the possession, use and transfer of select agents. Please contact the BSO immediately if you currently possess or plan to acquire any of these agents. Failure to provide notice may result in civil and criminal liability for individual researchers and/or the University. If you have questions, you may contact the BSO, or visit the CDC's Select Agent website, [www.selectagents.gov](http://www.selectagents.gov), which provides BSAT program information.

## 2.6 Non-Human Primate (NHP) Materials

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

Work with any NHP primary cell cultures or unfixed tissues must be registered with the IBC, and lab personnel must be trained in the safety procedures required for handling NHP material prior to beginning the research. Sharps use with these materials should be eliminated or restricted.

# III. Classification of Potentially Infectious Agents

Procedures and facilities involved in protecting laboratory workers, the public, and the environment from laboratory biological hazards are governed by federal and state regulations and guidelines. Many granting agencies require that grant recipients certify that they adhere to both the guidelines and the regulations.

The *NIH Guidelines* classifies pathogenic agents into one of four risk groups according to specific criteria.





### 3.1 Classification of Infectious Microorganisms by Risk Group

<i>Risk Group Classification</i>	<i>NIH Guidelines Definition</i>	<i>World Health Organization Definition</i>
Risk Group 1	Agents not associated with disease in healthy adult humans.	<i>No or low individual and community risk</i> A microorganism unlikely to cause human or animal disease.
Risk Group 2	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are <i>often</i> available.	<i>Moderate individual risk; low community risk</i> A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to lab workers, the community, livestock or the environment. Lab exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
Risk Group 3	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.	<i>High individual risk; low community risk</i> A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.	<i>High individual and community risk</i> A pathogen that usually causes serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

### 3.2 Microorganisms Capable of Causing Infection in Humans

Investigators must register work with human materials and non-recombinant work involving a Risk Group 2 or 3 agent with the BSO for assistance in conducting a risk assessment and establishing proper containment and work practices.

### 3.4 Genetically Engineered Microorganisms

Following receipt of the completed research registration form by the BSO, the laboratory will be surveyed to ascertain that it meets the containment requirements listed in the *NIH Guidelines* for the agent being studied. If the lab meets the requirements, the work will be reviewed and approved or disapproved by the IBC.

Work with all genetically engineered organisms must comply with the *NIH Guidelines*. These guidelines classify experiments into four levels of containment, based on the hazard of the microorganism and the procedures and quantities being used. Additionally, the United States Department of Agriculture (USDA) requires permits for field testing of genetically engineered plants. It is University of Oregon policy that all laboratories follow these guidelines.



## IV. Biosafety Containment Levels

The National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC) publish guidelines for work with infectious microorganisms. The publication, entitled *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, recommends that work be done using one of four levels of containment: Biosafety Level 1 (BSL1), BSL2, BSL3 and BSL4. The four biosafety levels, designated in ascending order by degree of protection provided to personnel, the environment, and the community, are combinations of laboratory practices, safety equipment, and laboratory facilities. All of these factors, including the organism's Risk Group, are considered when assigning a biosafety level to work.

Microbiological work at the University of Oregon is conducted at BSL1 or BSL2 containment. There are no BSL3 or BSL4 laboratories at the University.

### 4.1 Summary of Biosafety Levels

Below is a summary of each biosafety level; detailed criteria for each level are described in Section IV of *BMBL*.

#### ***Biosafety Level 1 (BSL1)***

Suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

#### ***Biosafety Level 2 (BSL2)***

Practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. Biosafety Level 2 builds upon BSL1. BSL2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL1 in that: 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves. Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

***Biosafety Level 3 (BSL3)***

Applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within BSCs or other physical containment devices. A BSL3 laboratory has special engineering and design features. The University of Oregon does not currently have a laboratory facility that meets BSL3 requirements.

***Biosafety Level 4 (BSL4)***

Required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory. The University of Oregon is not equipped for BSL4 work.

## 4.2 Summary of Biosafety Level Recommendations

***BSL 1***

*Agents:* Not known to consistently cause disease in health adults.

*Practices:* Standard Microbiological Practices.

*Primary Barriers & Safety Equipment:* None required.

*Secondary Barriers (Facilities):* Laboratory bench and sink required.

***BSL 2***

*Agents:* Associated with human disease. Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure.

*Practices:* BSL1 practices plus:

- Limited access
- Biohazard warning signs
- Restrict sharps use
- Biosafety manual defining any needed waste decontamination or medical surveillance



*Primary Barriers & Safety Equipment:* Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols. PPE: laboratory coats, gloves, face protection as needed.

*Secondary Barriers (Facilities):* BSL1 plus autoclave available.

### **BSL 3**

*Agents:* Indigenous or exotic agents with potential for aerosol transmission. Disease may have serious or lethal consequences.

*Practices:* BSL2 practices plus:

- Controlled access
- Decontamination of all waste
- Decontamination of laboratory clothing before laundering

*Primary Barriers & Safety Equipment:* Class I or II BSCs or other physical containment devices used for all work. PPE: protective laboratory clothing, gloves, respiratory protection as needed

*Secondary Barriers (Facilities):* BSL2 plus:

- Physical separation from access corridors
- Self-closing, double-door access
- Exhaust air not recirculated
- Negative airflow into laboratory

### **BSL 4**

*Agents:* Dangerous/exotic agents posing high risk of life-threatening disease. Transmission by aerosol route or unknown method of transmission.

*Practices:* BSL3 practices plus:

- Clothing change before entering
- Shower on exit
- All material decontaminated on exit from facility

*Primary Barriers & Safety Equipment:* All procedures conducted in Class III BSCs; or work performed in Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit.

*Secondary Barriers (Facilities):* BSL3 plus:

- Separate building or isolated zone
- Dedicated supply and exhaust, vacuum, and decontamination systems
- Others as outlined in BMBL



### 4.3 Animal Facilities

Four standard biosafety levels are also described for activities involving infectious disease work with commonly used experimental animals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4, and provide increasing levels of protection to personnel and the environment. The University of Oregon conducts work at ABSL1 and ABSL2 only.

One additional biosafety level, designated BSL3-Agriculture (or BSL3-Ag) addresses activities involving large or loose-housed animals and/or studies involving agents designated as High Consequence Pathogens by the USDA. BSL3-Ag laboratories are designed so that the laboratory facility itself acts as a primary barrier to prevent release of infectious agents into the environment. More information on the design and operation of BSL3-Ag facilities and USDA High Consequence Pathogens can be found in *BMBL*.

### 4.4 Clinical Laboratories

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

Except in extraordinary circumstances (e.g., suspected hemorrhagic fever), the initial processing of clinical specimens and serological identification of isolates can be done safely at BSL2, the recommended level for work with bloodborne pathogens such as HBV and HIV. The containment elements described in BSL2 are consistent with the OSHA standard, "*Occupational Exposure to Bloodborne Pathogens*." This requires the use of specific precautions with all clinical specimens of blood or other potentially infectious material (Universal Precautions). Additionally, other recommendations specific for clinical laboratories may be obtained from the Clinical Laboratory Standards Institute.

BSL2 recommendations and OSHA requirements focus on the prevention of percutaneous and mucous membrane exposures to clinical material. Primary barriers such as Class II BSCs should be used when performing procedures that might cause splashing, spraying, or splattering of droplets. Biological safety cabinets also should be used for the initial processing of clinical specimens when the nature of the test requested or other information suggests the likely presence of an agent readily transmissible by infectious aerosols (e.g., *M. tuberculosis*), or when the use of a BSC (Class II) is indicated to protect the integrity of the specimen. The segregation of clinical laboratory functions and limited or restricted access to such areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented.



## V. Emergency Procedures

### 5.1 Biological Spills

A spill kit should be kept in each laboratory where work with microorganisms is conducted. At a minimum, spill kits should contain concentrated disinfectant (such as chlorine bleach), absorbent material, household rubber gloves, biohazard bags, sharps container, and forceps to pick up broken glass.

### 5.2 Spill Clean-Up Protocol

Step	Procedure
1.	Any potentially contaminated clothing must be removed and placed in a biohazard waste bag for autoclaving. If the spill is inside a BSC, the BSC must remain running.
2.	Hands and any contaminated skin must be washed thoroughly with soap and water.
3.	Put on appropriate PPE (at a minimum: disposable gloves, eye protection and a lab coat). Obtain the spill kit. Everyone not needed for spill clean-up must be cautioned to stay away from the spill area. Signs may be posted if necessary.
4.	Any sharp contaminated objects must be removed from the spill area using mechanical means, never with hands.
5.	After all sharps are removed, cover the spill with absorbent materials.
6.	Use an appropriate disinfectant for the organisms present. If human blood or materials are involved in the spill, use freshly diluted 10% bleach. Disinfectant must be poured carefully around the edges of the spill, working from the outside of the spill toward the center. <ul style="list-style-type: none"><li>▪ If the spill is inside a centrifuge, the rotor and its contents should be moved to a BSC, if possible.</li><li>▪ If the spill is inside a BSC, the spill tray underneath the work area and the trough below the air intake grill must be cleaned as well. These are likely to be contaminated when the spill is large.</li></ul> Note: Alcohol is not recommended as a disinfectant for large spills.
7.	After initial clean-up, the affected area must be saturated with disinfectant and left to soak for at least 20 minutes (adequate contact time is important to ensure complete decontamination).
8.	Disinfectant can be absorbed with paper towels. A final wipe-down should be done with clean paper towels soaked with disinfectant. Laboratory personnel should be sure to disinfect any equipment, walls or other areas likely to have been splashed by the spill.
9.	All contaminated waste must be disposed of properly in the biohazard waste stream.
10.	Hands must be washed thoroughly with soap and water. If the spill is inside a BSC, the cabinet should be left running for at least 10 minutes before resuming use.
11.	Report the incident to Safety & Risk Services. Any incident of a spill or exposure



involving rsNA needs to be reported to Biosafety Officer. The NIH requires immediate reporting in the event of a significant research-related accident or problem.
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### 5.3 Procedures for Cleanup of Human Blood

*Only personnel enrolled in the UO Bloodborne Pathogens Program and appropriately trained may clean up blood spills. Follow the instructions outlined above, using a detergent solution for the initial cleanup and a 10% bleach solution for second round of disinfection.*

### 5.4 Injuries Involving Biological Materials

***For severe injuries:***

- Call 911 for assistance and transportation to the nearest emergency room.
- Accompany the injured person to the medical facility and provide information to personnel about the accident/exposure.
- Report accident to the PI and Environmental Health & Safety.

***For splash to the eye:***

- Immediately flush the eye in the eyewash station for 15 minutes. Hold the eyelid open. Be careful not to wash the contaminant into the other eye.
- Students should contact the UHC to obtain care. If UHC is closed, go to the nearest emergency clinic.
- Non-students should report to the nearest emergency clinic.
- Notify the PI and EHS, and seek additional medical assistance if necessary. More information can be found on the EHS website.

***For contamination of the hands or body:***

- Immediately remove contaminated clothing and drench skin with water. Wash with soap and water, and flush the area for 15 minutes.
- Students should contact the UHC at to obtain care. If UHC is closed, go to the nearest emergency clinic.
- Non-students should report to the nearest emergency clinic.
- Notify the PI and to EHS, and seek additional medical assistance if necessary. More information can be found on the EHS website.

Immediate evaluation by a medical professional is especially important after exposures to human blood. CDC recommends starting anti-retroviral drugs within two hours for significant exposures. The necessary appropriate prophylactic treatment can be started as soon as possible. Any relevant safety information about the pathogen should be brought with the patient. Referrals may be made to the workers' compensation medical provider for University of Oregon. The incident must be reported to EHS as soon as possible.



## 5.5 Fires in Biological Laboratories

Life safety is the highest priority. Without placing yourself in danger, put biological materials in secure location, such as incubator or freezer. Activate the building fire alarm and leave the building at once. Call the fire department from a safe location. Meet the fire department outside and direct them to the fire. Any individual who receives an exposure or potential exposure will be given a medical consultation and advised of available treatments.

# VI. Medical Surveillance

The Occupational Health Program provides medical surveillance for all personnel who are exposed to identified or regulated risks and for personnel with animal contact.

Workplace exposure to human blood and other potentially infectious materials (OPIM), as defined by the OSHA Bloodborne Pathogen Standard (29 CFR 1910.1030), requires medical surveillance and annual Bloodborne Pathogens Training. University of Oregon has a written Bloodborne Pathogen Exposure Control Plan, available on the EHS website.

## 6.1 Vaccinations

Personnel working with human blood or OPIM must be offered the Hepatitis B vaccination at no cost to them. Whether or not the employee wishes to be vaccinated, a Hepatitis B Immunization/Declination Form must be completed. A copy of the form will be maintained with EHS. Personnel choosing to receive the vaccine will receive instructions from EHS for vaccination at the University Health Center, and the department's index # will be billed.

The tetanus vaccine is recommended for those working with animals. Individuals enrolled in the Animal Occupational Health Program should consult with their physician for obtaining tetanus boosters every ten years, or as recommended.

Personnel who work with human pathogens should be offered the choice of receiving a vaccine, if available, and informed of the risks associated with the vaccine.

## 6.2 Preparing for potential exposures

Before beginning work with human pathogens, human blood or OPIM, all applicable safety information for a specific pathogen must be reviewed. If available, safety information will be sent to the PI with the IBC authorization form when the research project is approved. Familiarity with exposure routes, symptoms and treatment methods will provide better preparation for the possibility of exposure to the human pathogen, human blood or OPIM being used. If exposure to a human pathogen, human blood or OPIM occurs or is suspected to have occurred while at work, the appropriate medical treatment should be sought immediately.





## VII. Standard Microbiological Practices

### 7.1 Engineering Controls

Engineering controls are the preferred line of defense for protecting the worker and the environment from biohazards. Engineering controls include elimination of the hazard; facility design (e.g., directional airflow, self-closing doors, hands-free sinks); and safety equipment (e.g., biosafety cabinets) and enclosed containers, which are designed to remove or minimize exposures to hazardous biological materials. The biological safety cabinet (BSC) is the primary device used to provide containment of infectious droplets or aerosols generated by many microbiological procedures. Three types of BSCs (Class I, II, III) used in microbiological laboratories are described and illustrated in BMBL's Appendix A, "Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets". More information on BSCs is provided in the following section of this manual.

### 7.2 Work Practices

Safe lab practice is critical to preventing exposure when working with rsNA and infectious materials. The best laboratory and safety equipment in the world cannot provide protection without good work practices and adequate training. The Principal Investigator should establish a laboratory culture emphasizing safe and responsible work practices.

### 7.3 Training and Education

Anyone planning to work with rsNA and biohazardous materials must be adequately trained before beginning the work. Supervisors are responsible for ensuring that all personnel receive proper training. Annual refresher training is recommended to ensure continued safety. Information communicated in the training should include:

- a discussion of this manual and how it applies to activities conducted in specific work areas
- an explanation of the health hazards, signs and symptoms of exposure to rsNA and biohazardous materials used in specific work areas
- a description of actions personnel can take to protect themselves from exposure, such as special work practices, use of safety equipment, vaccinations, emergency procedures, etc.
- procedures to follow in case of an exposure or spill, including incident reporting and reporting requirement to outside entities (DEQ, OSHA, NIH for example)

Documentation of training sessions and/or competency should be maintained by the supervisor and individual receiving training.

### 7.4 Signs and Labeling

Anyone entering areas where rsNA and infectious materials are used must be aware of the potential hazards. Biohazard signs should be posted on doors to rooms where microorganisms, rsNA or biological toxins known to cause disease in humans are used, such



as microorganisms classified as Biosafety Level 2 or greater. Red or orange biohazard labels should be placed on containers and storage units (refrigerators, freezers, incubators, waste containers, etc.) that are used for microorganisms, rsNA or biological toxins causing disease in humans. Contaminated equipment must be labeled as well. Yellow animal biohazard signs should be posted where strict animal pathogens are used. Door signs may be requested from EHS.

## 7.5 Laboratory Practices and Techniques

Workplace-acquired infections do occur, and they are preventable. Information about the organism(s) should be gathered prior to commencing work with them. Good starting points for safety information about human pathogens are the Public Health Agency of Canada's Pathogen Safety Data Sheets and the Agent Summary Statements listed in the *BMBL*.

Infectious agents are transmitted through one or more of these routes of exposure:

- *Contact*: mucous membrane exposure (including the eyes, inside of the mouth and nose, and the genitals) or through broken skin (such as chapped or damaged skin through eczema, acne, etc.)
- *Injection*: via a puncture or cut with sharps (needlesticks, cuts with contaminated broken glass, etc.), also known as percutaneous exposure
- *Inhalation*: breathing in aerosols (microscopic solid or liquid particles small enough to remain dispersed and suspended in air for long periods; about 5 micrometers or less in diameter)
- *Ingestion*: by eating or drinking the contaminant

Using work practices that block routes of exposure can prevent workplace infection. Good microbiological techniques must always be used in the laboratory, as well as these standard practices:

- Eating, drinking, smoking, applying cosmetics or storing food in laboratories is strictly prohibited. Potentially contaminated hands should be kept away from the mouth, eyes, and non-intact skin.
- Hands should be washed frequently, even after wearing gloves, and scrubbed vigorously with soap and water for a full 30 seconds (as long as it takes to sing "Happy Birthday" or the "Oregon Fight Song"). The physical removal of organisms from the skin is just as important as using a disinfectant.
- Work surfaces and equipment must be decontaminated immediately after using rsNA or biohazardous materials, and routinely disinfecting items such as telephones and radio dials that may be handled by non-gloved hands.
- Wearing appropriate personal protective equipment (PPE) blocks potential routes of exposure.
- Keeping personal items such as cell phones and headphones out of lab areas.

More specific suggestions for common laboratory procedures used with rsNA or infectious materials follow. Each prevents rsNA or infectious materials from entering the body through



common exposure routes.

***Pipetting:***

The greatest risks with pipetting are the creation of aerosols and splashing. Mouth pipetting is prohibited; mechanical pipetting aids should be used instead. All rsNA or infectious materials should be pipetted in a biosafety cabinet. Cotton-plugged pipets should be used. Infectious or rsNA materials must never be forcibly discharged from pipets. “To deliver” pipets should be used instead of pipets requiring blowout. Disposable plastic pipettes are preferred, however if reusable pipettes are used, they should be placed horizontally in a pan filled with enough liquid disinfectant to completely cover them. The entire pan should be autoclaved before cleaning the pipets for reuse.

***Sharps:***

The greatest risks when using sharps are accidental injection. Needles and syringes should only be used when there is no alternative. Safety needles and syringes must be used when available and feasible. The sharp should be kept away from the fingers as much as possible. Sharps should never be bent, sheared, recapped, nor have needles removed from syringes after use. If a contaminated needle must be recapped or removed from the syringe, use a mechanical device, such as forceps, or recap using a one-handed scoop technique.

Sharps use also creates aerosols. Air bubbles should be minimized when filling a syringe. A pad moistened with disinfectant should be placed over the tip of the needle when expelling air. Work should be performed in a biosafety cabinet whenever possible. An appropriate sharps container must be kept close to the work area to avoid walking around with contaminated sharps. Care should be taken not to overfill sharps containers: they are considered full when they are 3/4 filled.

***Vortexing, Blending, Grinding, Sonicating, Lyophilizing:***

The greatest risk when using any of these devices is the creation of aerosols. This equipment should be operated in a biosafety cabinet whenever possible. Safety blenders should be used. Safety blenders are designed to prevent leakage from the bottom of the blender jar and to withstand sterilization by autoclaving. They also provide a cooling jacket to avoid biological inactivation. Avoid glass blender jars; if a glass jar must be used, it must be covered with a polypropylene jar to contain the glass in case of breakage.

A towel moistened with disinfectant must be placed over the top of the blender while operating. This practice can be adapted to grinders and sonicators as well. Aerosols must be allowed to settle for five minutes before opening the blender jar (or grinder or sonicator container). Lyophilizer vacuum pump exhaust should be filtered through HEPA filters or vented into a biosafety cabinet. Polypropylene tubes should be used in place of glass ampoules for storing biohazardous material in liquid nitrogen. Ampoules can explode, causing eye injuries and exposure to the biohazardous material.

## 7.6 Personal Protective Equipment

Appropriate PPE is chosen by considering the potential routes of exposure that need to be blocked to prevent exposure and infection. It is essential that PPE be removed before leaving



the laboratory or animal room. PPE must never be taken home. It should be disposed of or laundered in the work area where it is used.

***Lab Coats and Closed Shoes:***

Lab coats, scrub suits, gowns, and closed shoes prevent hazardous materials from reaching skin, and more importantly, any cuts, dermatitis, etc. that may be present. They also protect street clothing from needing decontamination, as well as preventing contamination of laboratory cultures from the normal flora present on the skin. At minimum, a long-sleeved lab coat and closed-toe shoes must be worn in any microbiology laboratory. Long sleeves minimize contamination of skin and street clothes and reduce shedding of microorganisms from the skin. Closed shoes protect the feet from spills and injuries from dropped sharps.

Elastic-cuffed labcoats help prevent spills caused by catching the cuff on laboratory equipment. When working with rsNA or infectious materials inside a biosafety cabinet, elastic cuffs prevent contaminated air from being blown up the lab coat sleeve into the breathing zone. Lab coats must remain in the laboratory when personnel go home or to non-laboratory work areas. This keeps any contamination in the laboratory instead of spreading it to other work areas or homes. Lab coats must not be taken home to be laundered. Lab coat laundering is available through EHS; costs are paid by the lab's department.

***Gloves:***

Gloves prevent exposure of the skin, and any cuts, dermatitis, etc. that may be present, to infectious materials. Nitrile gloves will prevent exposure to microorganisms. However, gloves must be compatible with the chemicals being handled, as well as offering protection from rsNA or infectious materials. EHS can provide assistance with choosing appropriate gloves. For the best protection, the cuffs of the gloves should overlap the cuffs of the laboratory coat. Disposable gloves must not be reused. They are designed for disposal after one use or if exposed to a chemical (they offer limited chemical protection). Utility gloves, such as rubber dishwashing gloves may be disinfected for re-use if they do not show signs of wear or degradation. EHS can aid finding an alternative for those allergic to gloves (most common with latex) and/or the powder they contain.

***Eye and Face Protection:***

Eye and face protection prevent splashes into the eyes, nose and mouth (mucous membrane exposure), and onto the skin. Goggles or safety glasses should be worn to protect the eyes. Full-face shields should be worn to protect facial skin, particularly when handling liquid nitrogen. Face masks protect against splashes, but do not prevent inhalation of aerosols. Face masks are also useful in preventing lab exposures through splashes or accidental touching of the face.

***Respirators:***

Respirators prevent the inhalation of aerosolized microorganisms (inhalation exposure) when safety equipment designed to contain infectious aerosols, such as a biosafety cabinet, is not available. EHS can aid in determining the appropriate respirator needed. Respirator training and fit-testing is required for certain respirators; the Respiratory Protection Program provides details. Respiratory protection as an alternative to engineering controls is not ideal and should



only be implemented under the guidance of EHS.

## 7.7 Laboratory Safety Surveys

The UO Biosafety Checklist, used for surveys completed by EHS, includes criteria for work with infectious agents and for work with rsNA. Periodic self-auditing using the criteria in Appendix G of the *NIH Guidelines* and Section IV of *BMBL* will help ensure that good laboratory safety practices are being used.

## 7.8 Animal Handling

The spread of infectious agents between laboratory animal populations can be prevented and laboratory personnel can be protected from zoonotic agents by adhering to the following basic guidelines, required by Animal Care Services wherever animals are housed or used on campus. Safe practices that apply to all animal areas, regardless of biosafety level, include:

- Shoe covers must be worn, when specified, upon entering an animal room.
- All animal room doors must remain closed at all times, except for entering and exiting.
- Disposable gloves must be worn when handling animals, bedding or soiled cages.
- Disposable or washable outer garments (such as lab coats, gowns, coveralls) must be worn to protect personal clothing from contamination.
- Eating, drinking smoking, applying cosmetics and handling contact lenses in animal rooms or procedure rooms is prohibited.
- Hand contact with the nose, eyes, or mouth is strongly discouraged when working with animals.
- Hands must be washed with soap and water immediately after handling any animals or animal equipment, and before leaving the animal facility or laboratory.
- Extra caution must be taken with needles or other sharp equipment used with animals. Needles shall remain capped until ready to use, and then promptly and properly disposed.
- Handle only those species for which proper handling training has been provided can prevent injury.
- Any bites or other wounds must be washed immediately with soap and water and appropriate medical attention sought.
- Unauthorized persons are prohibited from entering animal rooms. Additional requirements may be specified for certain research studies.

Fieldwork involving wild animals requires adapting the basic animal infection control guidelines to the particular situation in the field. One of the major concerns with fieldwork is exposure to wild populations that might carry one or more zoonotic diseases. Personnel working in areas where they are likely to be exposed to wild rodents or their nesting areas must consult with EHS on occupational health and safety considerations.

## 7.9 Cell and Tissue Culture

Cell cultures may contain viruses, and no cell culture line can be definitively determined to be free of any and all potential pathogens. It is prudent to consider all cell lines to be



potentially infectious. Most cell cultures can be safely manipulated using BL2 practices and containment. If cells are known or suspected to contain a specific pathogen or oncogenic virus, appropriate biosafety practices for handling that virus must be used when working with the cell culture. All other primary and permanent cell lines must be handled using BL2 practices and containment.

## VIII. Biosafety Equipment

### 8.1 Biological Safety Cabinets (BSCs)

The BSC is designed to provide protection to the product, the user, and the environment when appropriate practices and procedures are followed. Three types of BSCs (Class I, II, III) and the horizontal laminar flow cabinet are described below. The common element to all classes of BSCs is the high efficiency particulate air (HEPA) filter. This filter removes particles of 0.3 microns with an efficiency of 99.97%. However, it does not remove vapors or gases; this is why BSCs should never be used for work with volatile chemicals or flammable gases.

The BSC requires regular maintenance and certification by an NSF-accredited technician to ensure that it protects you, your experiments, and the environment. Each cabinet must be certified after installation, each time it is moved or repaired, and at least annually. If a BSC needs to be relocated, it must first be decontaminated by our contractor. Once the BSC has been moved, it must be recertified before use and annually thereafter. Additionally, repairs must only be made by contracted technicians. EHS administers this program for the University. Contact the BSO to confirm that your cabinet is included in this program.

### 8.2 Types of Biosafety Cabinets

<b>BSC Type</b>	<b>Features</b>
Class I Biosafety Cabinets	Protect personnel and the environment, but not research materials. They provide an inward flow of unfiltered air, similar to a chemical fume hood, which protects the worker from the material in the cabinet. The environment is protected by HEPA filtration of the exhaust air before it is discharged into the laboratory or ducted outside via the building exhaust.
Class II Biosafety Cabinets (Types A1, A2, B1, B2)	Provide personnel, environment, and product protection. Air is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air within the cabinet provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air passes through the exhaust HEPA filter, it is contaminant-free (environmental protection), and may be recirculated back into the laboratory (Type A) or ducted out of the building (Type B).



Class III Biosafety Cabinets (sometimes called Class III glove boxes)	These were designed for work with infectious agents that require BSL4 containment, and provide maximum protection to the environment and the worker. The cabinet is gas-tight with a non-opening view window, and has rubber gloves attached to ports in the cabinet that allow for manipulation of materials in the cabinet. Air is filtered through one HEPA filter as it enters the cabinet, and through two HEPA filters before it is exhausted to the outdoors. This type of cabinet provides the highest level of product, environmental, and personnel protection.
Laminar flow "clean air benches"	These are not BSCs. They discharge HEPA-filtered air across the work surface and toward the user's face, providing only product protection. They can be used for certain clean activities, such as dust-free assembly of sterile equipment or electronic devices. However, they should never be used when handling cell culture materials or potentially infectious materials, or as a substitute for a BSC in research laboratories.

### 8.3 Operation of Class II BSCs

Step	Procedure
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1. Turn on cabinet fan 15 minutes before beginning work. Put on appropriate PPE: gloves at a minimum, and preferably a lab coat and safety glasses as well.
2. Disinfect the cabinet work surface with 70% ethanol or other disinfectant.
3. Place supplies in the cabinet. Locate container inside the cabinet for disposal of pipettes and other waste. (Movement of hands in and out of the cabinet to discard items into an outside container disrupts the air barrier that maintains sterility inside the cabinet.) Work as far to the back (beyond the air split) of the BSC work space as possible. Always use mechanical pipetting aids. Do not work in a BSC while a warning light or alarm is signaling.
4. Locate liquid waste traps inside cabinet and use a hydrophobic filter to protect the vacuum line. If traps must be located on the floor, place them in a secondary container (such as a plastic bin) to prevent spilling.
5. Keep the work area of the BSC free of unnecessary equipment or supplies. Clutter inside the BSC may affect proper air flow and the level of protection provided. Also, keep the front and rear grilles clear.
6. While working in a BSC, use slow, deliberate motions and minimize entering and exiting the cabinet. All waste and/or disinfecting containers must be kept inside the cabinet while they are being used.
7. When work is completed, remove equipment and supplies from the cabinet. Wipe



the work area with 70% ethanol and allow cabinet to run for 15 minutes.

Additionally, the following guidelines should be followed with regard to proper BSC use:

- Some BSCs are equipped with ultraviolet (UV) lights. However, if good procedures are followed, UV lights are not needed. In fact, UV disinfection is discouraged because it is often impeded by shadows, dust, and inadequate intensity. UV radiation should never take the place of a disinfectant for disinfection of the cabinet interior. The UV lamp should never be on while an operator is working in the cabinet.
- Minimize traffic around the BSC and avoid drafts from doors and air conditioning.
- Do not put your head inside the BSC. This compromises the sterility of the environment and, more importantly, could expose you to infectious pathogens.
- Do not tamper with the BSC or interfere with its designed function. It was engineered to operate optimally with no obstructions around the sash or grilles.
- Open flames are not necessary in a BSC. On an open bench, flaming the neck of a culture vessel will create an upward air current that prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence, which disrupts the pattern of HEPA-filtered air supplied to the work surface. Therefore, the **use of open flames and gas burners is strongly discouraged in biosafety cabinets**. When deemed absolutely necessary, touch-plate micro-burners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops can also be used.

## 8.4 Vacuum Lines

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

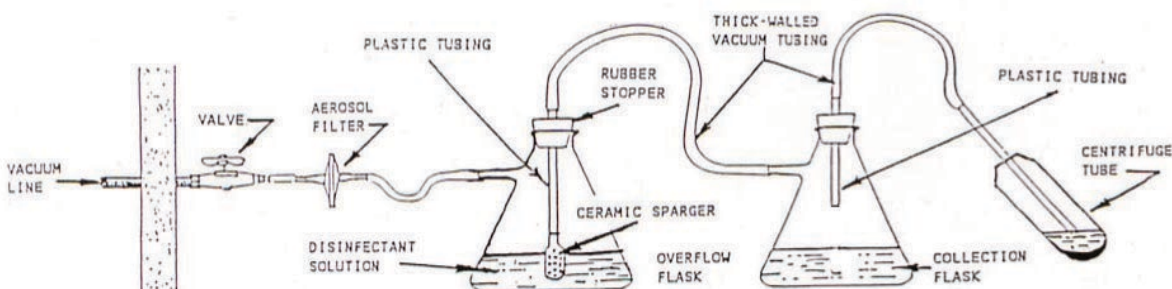
### ***Collection and Overflow Flasks***

- Collection tubes should extend at least 2 inches below the sidearm of the flask.
- Locate the collection flask inside the biosafety cabinet instead of on the floor, so the liquid level can be seen easily and the flask emptied before it overflows. The second flask (overflow) may be located outside the cabinet.
- If a glass flask is used at floor level, place it in a sturdy cardboard box or plastic container to prevent breakage by accidental kicking.
- In BSL2 laboratories, the use of Nalgene flasks is recommended to reduce the risk of breakage.



### ***Vacuum Line Filter***

A hydrophobic filter will prevent fluid and aerosol contamination of central vacuum systems or vacuum pumps. The filter will also prevent microorganisms from being exhausted by a vacuum pump into the environment. Hydrophobic filters such as the Vacushield are available from several scientific supply companies (Fisher Scientific, catalog #NC9032958, and VWR, catalog #55095-006).



## 8.5 Centrifuges

The greatest risk with centrifuging is the creation of aerosols. Centrifuge safety buckets and sealed rotors protect against release of aerosols. To avoid spills from broken tubes, the tubes, O-rings and buckets should be inspected for damage before each use. Never overfill centrifuge tubes since leakage may occur when tubes are filled to capacity. Fill centrifuge tubes no more than 3/4 full. The outside of the tubes should be wiped with disinfectant after they are filled and sealed. Rotors and centrifuge tubes should be opened inside a biosafety cabinet. If a BSC is not available, a minimum of 10 minutes' settling time should be allowed before opening.

## 8.6 Autoclaves

Autoclaves are classified as pressure vessels and must be inspected at least annually according to Oregon Administrative Code, Section 875, Chapter 209. Repairs to most autoclaves on campus are done by Campus Planning & Facilities Management. Because an autoclave uses saturated steam under high pressure to achieve sterilizing temperatures, proper use is important to ensure operator safety. Injuries can be prevented when using the autoclave by observing the following rules:

- Heat-resistant gloves, eye protection and a lab coat must be worn, especially when unloading the autoclave.
- Steam burns and shattered glassware can be prevented by ensuring that the pressure in the autoclave chamber is near zero before opening the door at the end of a cycle. The autoclave door should be cracked open slowly to allow the steam to escape gradually.
- Items must be allowed to cool for 10 minutes before removing them from the autoclave.



- Sealed containers must never be put in an autoclave. They can explode. Large bottles with narrow necks may also explode if filled too full of liquid.
- Solvents, volatile or corrosive chemicals (such as phenol, chloroform, bleach, etc.), and radioactive materials must never be placed in an autoclave. EHS can provide assistance with any questions about proper disposal of these materials.
- Autoclave components must be inspected regularly. In particular, cleaning the drain screen frequently will help to prevent operation problems and down time. If a problem is discovered, repair must be initiated. An autoclave should never be operated until it has been properly repaired.

Due to infectious waste management requirements under the Oregon Health Authority, autoclaves at UO cannot be used to decontaminate infectious or biohazardous materials prior to disposal. These materials **MUST** be incinerated. Contact EHS to obtain incineration boxes and biohazard bags.

## IX. Disinfection

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

The UO [Hazardous Materials Guide](#) specifies proper procedures for treatment and disposal of rsNA or infectious waste, according to applicable federal, state, local and university policies. The guidelines may be copied and posted near waste handling areas in the laboratory for quick reference.

### 9.1 Chemical Disinfectants

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

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



## 9.2 Disinfectants Commonly Used in the Laboratory

### ***Hypochlorites (bleach)***

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

### ***Iodophors (iodine, Wescodyne)***

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

### ***Alcohols (ethanol, isopropanol)***

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

## 9.3 Dilution of Disinfectants

### *Chlorine compounds (Household Bleach)*

<b>Dilution in Water</b>	<b>% Available Chlorine</b>	<b>Available Chlorine (mg/l or ppm)</b>
Undiluted	5.25	50,000
1:10	0.5	5,000
1:100	0.05	500

Bleach solutions decompose at room temperature and should be made fresh **daily**. However, if stored in tightly closed brown bottles, bleach solutions retain activity for 30 days. The use concentration is dependent on the organic load of the material to be decontaminated. Use a 1% solution to disinfect clean surfaces, and 10% solution to disinfect surfaces contaminated



with a heavy organic load. To disinfect liquid biological waste before disposal, add concentrated bleach to a final concentration of 1%. Bleach use on stainless steel surfaces should be followed by a rinse and wipe using 70% ethanol to remove corrosive residues. Shelf-stable pre-diluted bleach solutions may be preferable for ease of use and storage. Bleach-Rite is one available product.

#### *Iodophors*

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

#### *Alcohols*

Ethyl alcohol and isopropyl alcohol diluted to 70 - 85% in water are useful for surface disinfection of materials that may be corroded by a halogen or other chemical disinfectant. Surprisingly, stronger solutions of ethanol (90%) are *less* effective.

## **X. Biological Waste Disposal Procedures**

Lab staff must request pickup of hazardous wastes through EHS. The University of Oregon is unique in that the wastes generated are of an extremely varied nature. Researchers, instructors, and support services generate much of the University's hazardous waste. Everyone at the University who generates hazardous waste has the responsibility to ensure the success of the Hazardous Waste Management Program. The priorities of the program include:

- Reducing the quantity of hazardous waste generated
- Managing hazardous waste in a manner which protects the health and safety of students, staff, and faculty at the University, as well as the surrounding community
- Managing hazardous waste using the most environmentally sound and responsible methods practical
- Lowering the potential for a release of hazardous waste into the environment
- Complying with governmental regulations regarding hazardous waste management

### 10.1 Biological Waste

Decontamination prior to disposal is required for materials that are pathogenic or recombinant. If there is a question as to if a material is biohazardous, please contact EHS for clarification. These materials must be directly disposed into double-bagged incineration boxes obtained from EHS. When biohazard containers are almost full, submit an online waste pickup request. Alternatively, the groups may collect these materials in sturdy trash bins labeled with the biohazard symbol, and lined with bags printed with a biohazard symbol. These bags are picked up by EHS.

### 10.2 Multi-hazard or Mixed Waste

Mixed waste is defined as waste containing more than one hazard. Avoid generating mixed waste if possible, and keep volume to minimum. Do not autoclave mixed waste.



When discarding waste containing an infectious agent and radioactive material, inactivate the infectious agent first, then dispose as radioactive waste. Seek advice from the RSO before beginning inactivation procedures.

When discarding waste containing an infectious agent and a hazardous chemical, dispose as chemical waste. Contact EHS for advice.

### 10.3 Animal Tissues, Carcasses and Bedding

Contain animal carcasses and tissues in double bags, using biohazard bags if animals were infected with pathogenic or recombinant materials. Store the bags in freezers or refrigerators designated for this purpose within the laboratory. Request pickup through EHS by submitting an online request. Bedding from infected animals should be disposed of as “Biohazard” as described above.

### 10.4 Autoclaving rsNA Waste

Autoclaves are to be used at **UO only for destroying non-pathogenic recombinant materials, NOT biohazardous materials**. If you are unsure how your waste should be decontaminated, please contact the UO Biosafety Officer.

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

#### ***Container Selection***

Polypropylene bags, commonly called biohazard or autoclave bags, are able to withstand autoclaving and are tear resistant, but can be punctured or burst during autoclaving. Therefore, **place bags in a rigid container such as a polypropylene or stainless-steel pan during autoclaving**. Bags are available in a variety of sizes, and some are printed with an indicator that changes color when processed. Polypropylene bags are impermeable to steam, and for this reason should not be twisted and taped shut, but gathered loosely at the top and secured with a large rubber band or autoclave tape. This will create an opening through which steam can penetrate.

Always place bags into bins before loading into the autoclave. Polypropylene containers and pans are a plastic capable of withstanding autoclaving, but are resistant to heat transfer. Therefore, materials contained in a polypropylene pan will take longer to autoclave than the same materials in a stainless-steel pan. To decrease the time required to sterilize material in these containers,

- remove the lid (if applicable)
- turn the container on its side when possible



- select a container with the lowest sides and widest diameter possible for the autoclave.

Stainless steel containers and pans are an alternative to polypropylene bins. Stainless steel is an efficient conductor of heat and is less likely to increase sterilizing time, though it is more expensive than polypropylene.

#### ***Preparation and Loading of Materials***

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

#### ***Cycle Selection***

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

#### ***Time Selection***

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

#### ***Removing the Load***

- Check that the chamber pressure is zero.
- Wear lab coat, eye protection, heat insulating gloves, and closed-toe shoes.
- Stand behind door when opening it.
- Slowly open door only a crack. Beware of rush of steam.
- After the slow exhaust cycle, open autoclave door and allow liquids to cool for 20 minutes before removing.
- All autoclaved bags of waste must be put into plain opaque household trash bags before being put into the dumpster.

#### ***Monitoring***

Autoclaves used to decontaminate laboratory waste should be tested periodically to assure

effectiveness. Two types of tests are used: 1) a chemical indicator that fuses when the temperature reaches 121°C, and 2) heat-resistant spores (*Bacillus stearothermophilus*) that are killed by exposure to 121°C for approximately 15 minutes. Both types of tests should be placed well down in the center of the bag or container of waste, at the densest point.

The chemical test should be used first to determine that the temperature in the center of the container reaches 121°C. Ampules of heat-resistant spores should be used in subsequent test runs to determine the length of time necessary to achieve sterilization.

### ***Autoclaving Reusable Labware***

Items such as culture flasks and centrifuge bottles are decontaminated by lab personnel before washing by either 1) autoclaving items in an autoclavable container, or 2) chemically disinfecting items by soaking in diluted disinfectant for one hour before washing.

## 10.5 Sharps

Sharps include all materials capable of puncturing the skin. Examples include needles, razor blades, scalpels, glass (Pasteur pipettes, slides and coverslips, etc). Sharps are a leading cause of lab injuries and should be eliminated from lab procedures whenever possible. When this is not feasible, consider replacements: using plastic materials instead of glass and using safer sharps devices. To prevent needlestick injuries:

- Eliminate the use of sharps in your work, or replace glass with plastic.
- Do not bend, break, or otherwise manipulate needles by hand.
- Do not recap needles by hand.
- When needles must be removed from syringes, do not remove them by hand; use hemostats.
- Use care and caution when cleaning up after procedures that require the use of syringes and needles.
- Use extra care when two persons are working together. Locate sharps container between the workers when possible.

### ***Recapping***

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



**All Other Sharps**

The State of Oregon defines “sharps” to include the following:

- Needles
- IV tubing with needles attached
- Scalpel blades (including razor blades)
- Lancets
- Glass tubes that could be broken during handling (e.g., capillary tubes, thin-walled test tubes, Pasteur pipettes)
- Syringes that have been removed from their original sterile containers

Additionally, a “syringe” is an instrument that consists of a hollow barrel fitted with a plunger and a hollow needle.

UO requires that all sharps be collected in one of the following containers:

1. *In areas used for patient care, Animal and Biosafety Level 2 laboratories, or otherwise used with human or non-human primate source materials, a standard sharps container containing a biohazard symbol, as depicted below.*



2. *In all other areas, such as Animal and Biosafety Level 1 laboratories, chemistry laboratories, arts/theater, and facility maintenance use, the sharps containers depicted above are optional. Alternatively, groups may collect sharps in an EHS-approved leakproof, rigid, puncture-resistant container that can be securely closed once filled. The container must clearly be marked “SHARPS” and conflicting information removed or marked through. Examples include plastic laundry detergent bottles, coffee cans, or containers intended for non-hazardous sharps (below)*





When containers are  $\frac{3}{4}$  filled, close the lid securely and request pickup from EHS. **ALL** sharps must be picked up by EHS for incineration, with the only exceptions being groups contracting directly with a waste disposal vendor for incineration. Do not place sharps in the dumpster.

Syringes without attached needles may be disposed of in the biohazard (red bag) waste stream. However, removing needles from syringes for the express purpose of preferred waste disposal is prohibited.

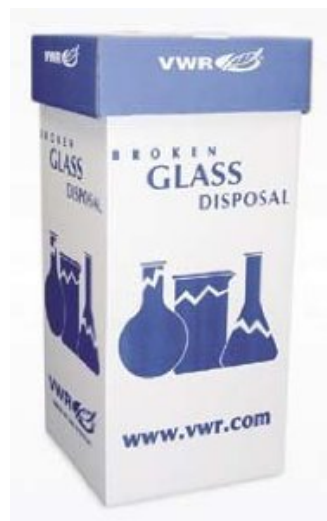
## 10.6 Glass

Laboratory glassware (e.g., beakers, Erlenmeyer flasks) and broken glass that is not contaminated with hazardous materials must be disposed of in standard “Broken Glass” boxes. These boxes may be obtained from Science Stores or scientific supply companies. The boxes should be securely taped at the bottom to prevent heavy contents from breaking through, and the plastic bag liner must be in place. Use of homemade cardboard boxes or other containers is only permitted with express approval from EHS.

Glass contaminated with infectious or recombinant materials may be decontaminated with appropriate disinfectant and then disposed in the “broken glass” box IF the glass can be safely handled. Alternatively, it may be disposed of in the sharps container.

If glassware is contaminated with chemical or radiological material, please consult with EHS for disposal options.

When the box is filled, please tape it shut securely and dispose into the nearest dumpster. Unbroken, empty reagent containers are not considered laboratory glassware, and may be disposed directly to garbage dumpsters.



## XI. Lentiviral Vectors

The use of lentiviral vectors has been increasing because the vector system has attractive features; however, they are not risk-free. It is important to remember that **replication-competent does not mean non-infectious**.

The major risks to be considered for research with HIV-1 based lentivirus vectors are the potential for generation of replication-competent lentivirus (RCL), and the potential for oncogenesis via random chromosomal integration.

The nature of the transgene must also be considered in assessing risk. These risks can be



mitigated by the nature of the vector system (and its safety features) or exacerbated by the nature of the transgene insert encoded by the vector (e.g., expression of a known oncogene with a constitutive strong promoter may require heightened safety precautions).

The potential for generation of replication-competent lentivirus from HIV-1 based lentiviral vectors depends upon several parameters, the most important of which are the number of recombination events necessary to reassemble a replication competent virus genome and the number of essential genes that have been deleted from the vector/packaging system. On this basis, later generation lentiviral vector systems are likely to provide a greater margin of personal and public safety than earlier vectors, because they use a heterologous coat protein (e.g., VSV-G) in place of the native HIV-1 envelope protein, thus reducing the risk of RCL generation. (It should be noted, however, that pseudo typing with coat proteins such as VSV-G may broaden the host cell and tissue tropism of lentiviral vectors, which will be considered in the overall safety assessment by the IBC). Later generation vector systems also separate vector and packaging functions onto three or four plasmids and they include additional safety features such as the deletion of Tat, which is essential for replication of wild-type HIV-1, and altered 3' LTR that renders the vector "self-inactivating." In contrast, earlier vector systems (such as two-plasmid vector systems) may have a higher potential for becoming replication-competent.

The most probable route of exposure for this work would be percutaneous via sharps (needlesticks), absorption through exposed scratches or abrasions on skin, or mucous membrane exposure of the eyes, nose, and mouth. Another route would be inhalation of aerosols depending on the use of equipment such as centrifuges or vortex mixers. Care must be taken when pipetting in order to avoid splashing or generation of aerosols. There may be increased risk potential for HIV positive individuals whose native virus may recombine with the recombinant virus. Such individuals are encouraged to discuss this with their physician. Immunocompromised individuals should not work with lentivirus.

You will need to provide a comprehensive risk assessment considering the nature of the vector system, transgene insert, vector propagation, and if applicable, animal hosts and manipulations, as part of your IBC registration. For many experiments, it is appropriate to use either BSL-2 or enhanced BSL-2 containment and practices with elimination/restrictions on sharps use.

## **XII. Transporting Infectious & rsNA Materials**

### 12.1 General Information

You must attend a training class before you package infectious substances (human or animal pathogens) for transport by commercial carrier. The U.S. Department of Transportation (DOT) and the International Air Transport Association (IATA) regulate shipment of human and animal pathogens. The regulations are complex and exacting. They require that researchers who prepare infectious materials for shipment receive training every two years. In addition, packages must be marked and labeled exactly as the regulations specify, and packaging materials must have been tested and certified to withstand certain durability and pressure



tests. Cardboard boxes in which supplies have been received cannot be used to ship infectious materials. Recent events have led to greater scrutiny for compliance with these regulations.

## 12.2 Permits

Permits are required from the Centers for Disease Control and Prevention (CDC) to **import or transport** 1) any microorganism that causes disease in humans; 2) biological materials, such as blood and tissues, when known or suspected to contain an infectious agent; 3) live insects, such as mosquitoes, known or suspected of being infected with any disease transmissible to humans; and 4) any animal known or suspected of being infected with any disease transmissible to humans. Importation permits are issued only to the importer, who must be located in the U.S. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the U.S. Public Health Service Division of Quarantine and release by U.S. Customs. Transfers of previously imported material within the U.S. also require a permit. Application for the permit should be made at least ten working days in advance of the anticipated shipment date. Further information and application forms may be obtained by calling the CDC at (404) 639-3235, or through the CDC web site at <http://www.cdc.gov/od/eaipp/>.

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

Permits are also required from the USDA/APHIS for **interstate movement, importation, or release into the environment (i.e., field tests)** of genetically engineered organisms that are **plant pests**, or that contain portions (plasmids, DNA fragments, etc.) of **plant pests**. Application should be made at least 120 days in advance of the anticipated release or shipment date. Information and application forms may be obtained through the [APHIS web site](#).

A validated license is required by the Department of Commerce for **export** of certain microorganisms and toxins (listed in [15 CFR Part 774](#)) to all destinations except Canada. Information may be obtained by calling (202) 482-0896.

## 12.3 Packaging

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



- **Infectious Substance:** a viable microorganism, or its toxin, which causes or may cause disease in humans.
- **Diagnostic Specimen:** any human or animal material including blood, tissue, and tissue fluids, shipped for the purpose of diagnosis.
- **Biological Product:** a product for human or veterinary use, such as vaccines and investigational new drugs.

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

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

## 12.4 Genetically Modified Microorganisms

The *International Air Transport Association's Dangerous Goods Regulations* outlines requirements for shipment of GMOs. Genetically-modified Category A agents must be shipped as Category A. Genetically-modified Category B agents must be shipped as Category B. Non-pathogenic GMOs are exempt from the shipping regulations.

## 12.5 Human Clinical Materials

The OSHA Bloodborne Pathogens Standard requires that all packages containing human blood and other potentially infectious materials be labeled with the universal biohazard symbol or color-coded. Clinical samples with a reasonable likelihood of carrying pathogens fall under the IATA regulations as well. Various carriers may have additional requirements.

## 12.6 On-Campus Transport

Any infectious or rsNA materials transported between laboratories or buildings on campus should be contained, as they would be in the laboratory, to prevent release of the materials into the environment. Transport containers should be labeled with the biohazard symbol and the identity of the material inside. For example, to transport a rack of test tubes containing Risk Group 2 organisms from a laboratory in Streisinger to Klamath, the tubes should be capped and placed inside a sealed, puncture-resistant, unbreakable secondary container with a biohazard label. The secondary container must remain intact in the event it is dropped.

## 12.7 Select Agent Human Pathogens and Biological Toxins

The Department of Health and Human Services rule "Additional Requirements for Facilities Transferring or Receiving Select Agents," which expanded the regulations that were already in existence, went into effect in 1997. Facilities sending out or receiving certain designated Select Agents, such as certain specified viruses, bacteria, rickettsia, fungi and biological toxins, are now required to apply for and receive a Site Registration Number from the CDC



before any shipments occur. Substantial criminal penalties apply to both individuals and organizations that do not comply with the regulation requirements. The University of Oregon currently does not hold, use or transfer threshold quantities of Select Agent Toxins. Any Principal Investigator anticipating use of select agents or greater-than-permissible amounts of select toxins must contact EHS *immediately* for initiation of a select agent program at UO. This process is extensive and complicated, and requires at least a year of lead time prior to receipt of the material.

## 12.8 Off-Campus Transport by Non-Commercial Methods

Please refer to UO's guidance on Hazardous/Regulated Materials Transportation. Materials must be classified, packaged, and labeled the same as if they were being transported via commercial courier. UO personnel may transport infectious or rsNA materials by non-commercial routes only in university-owned vehicles. Personal vehicles may not be used due to insurance coverage limitations. Transport by non-commercial routes may only be done within the state of Oregon.

## XIII. Laboratory Security Considerations

Although most microbiology laboratories contain a variety of dangerous biological, chemical and radioactive materials, these materials have rarely been used to intentionally injure anyone. However, there is growing concern about the possible use of biological or rsNA, chemical and radioactive materials by terrorists. In response to these concerns, the CDC has included guidelines to address laboratory security issues in the current edition of *Biosafety in Microbiological and Biomedical Laboratories*. Security is most critical for laboratories using biological agents, rsNA or toxins capable of causing serious or fatal illness to humans or animals, or causing serious damage to indigenous plants. All laboratories, however, should consider the following basic points of security and how they might apply to their individual situations. All laboratory personnel are responsible for:

- controlling access to areas where infectious agents, rsNA or toxins are used and stored, and for locking refrigerators/freezers used to store agents
- knowing who is in the laboratory
- knowing what materials are brought into the laboratory
- knowing what materials are removed from the laboratory
- reporting any undocumented visitors; missing biological, rsNA, chemical or radioactive materials; unusual or threatening phone calls; and suspicious persons or packages to the laboratory supervisor, EHS, and campus police



## Appendix A - Applicable Regulations and Guidelines

The following federal and international regulations and guidelines apply to work performed with biological materials:

- NIH Guidelines for Research Involving Recombinant DNA Molecules (04/2019):  
<http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines>
- NIH Office of Science Policy: <https://osp.od.nih.gov/>
- Biosafety in Microbiological and Biomedical Laboratories (6<sup>th</sup> edition):  
<http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>
- University of Oregon Biological Research Registration Forms:  
<https://safety.uoregon.edu/institutional-biosafety-committee>
- Oregon OSHA Bloodborne Pathogen Standard:  
<http://osha.oregon.gov/Pages/topics/bloodborne-pathogens.aspx>
- USDA/APHIS Permitting Requirements:  
<https://www.aphis.usda.gov/aphis/resources/permits>
- CDC Import Permit Program: <http://www.cdc.gov/od/eaipp/>
- Federal Select Agent Program: <http://www.selectagents.gov/>
- Export Administration Regulations Commerce Control list (15 CFR Part 774):  
<http://www.ecfr.gov/cgi-bin/retrieveECFR?gp=1&SID=02b534f76f96c9c5f13ald1be8edf8d4&ty=HTML&h=L&n=15y2.1.3.4.45&r=PART>
- NSF Standard 49 for the Evaluation of Class II Laminar Flow Biological Safety Cabinets:  
[https://static1.squarespace.com/static/586e9ae1be6594236ee585ba/t/5c067f780ebbe8abbbaa86623/1543929722473/NSF\\_49-2018+Annex+E.pdf](https://static1.squarespace.com/static/586e9ae1be6594236ee585ba/t/5c067f780ebbe8abbbaa86623/1543929722473/NSF_49-2018+Annex+E.pdf)