GENERAL BIOSAFETY MANUAL
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I. INTRODUCTION

1.1 Biohazard Definition

Biohazards include infectious or etiologic (disease causing) agents of humans, animals and plants, toxins of biological origin, human-derived materials, recombinant DNA and materials potentially contaminated by them. Biohazardous agents may include but are not limited to: certain bacteria, fungi, viruses, parasites, recombinant products, allergens, cultured human or animal cells and the potentially infectious agents these cells may contain and other infectious agents as outlined in laws, regulations, or guidelines.

Recombinant DNA (rDNA) is defined as a form of DNA that does not exist naturally, which is created by combining DNA sequences that would not normally occur together. The NIH Recombinant Advisory Committee addresses concerns that genetically engineered infectious agents could compromise human health, either as an occupational hazard or as a public health risk. If the synthetic DNA segment is not expressed in vivo as a biologically active polynucleotide or polypeptide product, the OHSU IBC committee can designate it exempt from the NIH Guidelines.

1.2 Purpose

This manual provides biosafety guidelines for those working at Oregon Health & Science University (OHSU), including any work that involves the handling of infectious microorganisms, recombinant DNA, human or animal tissues or body fluids, or research animals. This manual is meant to be a reference and provide guidance for addressing biosafety issues. This document is not meant to provide all biosafety requirements for highly specialized tasks, projects or locations at OHSU. Individuals may perform tasks that require more stringent precautions than the general biosafety principles covered in this manual and will need to evaluate such procedures and develop task-, project-, location- and/or device-dependent health and safety protocols to meet those requirements. Questions concerning biosafety practices or the development of specific protocols should be directed to the Biosafety Officer (BSO) in the campus Research Safety Program (RSP) office.

1.3 Roles and Responsibilities

Ensuring biosafety is a cooperative effort between the Research Safety Program at OHSU, the Principal Investigator or Area Supervisor, and their employees.

Biosafety at OHSU is operated under the direction of the OHSU Research Integrity Office (ORIO) by the Research Safety Program and the Institutional Biosafety Committee (IBC). The IBC reviews all research involving recombinant DNA and infectious agents, including research with animals involving infectious agents or recombinant DNA. ORIO supports full-time Biosafety Officers (BSO) for consultation to the OHSU research community regarding all aspects of work with biohazardous materials, as well as assistance with response to accidents involving biohazardous materials. In addition, Biosafety Officers verify compliance with NIH biosafety guidelines.

Principal Investigators and supervisors are responsible for maintaining a safe work environment, which will ensure the health and safety of their personnel, students, research participants, visitors, the public
and the environment. They are also responsible for providing the training, PPE, and safety equipment necessary for employees to perform their tasks safely.

Individuals that work with biohazardous materials have a responsibility to follow the guidelines in this manual and to consult with their Supervisor or a Biosafety Officer if they ever have questions regarding the risks and safety of their assigned tasks.

1.4 Regulations and Guidelines

National Institute of Health (NIH): Guidelines for Research Involving Recombinant DNA Molecules. These guidelines address the safe conduct of research that involves construction and handling of recombinant DNA molecules and organisms containing them. Included in the Guidelines is a requirement for the institution to establish an Institutional Biosafety Committee (IBC) with authority to approve or disapprove proposed research using the NIH Guidelines as a minimum standard. For more information, please refer to the Biosafety in Research website: [http://oba.od.nih.gov/rdna/nih_guidelines_oba.html](http://oba.od.nih.gov/rdna/nih_guidelines_oba.html)

Centers for Disease Control and Prevention (CDC) and the National Institute of Health (NIH) Guidelines on: Biosafety in Microbiological and Biomedical Laboratories (BMBL). The BMBL describes combinations of standard and special microbiological practices, safety equipment, and facilities that constitute Biosafety and Animal Biosafety Levels 1-4 and is commonly seen as the standard for biosafety. The BMBL is accessible at: [http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.html](http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.html)

The Oregon Occupational Safety and Health Division (OR-OSHA) regulations specify the minimum requirements for exposures to human tissues/body fluids in OAR 437-002-0360 and OHSA 29 CFR 1910.1030, the Bloodborne Pathogen Standard.
2 TRAINING

All researchers at OHSU must be adequately trained to perform research in a safe manner. While the PI is ultimately responsible to ensure that researchers under his/her direction are properly trained for the specific procedures carried out in the PI’s laboratory, general training is provided online through the Big Brain portal. OHSU has set up requirements for particular training modules, corresponding to the duties and potential exposures of individual researchers.

2.1 Big Brain

All research personnel are required to complete the following Big Brain training modules: General Safety and Laboratory Safety, Integrity Education Booster, and RCR for All. Most laboratory personnel should also take RCR Involving rDNA & Infectious Agents, since almost all current biological research involves some form of recombinant DNA. Optional courses that are strongly recommended include RCR Involving Human Subjects for personnel working on clinical trials or who work with primary human tissues or cells, and RCR Involving FDA Regulated Products for personnel conducting research with experimental drugs or toxins. In addition, the Dangerous Goods Shipping course is required for anyone who wishes to ship biological materials or toxins.

In compliance with OSHA requirements, all personnel conducting research that involves human tissue or cells, non-human primates, tissue or cells, or other sources of bloodborne pathogens must complete the Big Brain course on Bloodborne Pathogens. A refresher course must be completed annually.

2.2 Lab-Specific Training

It is the responsibility of PIs to ensure that all research personnel under their direction are trained to work safely. Not only should training include safe microbiological practices in accordance with NIH guidelines, but training in the appropriate response to spills and accidents as well. This responsibility may be delegated at the discretion of the PI, but the PI may be held responsible for laboratory-acquired infections where the root cause was lack of training. Most of the elements for training are provided in this manual; however, there is no substitute for hands-on, one-on-one instruction. On the other hand, it is also the responsibility of personnel to make sure they understand procedures before attempting them.

2.3 Animal Handling

In addition to enrollment in the OHSU Occupational Health and Safety Program and prior IACUC approval, all personnel who will be working with live animals must complete training. Individuals that work with animals also must complete the Big Brain training module RCR Involving Animal Subjects.

For the Central and Waterfront campuses the Department of Comparative Medicine offers periodic training sessions in mouse and rat handling, ABSL2 training, and can provide specialized training for procedures or other handling of other animal species. Further information about services provided by DCM is available on their website: http://www.ohsu.edu/research/rda/cm/.

At the OHSU – West Campus research involving animals requires animal species specific training. Research involving small animals such as rodents requires Rodent Biosafety and Tier 1 animal handling that is
offered through the Division of Animal Research. Work with non-human primates requires a NHP Biosafety class provided by the West Campus Research Safety Program, Tier 1 NHP welfare training through the Office of Integrity, and animal handling training from DAR.
3 PRINCIPLES OF BIOSAFETY

Central to any discussion involving biosafety is the concept of containment of infectious agents to prevent contamination of the worker, nearby workers, or the environment. Containment is also utilized to prevent contamination of research samples or animals. There are three general elements of containment:

1. Laboratory practices and techniques,
2. Safety equipment,
3. Facility design.

The \textit{Biosafety in Microbiological and Biomedical Laboratories} (BMBL) is published by the United States Department of Health and Human Services, is the definitive reference on biosafety and should be read and followed by all OHSU personnel working with potentially infectious agents. This publication can be accessed on the Centers for Disease Control and Prevention (CDC) website.

\url{http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm}

3.1 Biosafety Levels

There are four levels of biosafety assigned to operations conducted in laboratories. Assignment of a Biosafety Level (BSL) to a given project is determined by criteria such as the scale of production, concentration, pathogenicity, and route(s) of transmission associated with a specific biological agent. For those operations that involve the use of animals there are also four levels of biosafety distinguished as animal biosafety levels (ABSL).

\textbf{BSL1} is appropriate for agents not known to consistently cause disease in healthy adult humans. These agents are of minimal potential hazard to laboratory personnel and the environment. Examples of agents commonly found in research labs are the \textit{E. coli} K12 strains, yeast \textit{S. cerevisiae} and \textit{S. pombe}, insect SF9 cells, and helper-free adeno-associated viral (AAV) vectors.

\textbf{BSL2} is applicable for agents that have a moderate potential hazard to cause disease in healthy adult humans and pose a moderate risk to the environment. If a worker contracts a disease related to BSL-2 agents, treatment is generally available. Culturing primary human cells requires BSL-2 practices, as does work with adenoviral, amphotrophic retroviral, and lentiviral vectors.

\textbf{BSL3} is used for agents that may be indigenous or exotic and are an aerosol transmission hazard. Diseases in this category may have serious health effects and treatment may or may not be available. Examples are: \textit{Mycobacterium tuberculosis} (TB), \textit{Coxiella burnetii}, and West Nile Virus.

\textbf{BSL4} is required for agents that are dangerous or exotic and pose a high risk of life threatening disease, are aerosol transmissible, or are related agents with unknown risk of transmission. Treatment for infections by these agents is generally not available. Examples are: Marburg virus and Ebola virus. There are no BSL4 labs at OHSU.
For guidance, very basic definitions of the different biosafety levels are given in this adapted table from BMBL 5th edition. An extensive list of agents assigned to BSL levels 1-4 is provided in the Appendix B of the NIH guidelines: http://oba.od.nih.gov/oba/rac/guidelines_02/NIH_Guidelines_Apr_02.htm.

### 3.2 Laboratory Practices and Techniques

While laboratory acquired infections are rare, studies have indicated that over 80% of laboratory infections cannot be traced back to an overt accident\(^1,2\). Most exposures and subsequent infections probably occur while performing routine procedures and techniques. Understanding how and infectious agent can be transmitted can help prevent laboratory acquired infections. The route of exposure for an infectious agent may be via one or all of these mechanisms:

- Sharps injuries (needle sticks, cuts from sharp objects, also known as parenteral exposure)
- Ingestion (commonly via the fecal-oral transmission, do not forget to wash your hands)
- Mucous membrane exposure (eyes, inside of nose and mouth, open wounds)
- Inhalation of aerosols (small solid or liquid particles of approximately 5 µm in diameter that can be suspended in air and are not affected by gravity)

Strict adherence to standard microbiological practices and techniques is essential for successful containment. Work practices should be developed to block potential routes of exposures, including:

- Selection and use of appropriate personnel protective equipment.
- Refraining from eating, drinking, chewing tobacco, applying cosmetics, or storing food in laboratory or animal areas.
- Practices such as routine hand washing at each available opportunity can be very successful in preventing contamination of more susceptible regions of the body as well as inanimate surfaces.
- Decontamination of work surfaces and equipment after using biohazardous materials.
- Reduction of aerosols.
Aerosol formation has the potential to contaminate work surfaces, exposed skin and garments, and air. Thus, aerosols can result in topical, oral, and respiratory exposures for workers. Manipulation of a biological sample has the potential for releasing a portion of the sample in microdroplet form to the air and work surfaces.

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

Aerosols Created by Common Laboratory Procedures

<table>
<thead>
<tr>
<th>Technique</th>
<th>Average Colonies Recovered from Air During Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipetting 10 ml culture into 1,000 ml broth</td>
<td>2.4</td>
</tr>
<tr>
<td>Drop of culture falling 12 in. (30 cm) onto:</td>
<td></td>
</tr>
<tr>
<td>Stainless steel</td>
<td>49.0</td>
</tr>
<tr>
<td>Painted wood</td>
<td>43.0</td>
</tr>
<tr>
<td>Hand towel wet with 5% phenol</td>
<td>4.0</td>
</tr>
<tr>
<td>Re-suspending centrifuged cells with pipette</td>
<td>4.5</td>
</tr>
<tr>
<td>Blowing out last drop from pipette</td>
<td>3.8</td>
</tr>
<tr>
<td>Shattering tube during centrifuging</td>
<td>1,183.0</td>
</tr>
<tr>
<td>Inserting hot loop into broth culture</td>
<td>8.7</td>
</tr>
<tr>
<td>Streaking agar plates</td>
<td>0.2</td>
</tr>
<tr>
<td>Withdrawing syringe and needle from vaccine bottle</td>
<td>16.0</td>
</tr>
<tr>
<td>Injecting 10 guinea pigs</td>
<td>16.0</td>
</tr>
<tr>
<td>Making dilutions with syringe and needle</td>
<td>2.3</td>
</tr>
<tr>
<td>Using syringe/needle for intranasal inoculation of mice</td>
<td>27.0</td>
</tr>
<tr>
<td>Harvesting allantoic fluid from 5 eggs</td>
<td>5.6</td>
</tr>
</tbody>
</table>

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

**Pipetting** can introduce aerosols and splashes. Micropipettors can also introduce aerosols.
- √ Mechanical pipettors should be used. No mouth pipetting.
- √ Using pipette tips with cotton plugs when transferring biohazardous material.
- √ “To deliver” pipettes should be used instead of pipettes requiring blowout.
- √ To avoid splashes the material should be dispensed such that the tip of the pipette is placed against the wall of the receiving container.

**Centrifugation** can introduce aerosols.
- √ Prevent leaks by not overfilling centrifuge tubes.
- √ Use sealed tubes, O-ring sealed rotors or safety buckets and check for damage before use.
- √ Rotors must be balanced before use.
Sharps can lead to accidental infections and can introduce aerosols.
- Safety needles and syringes must be used whenever possible.
- Sharps must never be bent, sheared, or recapped. Safety devices must not be modified.
- A sharps container must be available and used for their disposal. Do not overfill sharps containers.
- Air bubbles should be minimized when filling a syringe.

Blending, Grinding, Sonicating, Lypophilizing, and Freezing can all result in aerosol production.
- Whenever possible blenders, grinders, sonicators and similar equipment should be operated in a biosafety cabinet. Shielding should be used to minimize aerosols and splatters.
- Lypophilizer vacuum pump exhaust must be HEPA filtered or vented into a biosafety cabinet.
- Tubes placed in liquid nitrogen have the potential to explode or vent upon removal.

Open Flames can produce aerosols when used to sterilize inoculating loops. They are also a fire hazard.
- An electric incinerator with a shield should be used in place of an open flame.
- Consider using plastic disposable inoculating loops.
- Open flames can disrupt the airflow of a biosafety cabinet.

Biosafety Level 1 Minimum requirements
- The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, potential hazards, the necessary precautions to prevent exposures, exposure evaluation procedures, and applicable safety training.
- The laboratory supervisor must enforce the policies that control access to the laboratory.
- Personnel wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storage of food for human consumption is not permitted in laboratory areas.
- Mouth pipeting is prohibited; mechanical pipeting devices must be used.
- Policies are in place for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware.
- Procedures are in place to minimize the creation of splashes and/or aerosols.
- Work surfaces are decontaminated after completion of work and after any spill or splash of potentially infectious material.
- All cultures, stocks, and other potentially infectious materials are decontaminated before disposal.
- Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak-proof container and secured for transport.
- Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
- An effective integrated pest management program is required.
- An eyewash station is available.
- Laboratory doors should be self-closing and have locks.
- Laboratories must have a sink for hand washing. It should be located near the exit door.

Biosafety Level 2 Minimum Requirements
- BSL1 safety practices are followed.
• While personnel are working with BSL2 biohazardous materials in the lab, a biohazard sign must be posted at the entrance to the lab, with contact information of the PI, the lab supervisor, and the identity and biosafety level of the particular biohazard present.
• All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
• The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and lab-specific microbiological practices before working with BSL2 agents.
• Laboratory personnel must be offered medical surveillance and appropriate immunizations for agents handled or potentially present in the laboratory.
• A laboratory-specific biosafety manual must be prepared and adopted as policy.
• Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
• Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with the infectious agent.
• Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination and before repair, maintenance, or removal from the laboratory.
• Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in Chapter 8 of the Biosafety manual.
• Animals and plants not associated with the work being performed should not be present in the laboratory.
• All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.
• BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
• HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer’s recommendations.
• Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps should be utilized.
• A method for decontaminating all laboratory wastes should be available in the facility.

The minimum requirements for Biosafety Level 3 Work practices are covered in the agent specific manuals for personnel that work at that level and in specific training before working in a BSL3 facility. Biosafety Level 4 Work is not conducted at OHSU.

### 3.3 Safety Equipment

Safety equipment is often referred to as a primary barrier, since it generally represents the initial barrier(s) of protections downstream from standard microbiological practice. Safety equipment includes biological safety cabinet (BSCs), safety centrifuge cups, and enclosed containers. Safety equipment also includes PPE such as gloves, coats, coveralls, shoe covers, boots, respirators, face shields, safety glasses, and goggles.

Properly maintained BSCs, appropriate personal protective equipment, or other physical containment devices must be used whenever:
• Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicingating, opening containers of infectious materials, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.

• High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.

**Personal Protective Equipment**

Combinations of various types of safety equipment can be used to create more than one primary barrier. However, circumstances may make it impractical to use equipment such as BSCs or completely enclosed containers, leaving PPE as the only primary barrier between the worker and a sample containing an infectious agent. This again illustrates the importance of standard microbiological practices because of the potential for PPE or other safety equipment failure.

*Lab coats, scrubs, or uniforms* are to be worn while working with hazardous materials.
- ✓ They provide a barrier between you and the hazardous material, preventing contamination of your street clothes or exposure of your skin and any cuts or open sores to hazardous material.
- ✓ Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices).
- ✓ Laboratory clothing is not to be taken home for laundering. Make arrangements through your department for laundering.
- ✓ It is recommended that the end of the lab coat sleeve is tucked into your glove.

*Gloves* provide protection against exposures of biohazardous materials to the skin and any cuts or open sores.
- ✓ Glove selection should be based on an appropriate risk assessment, contact your campus Research Safety Program if you are uncertain about appropriate glove selection.
- ✓ Alternatives to latex gloves should be available.
- ✓ Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
- ✓ Do not wash or reuse disposable gloves. Dispose of potentially contaminated gloves with other contaminated laboratory waste.
- ✓ Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
- ✓ Gloves must not be worn outside the laboratory.

*Eye and face protection* (safety glasses, mask, face shield or other splatter guard) protect against splashes or sprays of infectious or other hazardous materials
- ✓ Most prescription eyewear does not function as safety eyewear.
- ✓ Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse.
- ✓ Eye, face and respiratory protection use is determined by the risk assessment. Signage indicating the required PPE will be posted.
Respirators protect against the inhalation of aerosolized infectious agents.
√ Respirator use is determined by risk assessment.
√ Personnel must complete a Medical Questionnaire and be fit tested before use.

Biosafety Cabinets

A biosafety cabinet (BSC) serves to protect the personnel, the environment, and the products being handled. BSCs are different from chemical fume hoods or laminar flow hoods. BSCs use airflow to create a barrier to airborne particulates. BSCs also utilize High Efficiency Particulate Air (HEPA) filters to mechanically decontaminate the air entering the work area of the BSC and the air being exhausted to the environment. This HEPA filtration removes biohazards from the air, but does not remove fumes from volatile chemicals.

3.4 Facility Design

Facility design is viewed as a secondary barrier to protect workers, both inside and outside the facility. Secondary barriers may include separation of the laboratory work area from public access, hand washing facilities, specialized ventilation systems to limit recirculation of air, and directional airflow into the lab. Most laboratory spaces at OHSU are designed to provide secondary containment for work at the Biosafety Level 1 and 2. Such laboratories are equipped for work with infectious agents or potentially infectious materials when the hazard levels are low and laboratory personnel can be adequately protected by standard laboratory practice. While work is often on the open bench, certain operations are confined to BSCs (especially those that produce aerosols).
4 Exposure sources

A risk assessment is conducted prior to use of a pathogen and assists in determining the proper equipment and procedures required for safe research. The risk assessment for a given activity that includes work with infectious agents is a subjective process. Inherent in any risk evaluation of this nature is the extent of knowledge concerning the potential for transmission of a given agent while performing a specific activity. This clearly points to the need to do risk assessment on a case-by-case basis. A risk assessment is the rational application of safety principles to available options for handling hazardous materials. The following characteristics are to be considered when evaluating use of a potential pathogen:

- Nature of agent (risk group)
- Source of agent
- Route of infection
- Dissemination of agent

Each time an individual at OHSU works with any animal or biological sample that may be a reservoir for a pathogen that affects humans, that individual has carried out a risk assessment concerning the potential for disease transmission under a given set of circumstances. This is true regardless of that individual’s experience and educational background. The real issue is not whether or not a risk assessment has occurred, but rather how thoroughly the assessment has been conducted. **Supervisors are responsible for the safety of any assigned employees and should be consulted for assistance regarding specific hazards of the task.**

It is not the intent of this manual to create an exhaustive list of all pathogens that have the potential to cause laboratory-associated disease as a result of work involving human tissues/body fluids. However, there are some primary agents of concern, which include the following:

4.1 Clinical and Diagnostic Specimens/Bloodborne Pathogens

A variety of pathogens can reside in tissues/body fluids. There are many reports concerning laboratory-associated infections while working with these materials. Many of these instances were associated with research or clinical work focused on a specific infectious agent. BMBL (5th ed.) and Pike provide excellent references on agents that have been reported to cause disease in laboratory workers.

Due to the risk of infectious materials present in human and non-human primate tissues and fluids, all individuals handling such material are required to take a Bloodborne Pathogen training course and a yearly renewal. Researchers should assume that any tissue or fluid from a human or non-human primate is potentially contaminated and practice universal precautions in their handling. The CDC recommends a minimum of BSL2 standard and special practices, containment equipment and facilities for all activities involving all blood-contaminated clinical specimens, body fluids and tissues from all humans, non-human primates or laboratory animals inoculated with an infectious agent.

4.1.1 Human Tissues/Body Fluids
Personnel in laboratories and clinical areas handling human blood, body fluids, or tissues must practice universal precautions, an approach to infection control wherein all human blood and certain human body fluids and tissues are treated as if known to be infectious for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and other blood-borne pathogens. Federal regulation 29 CFR 1910.1030(c)(2)(i) requires that all workplaces with potential occupational exposures to human tissues or body fluids perform an Exposure Determination. OR-OSHA defines occupational exposures as reasonably anticipated skin, eye, mucous membrane or parenteral contact with human blood or other potentially infectious materials (OPIM) while performing occupational duties. OPIM includes any unfixed tissue or organ, other than intact skin, from a living or dead human, such as:

- Blood
- Organs
- Vaginal secretions
- Tissues
- Semen
- CSF
- Synovial fluid
- Saliva from dental procedures
- Amniotic fluid
- Pericardial fluid

**Hepatitis B Virus.** This virus can be present in blood, urine, semen, cerebrospinal fluid, saliva and tissues. Transmission is typically via accidental inoculation or direct exposure of mucous membranes or compromised skin to infectious material. All human tissues/body fluids should be handled with universal precautions to reduce the potential for exposure. The virus is quite stable and has been shown to survive several days in dried blood. Symptoms of infection may or may not be present. Symptoms may include fatigue, nausea, weakness, headache, chills, jaundice and liver disease. Currently in the U.S., there are approximately 5,000 deaths per year attributed to HBV infection. A prophylactic immune globulin and recombinant vaccine are both available.

**Hepatitis C Virus.** HCV is very similar to HBV in potential transmission routes and symptoms. All human tissues or fluids should be handled under universal precautions. According to the CDC, there are more cases of hepatitis C (10,000 deaths per year) than hepatitis B. By the time most cases are diagnosed, there is irreversible liver damage. There is no vaccine for HCV and treatment options are limited at this time.

**Human Immunodeficiency Virus (HIV).** Over one million Americans are believed to be seropositive for this retrovirus, yet very few are believed to have seroconverted due to occupational exposure. Of those cases, the most common means of transmission appears to have been percutaneous inoculation, direct mucous membrane exposure, and direct exposure of non-intact skin to infected body fluids or tissues. The cell-associated nature of the virus appears to limit the potential for airborne exposure. HIV has been found in blood, semen, saliva, tears, urine, cerebrospinal fluid, amniotic fluid, breast milk, vaginal secretions, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, and a number of different tissues. The virus appears to be quite fragile and succumbs quickly to drying and chemical disruption. Data on occupational HIV transmission in laboratory workers were collected through two CDC-supported national surveillance systems, for AIDS and for HIV-infected persons who may have acquired their infection through occupational exposures. For these purposes, laboratory/animal care workers are defined as those persons, including students and trainees, who have worked in a clinical or research HIV laboratory/animal care setting at any time since 1978. Among those with documented occupational transmission, the great majority had percutaneous or mucocutaneous exposures. The three non-clinical exposures involved exposure to concentrated virus in a laboratory.
Activities such as producing research-laboratory-scale quantities of HIV or SIV, manipulating concentrated virus preparations, and conducting procedures that may produce droplets or aerosols, are performed in a minimum of a BSL2 facility, but using the additional work practices and containment equipment recommended for BSL3 facility. Animals infected with HIV or SIV should be housed in a minimum of ABSL2 facilities using ABSL3 special practices and containment equipment.

4.1.2 Non-Human Primate Tissues/Body Fluids

**Macacine herpes virus I** (also called **Herpes virus simiae, Herpes B, Monkey B or B virus**). The disease is 58-70% fatal in humans without antiviral drug therapy. B-virus is a member of the herpes group of viruses that occur naturally in macaques and possibly in other Old World monkeys. Infection with B-virus produces very mild disease in the macaque. Most have no obvious evidence of infection, but the virus typically resides permanently in the macaque and may periodically reactivate and cause ulcerative lesions. During these periods of active lesions the virus resides in the animal’s tissues or body fluids, and may be shed by the macaque to the environment. Macaques without visible symptoms may shed the virus, so the Guidelines for Prevention of Herpesvirus simiae (B Virus) Infection in Monkey Handlers should be followed closely at all times when working with NHPs and NHP tissues/fluids. Transmission to humans occurs by exposure to contaminated macaque saliva or tissue/fluids of infected macaques. The most likely routes of transmission are bites and scratches, however transmission may also occur through cuts or other breaks in the skin, or through direct contact with eyes or mucous membranes when handling infected tissues/fluids. Those at risk of contracting this disease include animal caretakers, laboratory personnel or anyone who is exposed to macaques or macaque tissues/fluids.

**Simian Immunodeficiency Virus (SIV), Human Immunodeficiency Virus (HIV).** SIV and HIV have been isolated from blood, cerebrospinal fluid, and a variety of tissues of experimentally infected NHPs. Although the risk of occupationally acquired SIV/HIV is primarily through exposure to infected blood, it is also prudent to wear gloves when manipulating other body fluids such as feces, saliva, urine, tears, sweat and vomitus from experimentally infected NHPs. This reduces potential exposure to low levels of SIV/HIV as well as microorganisms that may cause other types of infections. In the laboratory, SIV/HIV should be presumed to be present in all blood or clinical specimens contaminated with blood, in any unfixed tissue or organ (other than intact skin) from an experimentally infected NHP (living or dead), in SIV/HIV cultures, in all materials derived from SIV/HIV cultures and in/on equipment and devices coming into direct contact with any of these materials. The skin (especially when scratches, cuts, abrasions, dermatitis or other lesions are present) and mucous membranes of the eye, nose and mouth should be considered as potential pathways for entry of SIV/HIV. Needles, sharp instruments, broken glass and other sharp objects must be carefully handled and properly discarded. Care must be taken to avoid spilling and splashing infected cell-culture media and other virus-containing or potentially infected materials.

4.2 Cell Culture

Unfixed primary human tissues and cells are considered to be other potentially infectious material (OPIM). Work with OPIM requires Blood Borne Pathogen training and the use of universal precautions at a BSL2 level.

4.3 Animals
Numerous risks may be present when animals are used in research. Research involving microorganisms, as well as research involving hazardous chemicals may increase the risk. These risks include, but are not limited to, the following:

- Inoculation from animal bites and scratches
- Exposure to animal excreta in cage bedding
- Exposure to animal allergens
- Self-inoculation from instruments and sharps
- Generation of aerosols during procedures
- Preparation and use of hazardous chemicals

Infections from animals may, on some occasions, produce significant disease in humans. These infections are called zoonotic diseases. They are transmitted from animals to humans by one, or a combination of, the following routes: contact, ingestion, inhalation, or percutaneous (skin penetrating) exposure. In many cases, the animal shows little, if any, sign of illness. One should always be aware of possible consequences when working with each type of animal and take precautions to minimize the risk of infection. Personnel who have suppressed immune systems are at an increased risk of zoonotic disease infection. The scope of possible zoonotic diseases is quite large. It is important to be informed of the specific diseases associated with the type of animals you may work with or around. Common zoonotic diseases, their symptoms, common animal reservoirs, and mini are listed in Appendix A.

Cross-infection occurs between laboratory animals and/or animal tissues/body fluids and personnel, usually via one of the following routes:

1. Primary exposures (bites, scratches, aerosols, topical exposure, accidental inoculation, etc.) from infected animals or their tissues/body fluids obtained directly from the wild or uncontrolled conditions (e.g., non-human primates, dogs, cats, farm animals).

2. Primary exposure to animals with latent infection (infections in a sub-clinical state, which manifest themselves during periods of stress), as well as exposure to animals inoculated with infectious agents.

When working around animals the following precautions should be followed:

1. Wash hands frequently, when leaving animal areas and before eating, drinking, or smoking.
2. Avoid the use of sharps whenever possible.
3. Keep hands away from the face.
4. Do not eat, drink, smoke, handle contact lenses, take medications, or apply cosmetics in animal areas.
5. Wear appropriate PPE.
6. Be aware of your proximity to the animal to avoid accidental direct contact.

All personnel must wear appropriate PPE to protect against animal related hazards. Common examples of PPE utilized in animal areas include: eye and face protection, hand and body protection, foot protection, and possibly respirators. Special practices may be employed when infections agents are used experimentally, or as circumstances require. Training is necessary on any special practices or precautions before work can begin. Signage will be posted outside the animal area indicating the minimum PPE that
must be worn in that area. The selection of PPE is based upon risk to the worker, based on the type of work performed and the infectious agents present. The employee’s supervisor is responsible for ensuring proper PPE is being worn. However, it is ultimately the individual’s responsibility to consistently follow the guidelines regarding PPE, and not to take short-cuts.

Access to animal housing, use, and support areas is limited to authorized, trained, and informed personnel. Only those individuals with work-related requirements, and who have had the appropriate training, may be in animal areas unescorted. All unescorted personnel and visitors must receive training on health and safety issues prior to beginning work in areas in which they may have contact with animals. Training should include information on the Occupational Health Program, zoonotic diseases of concern, disease transmission prevention, personal hygiene, PPE selection, and accident and exposure reporting procedures. Supervisors are responsible for ensuring that their workers have received adequate training. Visitors or service technicians who have not received training must be escorted by an OHSU representative trained in biosafety procedures at all times while in animal areas.

**Non-human Primates** (NHPs) are generally considered to be the species with the greatest risk of having chronic infections or being re-infected with zoonotic diseases. Humans are susceptible to a variety of infections carried, often in a sub-clinical state, by NHPs. Due to these inherent risks of working with NHPs, all NHPs are housed in ABSL2 facilities and with ABSL2 practices. Tissues or body fluids collected from NHPs may be hazardous, sometimes even after fixation, prolonged storage or subculture and be treated with universal precautions at a BSL2 level. Details of zoonotic pathogens, including Monkey Herpes B virus, found in NHPs are detailed in Appendix A. Further hazards are associated with the use of NHPs as host-models in experimental infections with human diseases.

Special considerations for working with NHPs:

- Appropriate PPE must be worn in all NHP housing rooms. Signs will be posted indicating the minimum required PPE for entering areas with NHPs and for working directly with the NHPs.
- Minimize direct handling.
- Work with at least one other person when handling NHPs.
- Immediately report all bites or scratches direct from the live animal, contaminated needle/sharps sticks and mucous membrane exposures involving macaques.
- Report any injury involving macaque tissues or body fluids that are associated with breaks in the skin or mucous membranes.

### 4.4 Laboratory Animal Allergens

Allergic reactions associated with handling animals are common. The potential for animal-care workers to develop allergic symptoms has been clearly demonstrated. Studies have also shown that animal care workers with preexisting allergic conditions, such as hay fever, are more likely to develop sensitivity to animal related allergens at work. Symptoms can even evolve into occupationally–related asthma.

Animal allergens are most often associated with urine or dander from a specific animal. The human immune system produces antibodies that are specific for each allergen as a result of initial exposures. During subsequent exposures, the allergen binds with these antibodies causing the release of histamines stored in cells closely associated with the antibodies. These chemicals, on contact with the surrounding
tissue (respiratory tract, etc.) can result in hives, nasal congestion, sneezing, nasal drainage, coughing, wheezing and shortness of breath. These symptoms can occur as quickly as 10-15 minutes after exposure.

Rats, mice, guinea pigs, gerbils, rabbits, cats and dogs have all been shown to be sources of allergen exposure to laboratory animal workers. However, NHPs have rarely been found to cause sensitization. The major sources of allergens in rats and mice appear to be urine and saliva. Guinea pigs also produce allergenic materials in dander, fur, saliva and urine, with urine appearing to be the major source. Rabbits produce an allergen that is primarily associated with the fur, although saliva and urine allergens do exist. The major cat allergen is produced by the sebaceous glands in the skin and coats the hair shaft. It is also produced in the saliva. The main sources of dog allergens appear to be saliva, hair and skin.

The primary exposure route for workers is through inhalation of allergens. Disturbance of contaminated litter and bedding results in the release of very small particles of litter containing the allergen. These particles are often small enough to stay airborne for extended periods of time and can easily be deposited in the airway. Studies have demonstrated that cage cleaning, weighing, shaving, injections, blood collection and surgery can release significant quantities of the allergens. Of these, cage cleaning represents a major source of exposure. However, the ultimate magnitude of exposure is directly proportional to the number of animals in a given work area. General ventilation may or may not be effective.

Thoughtful job assignment, careful work practices and training can serve to reduce the release of allergens and thus reduce the potential for exposure. Workers with known risk can be assigned to tasks with low risk of exposure to allergen. Task assignment is the first important step in minimizing exposures, especially for workers who have become sensitized. Minimizing exposure time in animal housing areas with potential for allergen release is another approach to reducing exposure. More important is to minimize manipulation/disturbance of animal litter and bedding after contamination.

PPE should be utilized in addition to engineering controls to reduce the potential for exposure. At a minimum, workers should wear dedicated lab coats, disposable gowns or coveralls, latex gloves and eye protection. In addition, a HEPA filtered dust/mist disposable respirator should be worn by individuals with known animal related allergies at all times while in the animal housing area. Before using an air filtering respirator you must complete a medical questionnaire and be fit tested. Hands and exposed skin areas should be washed prior to leaving the area.

All personnel should receive instruction prior to entering animal housing areas where allergen exposure is likely. Training should include at a minimum the following topics:

1. Animal allergen theory
2. Specific animals of concern
3. Symptoms
4. Work practices
5. PPE
5 Occupational Health

5.1 Preventative Medicine

_Tuberculosis screening._ All new employees are required to undergo Tuberculosis (TB) screening at the start of employment. Additionally, employees must have a current negative TB test prior to receiving access to animal areas. This protects the research animals, especially non-human primates, which are susceptible to acquiring TB from an infected individual. Individuals who do not participate in the Occupational Health Program will not be granted access to animal areas.

TB screening is:

1. Required upon hire or commencement of duty or schooling for all persons. Two step skin testing is required if a TB skin test has not been applied within the last twelve (12) months and the individual has no history of a positive skin test.

2. Required annually for persons who:
   - Have face-to-face contact with patients, or human or primate research subjects,
   - Handle respiratory secretions from humans or primates, or
   - Have a history of a positive skin test.

_Hepatitis B Vaccination._ In compliance with 29 CFR 1910.1030(f), OHSU will make the HBV vaccine and vaccination series available to all OHSU employees who have potential occupational exposure to primary human tissues/body fluids, and post-exposure evaluation and follow-up to all employees who have an exposure incident involving primary human tissues/body fluids. An exposure incident involves contact of human tissues/body fluids with your eye, mouth, other mucous membrane, non-intact skin or that a contaminated item that pierces your skin. All medical evaluations, procedures and laboratory tests associated with the vaccine and any exposure incidents will be provided at no cost to the employee and will be confidential.

HBV vaccination will be made available after each employee has received the bloodborne pathogen training, and within 10 working days of initial assignment, to all employees who have occupational exposure unless the employee has previously received the complete HBV vaccination series, antibody testing has revealed that the employee is immune, or the vaccine is contraindicated for medical reasons. If the employee initially declines HBV vaccination, but at a later date decides to accept the vaccination, the vaccination series will be made available. All employees who decline to accept the HBV vaccination must sign a vaccine declination statement. If a routine booster dose of HBV vaccine is recommended by the U.S. Public Health Service at a future date, such booster dose(s) will be made available.

_Other Work Related Vaccinations._ When appropriate, pre-exposure vaccination and testing may be provided for the following infectious agents: Rabies, Hepatitis A, Influenza, Tetanus, Varicella, Meningococcus, Pneumococcus, Measles, Mumps, Yellow Fever, Smallpox, Q-Fever and Rubella.

5.2 Post-Exposure Evaluation and Follow-up

Appropriate post-exposure procedures are of extreme importance in reducing the risk of disease transmission. Exposures involving infectious potentially infectious material require immediate attention.
A potential exposure incident is an incident that involves eye, mouth, other mucous membrane, non-intact skin or parenteral contact with potentially infectious material. All wounds (unless life-threatening) should be washed immediately with soap and water for 15 minutes using a massaging motion. For exposures to skin, regardless of whether they involve intact or broken skin, wash with soap and water continuously for a minimum of 15 minutes. If the exposure involves mucous membranes, rinse the area with water for 15 minutes. The potential for various disease infections depends upon a variety of circumstances, including time from exposure to thorough cleaning, route of transmission, immune status of the exposed individual, and characteristics and source of the infectious material. A medical evaluation may be necessary for some types of exposures.

Potential and overt exposures should be followed up with a Post-Exposure evaluation by Employee Health (Central and Waterfront Campuses) or the Occupational Health Nurse (West Campus). OHSU will obtain and provide the exposed employee with a copy of the evaluating health care professional’s written opinion. The health care professional’s written opinion for post-exposure evaluation and follow-up will be limited to documenting that the employee has been informed of the results of the evaluation and about any medical conditions resulting from exposure to the potentially infectious material which require further evaluation or treatment. If medically indicated, post-exposure prophylaxis will be offered at no expense to the employee. Counseling and evaluation of reported illnesses will also be made available to the exposed employee. All other medical findings or diagnoses will remain confidential and will not be included in the written report.

Exposures to Non-Human Primate tissues and fluids can be extremely serious and the Macaque Exposure Protocol should be followed. For exposures arising from work with other live animals, report the incident to the supervisor and the attending veterinarian. Human tissue related incidents should be reported to the supervisor and Occupational Health as a potential Bloodborne Pathogen exposure.

All incidents should also be reported on OHSU Incident Report forms. The purpose of reporting is to expedite and optimize post-exposure treatment as well as to alert OHSU management to conditions that could lead to further injuries or illnesses. The Incident Report form assists the safety committee in reviewing the incident for suggestions of risk reduction strategies. All work-related incidents and injuries should be reported to Risk Management “Worker and Student Injury Reporting System (WSIRS)” via their web portal: http://www.ohsu.edu/ohsuedu/central/risk/workers_compensation.cfm

Since many laboratory acquired infections cannot be traced back to a specific incident, it is important to be able to recognize symptoms that may signify the occurrence of an exposure, and to remain vigilant regarding those symptoms.

5.2.1 Information Provided to the Health Care Professional

OHSU will ensure that the health care professional evaluating an employee after an exposure incident is provided the following information:

2. A description of the exposed employee’s duties as they relate to the exposure incident.
3. Documentation of the routes(s) of exposure and circumstances under which exposure occurred.
4. Results of the source individual’s blood testing, if available.
5. All medical records relevant to the appropriate treatment of the employee, including vaccination status.
5.2.2 Post Exposure Evaluation for Specific Bloodborne Pathogens

In the case of a potential exposure to Hepatitis B, Hepatitis C, or HIV the source individual will be identified and documented unless such identification is not feasible. The source individual’s blood will be tested as soon as feasible and after consent is obtained in order to determine HCV, HBV and HIV infectivity. If consent is not obtained, OHSU will establish that legally required consent cannot be obtained. When the source individual’s consent is not required by law, the source individual’s blood, if available, will be tested and the results documented. When the source individual is already known to be infected with HCV, HBV or HIV, testing for the source individual’s known HCV, HBV or HIV status need not be repeated. Results of the source individual’s testing will be made available to the exposed employee, and the employee will be informed of applicable laws and regulations concerning disclosure of the identity and infectious status of the source individual. The exposed employee’s blood will be collected as soon as feasible and tested after consent is obtained. If the employee consents to baseline blood collection, but does not give consent at that time for HIV serologic testing, the sample will be preserved for at least 90 days. If, within 90 days of the exposure incident, the employee elects to have the baseline sample tested, such testing will be done as soon as feasible.

The OR-OSHA Bloodborne Pathogen Standard requires employers to establish and maintain an accurate record for each employee that sustains an occupational exposure to human tissues and/or body fluids. The Employee Health and Risk Management Departments are responsible for keeping records on human tissue/body fluid exposures. This record consists of the following:

1. The name and social security number of the employee.
2. A copy of the employee’s HBV vaccination status, including the dates of all the HBV vaccinations and any medical records relative to the employee’s election to receive vaccination.
3. A copy of all results of examinations, medical testing, and follow-up procedures as noted in the OHSU Exposure Control Plan.
4. The employer’s copy of the health care professional’s written opinion post-exposure.
5. A copy of the information provided to the health care professional post-exposure.

OHSU must ensure that employee medical records are kept confidential and not disclosed or reported, without the employee’s express written consent, to any person within or outside the workplace except as required by law. OHSU will maintain these records for the duration of employment plus 30 years.

5.3 Central and Waterfront Campus Employee Health

The Department of Employee Health, located in Multnomah Pavilion, Room 1110, (503-494-5271), is the primary resource for both preventive care and follow-up on non-life-threatening injuries and exposures to infectious agents for employees working at Central and Waterfront Campuses. Preventive care includes work related vaccinations and TB screening. Post-exposure follow-up for exposures to infectious agents includes specific titers and health monitoring related to the agent in question. It is important to contact Employee Health in the event of an injury or exposure, so that the incident can be documented. Employee Health can help you determine whether you might need a specific vaccine for your work, or a baseline blood test. They can also provide discrete counseling on occupational risks for employees who may be pregnant or immune-compromised. Employee Health is required to maintain health records of employees for a minimum of 30 years from the last day of work.
The Employee Health website is located at: [http://ozone.ohsu.edu/employeehealth/](http://ozone.ohsu.edu/employeehealth/)

The working hours for Employee Health are 6:30 am - 4 pm Monday-Friday, with walk-ins accepted on Wednesdays only. The OHSU Hospital Emergency Department is always available for life-threatening injuries, and for serious injuries outside normal working hours.

In the event of an exposure or injury the following steps should be followed:

1. Determine the severity of the injury/exposure. If it is life-threatening, call Public Safety immediately **4-4444**. They will help you decide whether to go directly to the ER or wait for emergency responders. Please provide any relevant information about infectious agents to which you have been exposed. In the event of a potential exposure to an infectious agent, also report the incident to a Central Campus Biosafety Officer **4-0655 or 4-2580**.

2. For non-life threatening exposures, immediately wash the area thoroughly with soap and water. Use only water if it is a mucosal area.

3. If medical attention is necessary or your injury involves a potential exposure, call Employee Health at **4-5271**. Over the phone, they can help you with first-aid advice, and also help you decide whether to go to Employee Health or the OHSU Emergency Department for follow-up treatment. You may also contact your primary care physician. In the event of a potential exposure to an infectious agent, report the incident to a Central/Waterfront Campus Biosafety Officer.

4. If you are injured/exposed and need to leave the lab for treatment, delegate responsibility to a colleague for any clean-up or decontamination that may be necessary. Have the colleague document what happened.

### 5.4 West Campus Occupational Health

To meet the unique needs of the OHSU – West Campus, ONPRC has established an Occupational Health Program staffed by an on location Occupational Health Nurse. The Occupational Health Nurse provides preventive care including standard vaccinations, vaccinations that are work-specific, TB screening, and post-exposure follow-up for incidents involving infectious agents and/or research animals.

In the event of an exposure or injury the following steps should be followed:

1. If your injury is severe or life threatening, call **9-911** to report the incident and location. Tell them you will have to call them back. Dial **503-690-7777** to alert the ONPRC Emergency Response team to escort the emergency responders to your location on campus.

2. For non-life threatening exposures, immediately wash the area thoroughly with soap and water. Use only water if it is a mucosal area.

3. If medical attention is necessary or your injury involves a potential exposure, visit the ONPRC Occupational Health Nurse or call the office at 503-629-4031. If the Nurse is unavailable or it is after hours, report to Cascade Occupational Medicine at Tuality Health Place, 1200 NE 48th Ave Ste 1000, Hillsboro, OR 97214, 503-726-1021. You may also contact your regular physician.

4. Notify your supervisor of the incident. In the event of a potential exposure to an infectious agent, report the incident to a West Campus Biosafety Officer (503-690-5312; 503-690-5310; 503-690-5368).
**NHP Exposure Procedures.** Due to the unique risks involved in working with Macaques, the Macaque Exposure Protocol must be followed. All individuals that work in non-human primate areas or with non-human primate products receive specific training for Macaque Exposures and the Macaque Exposure Protocol is posted in those areas. All skin breaking or mucous membrane exposures received from NHPs, injuries inflicted while handling contaminated NHP equipment such as cages, needles and other sharps, and injuries involving NHP tissues and body fluids when these injuries or wounds penetrate the skin are potential exposure to Monkey B virus. This also includes superficial or minor wounds that break the skin and cause bleeding, and for eye or nasal/mouth exposures that are directly associated with macaques or laboratory manipulation of macaque tissues or body fluids but are not life-threatening, including bites, scratches, contaminated needle/sharps sticks and all mucous membrane exposures.
6 Decontamination, Spills, and Incident Response

6.1 Decontamination

Decontamination methods play a role in the control of infectious diseases and neutralization of biohazardous materials. Decontamination is the reduction of removal of infectious material to make an item suitable for re-use or safe disposal. Disinfection is the process that reduces the number of infectious organisms below the level necessary to cause infection. The process of completely removing all organisms is sterilization.

Decontamination can be achieved by mechanical, chemical, or physical means. Mechanical decontamination involves measures to remove, but not necessarily neutralize an agent. An example would be filtration of water to remove *giardia*. Chemical decontamination renders biohazardous materials harmless by the use of disinfectants. Chemical disinfectants can be harmful to humans, animals, the environment and/or materials. Autoclaving or dry heat are physical means of rendering an agent harmless through heat and steam exposures. Dry heat at 160° C for two hours is another physical means of rendering biohazardous materials harmless.

The following items should be autoclaved or chemically disinfected before disposal:

1. Animal tissues not meeting the definition of pathological waste. (Pathological waste must be incinerated.)
2. Infectious agents not meeting the definition of cultures and stocks, propagated experimental agents, and any agents isolated from animals.
3. Specific contaminated solid waste to be autoclaved is limited to the containers and transferring devices used in procedures associated with #1 and #2 above.
4. Materials saturated with body fluids, tissue culture fluids and other infectious material.

The following are additional guidelines to help the worker decide the most appropriate means for decontamination. Any questions should be directed to their campus Research Safety Program.

6.1.1 Chemical Disinfection

Chemical disinfecting agents can generally be split into two categories; chemical mixtures that are made to clean and disinfect surfaces and chemical mixtures that are made for terminal disinfection of inanimate surfaces. Soap or detergent mixtures including disinfectants are made to clean dirty surfaces. These cleaners contain a soap or detergent to suspend gross contaminants into solution until they are rinsed off. A disinfectant is often added to help start the process of decontamination. Mixtures formulated to do terminal disinfection on inanimate surfaces commonly contain no soap or detergents. These solutions are made to disinfect surfaces that are already clean. These disinfectants are not recommended for use on animals or human patients.

A general rule of thumb is that the chemical disinfect should be in contact with the surface for ten minutes for effective disinfection. Contact times of less than ten minutes may result in partial disinfection at best, and work only as a surface cleaner. Other variables that effect disinfection times are:

1. The amount or concentration of the biohazardous material.
2. The type of biohazardous material to be decontaminated, and the presence of additional proteinaceous material.
3. The dilution of the disinfectant.
4. The temperature (in general, colder temperatures require longer contact times).

Most disinfectants have directions that specify a dilution depending on the infectious agent/material to be disinfected and the type of surface to be disinfected. The directions for the disinfectant must be followed precisely for effective disinfection.

There are several common classes of chemical disinfectants:

**Bleach** is cheap, effective, but corrosive, so surface decontamination should be followed with rinse with 70% EtOH or water. Bleach is commonly used in vacuum traps to decontaminate aspirated supernatants from tissue culture. A 10% final concentration will eliminate viruses, bacteria, fungi, and spores. An effective recipe for a homemade surface decontaminant is 1% Bleach plus 0.7% non-ionic detergent (e.g. Triton X-100, Tween-20, etc.).

**70% Ethanol (EtOH)** is somewhat overrated for decontamination because it volatilizes quickly, thus reducing the actual time that it is in contact with microbes. Increasing the percentage of EtOH to 100% does not help. Ethanol must be diluted with water to allow enough contact time to disinfect prior to evaporation.

**Iodophor** containing products like Wescodyne are effective against many bacteria, viruses, and some fungi, and can be used for decontamination of hard surfaces, but are not recommended for large-scale decontamination, such as blood spills, because the iodophors bind non-specifically to proteins. Iodophors are corrosive, so stainless steel surfaces should be rinsed after the recommended contact time. Iodophors can also stain absorbent material, such as a lab coat.

**Phenolics**-based disinfectants, like Amphil, are effective against many bacteria, viruses and some fungi. They can be used in vacuum traps, and for surface decontamination. Because concentrated phenolic compounds can cause skin burns, care must be taken when using these.

**Quaternary ammonium compounds** are not recommended for spill clean-up, but may be utilized for surface decontamination. Manufacturer’s claims for newer formulations, like Coverage Plus NPD and Roccal D-Plus, indicate that these are effective against most bacteria, viruses, and some fungi. They are relatively non-corrosive, so may be useful for surface disinfection of sensitive instruments.

See tables in Appendix D for more information about decontaminants and their use in laboratories.

### 6.1.2 Autoclave Use and Requirements

OHSU requires that certain materials and items be autoclaved before leaving their location of generation and entering the waste stream. For decontamination, all autoclave users must develop written standard protocols for proper autoclave performance as described in Oregon Health Division regulation [OAR 333-56-0030(2)(b)](https://www.oregon.gov/oha/OSHA/PUBS/OSHA233/chapter-3-part-56.cfm). As a general rule, autoclaving should be done at 121°C/250°F for a minimum of 20 minutes at one atmosphere of overpressure (15 lbs. per square inch), depending on the size and density of the load.
Waste must be placed into an autoclavable bag and a secondary autoclavable container (e.g., a Nalgene tub) sufficient to contain the waste in the event the primary bag/container fails. The bag and the secondary container must be able to withstand temperatures from 250°F to 270°F. An autoclavable indicator that reacts to both duration and contact with steam and heat should be used to indicate effective decontamination.

Steam autoclaving may be used for decontamination, as long as:

1. the waste does not have volatile or reactive organics, or strong oxidizing agents that could react with heat and steam,
2. the waste quantity does not exceed the capacity of the autoclave to decontaminate,
3. the waste can be contained in some way such that it will not grossly contaminate the interior of the autoclave, and
4. the waste does not contain volatile radionuclides.

It is suggested that autoclaves be dedicated to sterilization or decontamination, and not be used for both. If both decontamination and sterilization must be done with the same autoclave then an empty cycle should be run between a decontamination cycle and a subsequent sterilization cycle to prevent residual cross contamination. Cycle times and temperatures are determined by the load size and the agent to be decontaminated. Minimally, the autoclave should run at 121°C/250°F for 20 minutes. Temperature calibration must be verified yearly. OAR 333-56-0030(2)(b) requires a monthly quality assurance run to ensure that autoclaving is effective. Methods that indicate an effective run after an appropriate contact time with heat and steam should be used, for example a biological indicator such as *B. stearothermophilus*. Steris Corporation markets several chemical indicators that are more informative than autoclave indicator tape. Records of tests must be kept for at least one year.

### 6.2 Procedures for Inactivation and Safety Containment of Toxins

Table 1 provides information about chemical inactivation of selected toxins. It is further recommended that cages and bedding from animals exposed to T-2 mycotoxin or brevetoxin be exposed to 0.25% NaOCl and 0.025 N NaOH for four hours. Exposure to 1.0% NaOCl for thirty minutes is an effective procedure for laboratory solutions, equipment, animal cages, working areas and spills for inactivation of saxitoxin, tetrodotoxin, microcystin, palytoxin, ricin, botulinum toxin or staphylococcal enterotoxins (SEB). Increasing the concentration of disinfectant will not allow for shorter contact times.

**Table 1**

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<th>Toxin</th>
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</tbody>
</table>
Note: household bleach contains approximately 5.25% NaOCl. For complete inactivation of T-2 mycotoxin and brevetoxin, it is recommended that all liquid samples, accidental spills and non-burnable waste be soaked in a solution of 2.5% sodium hypochlorite (NaOCl) with 0.25 N NaOH for four hours.  

All burnable waste from toxins should be incinerated at temperatures in excess of 1500° F. Autoclaving can be used for the protein toxins (ricin, botulinum toxin and SEB), but should not be used for any of the low molecular weight toxins. Table 2 provides information about heat/autoclave inactivation of selected toxins.

### Table 2

Inactivation of toxins by autoclaving, or 10 min. exposure to varying temperatures of dry heat

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Autoclaving</th>
<th>200°F</th>
<th>500°F</th>
<th>1000°F</th>
<th>1500°F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brevetoxin</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Botulinum toxin</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Microcystin</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Palytoxin</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ricin</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Saxitoxin</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>SEB</td>
<td>Yes?</td>
<td>Yes?</td>
<td>Yes?</td>
<td>Yes?</td>
<td>Yes?</td>
</tr>
<tr>
<td>T-2 mycotoxin</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Tetrodotoxin</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Tap water with normal chlorination is not a useful medium for inactivation of any of these toxins.

Procedures should be implemented to prevent contamination of personnel and equipment with these toxins. If the skin is accidentally exposed to toxins, it is recommended that it be washed immediately with soap and water. All procedures that may generate aerosols of toxins must be performed in a class III BSC (see BMBL, 5th ed., Appendix A). Except for botulinum toxin, the class III BSC can be in a BSL-2 laboratory. Work with botulinum toxin must be contained by a class III BSC in a BSL-3 laboratory.

### 6.3 Spills and Spill Kits

For many accidental spills in the lab, the appropriate response requires more forethought and planning than “Just clean it up”. The response for biohazardous spills depends upon knowledge of the infectious material involved, in this case: its concentration, location, volume, pathogenicity, mode of transmission, persistence, to name a few considerations.

Since the personal safety of the individual is paramount, the immediate response to a spill should be to check for direct exposure to an infectious agent. Appropriate post-exposure procedures are of extreme importance in reducing the risk of disease transmission and require immediate attention. For exposures to skin, regardless of whether they involve intact or broken skin, wash with soap and water continuously for a minimum of 15 minutes. For exposures involving the eyes, or other mucous membranes, flush with clean water for 15 minutes.
OHSU does not require laboratories to have a spill kit, but assumes that labs conducting BSL2 research will have the basic components readily available to initiate an immediate response to a spill. The general components of a spill kit include: concentrated household bleach; a spray bottle for making 10% bleach solutions; forceps, broom and dust pan, or other mechanical device for handling sharps; paper towels or other suitable absorbent; biohazard autoclave bags for the collection of contaminated spill clean-up items; gloves and eye/face protection.

The following procedures are included as guidelines for incidents at BSL1 and BSL2, respectively. These address the following:

**BSL1 Spills**

These come closest to the “Just clean it up” approach. Administrative follow-up is not required, unless the spill involves personal injury.

1. Cover spill with paper towels.
2. Carefully pour disinfectant onto the paper towels, starting at the periphery and working inward toward the center. Allow sufficient contact time for disinfectant.
3. If sharps are involved do not use hands to pick up; rather, use forceps or a brush and dustpan.
4. Transfer to appropriate waste container.

**BSL2 Spills**

The spill response depends upon location and volume. Spills inside the biosafety cabinet are considered “contained”. Spills outside the BSC are of much greater concern, since there is a risk of exposure to an infectious agent via aerosols or possible skin exposure via a splash. Categories of spills are also subdivided into “Minor Spills” and “Major Spills”. “Minor spills” are somewhat arbitrarily defined as spills of 10 ml or less, where there is little chance that a splash could get out of control before it could be contained with absorbent material. A “Major Spill” is anything over 10 ml, where there is a risk that the liquid is not easily contained. Special considerations are required for centrifuge accidents, biohazard spills involving radioactivity (decontaminate first, then clean up as for radioactive spills), and biohazards with toxic chemicals. In addition, labs working with biological toxins must have SOPs for decontamination of those agents.

Appendix E contains general standard operating procedures (SOPs) for clean-ups of various types of spills. These can be adapted for laboratory-specific SOPs included in each lab’s Laboratory Biosafety Manual.

### 6.3.1 Incident Follow-up

Spills of biohazardous materials may or may not involve injury or overt exposure. Spills of infectious agents can also present scenarios in which there is a possibility of exposure via aerosols or inadvertent contact during spill decontamination. Generally, only spills that involve acute injury or overt exposure need to be reported on the OHSU Risk Management Incident Report Form. However, if you start to feel symptoms consistent with an infectious agent that you recently spilled, this should be reported. For detailed information on follow-up after an injury or overt exposure, refer to Chapter 8.

**NIH Requirements**
Additional reporting requirements are imposed by NIH/OBA for incidents involving recombinant DNA or BSL2 and BSL3 infectious agents. The IBC has a policy for reporting spills, accidents, and exposures as required by NIH/OBA. The IBC acts as an intermediary in filing reports to the NIH/OBA, and has developed its own IBC Protocol Deviation Form. The timetable for notification is given below. Please note that incidents involving documented exposure to a BSL2 agent, potential or documented exposure to a BSL3 agent, or a spill of a BSL3 agent outside the BSC MUST BE REPORTED TO A BIOSAFETY OFFICER IMMEDIATELY. This is mandated by the NIH.

<table>
<thead>
<tr>
<th>Type of spill or exposure</th>
<th>Reporting time frame</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spill of a BSL2 agent outside the BSC</td>
<td>Incident Report Form must be submitted to the IBC within 10 days.</td>
</tr>
<tr>
<td>Documented exposure to a BSL2 agent</td>
<td>Report immediately to the Biosafety Officer (preferably by phone call). Submit Incident Report Form to the IBC within 5 days.</td>
</tr>
<tr>
<td>Potential or documented exposure to a</td>
<td></td>
</tr>
<tr>
<td>BSL3 agent</td>
<td></td>
</tr>
<tr>
<td>Spill of a BSL3 agent outside the BSC</td>
<td></td>
</tr>
</tbody>
</table>

Note:
Some incidents that do not require reporting to the IBC, such as a spill inside a BSC that is properly decontaminated, should nevertheless be reported to the PI/supervisor in order to review or revise SOPs so as to minimize recurrence of the event, or to prompt refresher training of personnel.

6.4 Lab Close-Outs: Moves and Retirements

If a lab moves or shuts down completely, equipment and the lab space must be decontaminated before new occupants can move in or before renovation can begin. The PI has the responsibility to ensure that decontamination has been carried out and the campus Research Safety Program is responsible for verifying that the PI has completed the decontamination. This is accomplished by a site visit and an interview to establish which method of decontamination was used, and whether this was appropriate for the biohazardous material that was used in the lab. Once the Biosafety Officer is satisfied that all significant biohazards have been contained/eliminated, they will sign and date a form which will be posted in the lab stating that the space has been cleared of biohazardous material.

Labs that were using radioactivity must also ensure that all radioactive waste and remaining radioactivity are properly taken care of by their campus Radiation Safety Officer.

All equipment used for biohazardous material must be decontaminated prior to shipment, repair, or surplus. Examples most often encountered are defunct freezers and refrigerators, obsolete clinical centrifuges, incubators, and occasionally BSCs.
7 Biohazardous Waste

Management of biohazardous/medical waste is an important aspect of biosafety. Biohazardous waste is waste that has been generated as a consequence of patient diagnosis, treatment or immunization as well as waste associated with laboratory manipulation of recombinant DNA, infectious materials, or human or animal tissues, blood, or body fluid, including:

- Blood and blood products, excretions, exudates, secretions, aspirates and other body fluids that cannot be directly discarded into the municipal sewer system, and waste materials saturated with blood or body fluids, but does not include diapers soiled with urine or feces. In addition, biohazardous waste does not necessarily include articles contaminated with fully absorbed or dried blood, such as gauze, paper towels and sanitary napkins. The term fully absorbed is interpreted to mean not dripping or not capable of releasing blood or body fluids if compressed.

- Cultures and stocks, which includes infectious agents, recombinant DNA, and associated materials, including specimen cultures, dishes and devices used to transfer, inoculate and mix cultures; wastes from production of biologicals; serums that have not been decontaminated and discarded; live and attenuated vaccines.

- Pathological waste, which includes biopsy materials and all human tissues, anatomical parts that emanate from surgery, obstetrical procedures, autopsy and laboratory procedures and animal carcasses exposed to pathogens in research and the bedding and other waste from such animals.

- Contaminated solid waste (paper, paper towels, table liner, latex gloves, various plastics), as described in #1, unless the item is saturated with blood or body fluids.

- Sharps, which includes needles, IV tubing with needles attached, scalpel blades, lancets, glass tubes that could be broken during handling, and syringes, with and without needles, that are either clean or contaminated.

Additional consideration should be given when handling Putrescible Waste - solid waste containing organic material that can be rapidly decomposed by microorganisms, which may give rise to foul-smelling, offensive products during such decomposition, or which is capable of attracting or providing food for birds and potential disease vectors such as rodents and flies.

Some of the NIH and CDC guidelines are open to interpretation. Below are two examples where OHSU has interpreted the guidelines in the BMBL 5th edition for BSL1 and BSL2 biosafety practices:

- “Dispose of used gloves with other contaminated laboratory waste.”

Obviously, disposable gloves are used for many purposes in research labs, like weighing hazardous chemicals, setting up PCR, or RNA purification, where there is no risk of contamination with an infectious agent. Disposable gloves should be disposed of according to the nature of any hazardous substance contaminating them; e.g., ethidium bromide-stained gloves go into ethidium bromide solid waste. One can assume, however, regarding the disposal of gloves as contaminated laboratory waste, that the BMBL guideline above refers primarily to gloves that might be contaminated with potentially infectious material, such as those worn while doing tissue culture.
• “Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method.”

7.1 Biohazardous Waste Stream Procedures

Biohazardous or infectious waste must be segregated from other waste at the point of generation. Biohazardous waste can be liquid or solid. Storage containers used for biohazardous wastes need to be closed to prevent access by or exposure to third parties, and must be marked with the universal biohazard symbol. Bags used to hold biohazardous waste must be either red or orange in color, autoclavable, and be marked with the universal biohazard symbol.

**Liquid waste** containing biohazardous material should be decontaminated with bleach or another effective disinfectant or autoclaved (see autoclave use and requirements for more details) prior to disposal into the sanitary sewer (exceptions would be radioactive waste, where drain disposal limits must not be exceeded, or toxic compounds that must be handled as chemical waste). Liquid waste can be decontaminated by adding disinfectant to a final concentration that will inactivate the infectious agent in question. Typical disinfectants used to decontaminate liquids are (final concentration)

- Household bleach (10%)
- Wescodyne (2-5%, may not work with some viruses or spores)
- Amphyl (2.0%)

**Contaminated solid waste** may be autoclaved if appropriate and discarded in the normal solid waste stream. Any color bag except red or orange may be used for disposal of trash that has already been decontaminated by autoclaving. Use red (or orange) bags with the biohazard symbol for contaminated waste that you want custodians to pick up. **Solid or semisolid tissues** should be considered biohazardous waste.

**Sharps** (glass pipettes, serum tubes, syringes, needles, etc.) must be placed in rigid sharps containers immediately after use. In the rare circumstance where it is not possible to immediately place sharps into sharps containers, a temporary container may be used provided that no personnel exposure to the sharps can occur during the eventual transfer to a sharps container. Sharps containers must be sealed when they become no more than ¾ full and a new sharps container provided. In an effort to keep syringes out of regular landfills, *Oregon law defines any syringe as a sharp*. This means even syringes used without needles are still “sharps”, and must be disposed of in sharps containers. Sealed sharps containers will be picked up upon request to Facilities Custodial Services (Central and West Campus), and can be dropped off in the biohazardous waste staging areas at the Waterfront Campus.

**Animal carcasses** should be placed in an appropriately labeled bag, refrigerated or frozen as appropriate, and transported to the designated collection point for the area, as required by the Department of Comparative Medicine (DCM) or the Division of Animal Resources (DAR). Each campus has slightly different practices.

Handling of biohazardous waste at OHSU differs slightly in specific areas. This manual was written to provide guidelines for prudent biohazardous waste handling practices in compliance with local, state and federal regulations. The specialized needs of a given laboratory or animal area may require additional
evaluation of procedures. For assistance with questions regarding waste handling procedures, contact your campus Research Safety Program.

**Central Campus**

Custodians are instructed not to pick-up open biohazard bags. For disposal, biohazard bags must be closed with tape or rubber bands, and placed in a centralized biohazard container (usually a 32 gallon garbage can labeled with a biohazard sign), in a location that is determined by mutual agreement with the PI and Facilities Custodial Services. It is important not to close bags too tightly, to allow for steam penetration and to avoid having the bag burst in the autoclave. Custodial staff will pick up closed biohazard bags on weekday evenings. Labs working with BSL2 agents that may pose a risk of infection due to high concentration, a capacity to aerosolize, or persistence, should consider autoclaving their biohazardous waste prior to pick-up by the custodian.

Pathogen-free animal carcasses and tissues are placed in clear zip-lock bags and brought to a DCM freezer for incineration. ABSL2 carcasses contaminated with infectious agents must be delivered in clearly labeled, closed biohazard bags, with the infectious agent identified on the label. With some infectious agents, special protocols will be developed with DCM and the IACUC.

**Waterfront**

Custodial staff has been instructed not to pick-up open biohazard bags. For disposal, red biohazard bags are kept in biohazard bins or wastebaskets. When bags are full, they are closed by lab personnel and will then be collected by the custodian.

Animal carcasses and tissues are bagged in clear ziplock bags. Carcasses and tissues that have been exposed to infectious agents are bagged in red ziplock bags, with any infectious agents clearly identified on the bag, and brought to a freezer in CHH 14116.

**West Campus**

Waste protocols vary building to building at the OHSU West Campus. Most areas are responsible for autoclaving their own infectious waste before it enters into the conventional waste stream. If you dispose of biological waste in this manner, autoclaved biological waste should be in a clear bag and it must not have any biohazard warning signs associated with it. The exception is the VGTI where biohazardous waste can be placed in a red biohazard bag that must be closed with tape or a rubber band and is then picked up by custodial staff.

Small animal carcasses and tissues are bagged in clear bags. Carcasses and tissues that have been exposed to infectious agents are bagged in red bags, with any infectious agents clearly identified on the bag. Carcasses are brought to a freezer in the small animal vivarium.

Items routed to the normal waste stream, including waste that has been chemically decontaminated or previously autoclaved must be bagged in non-red bags and placed in the normal solid waste stream. Absolutely no red bags or obvious medical waste may be disposed of in the regular trash.

The waste-flow chart in Appendix F provides guidance on disposal of most research waste. Special consideration must be given to radioactive waste that is also biohazardous. This must be decontaminated
before it can be treated as normal radioactive waste, but the decontamination process must not aerosolize or volatilize the radionuclides. In general, radioactive material *may* be autoclaved, as long as radionuclides are not released in the process.
8 IMPORTATION, INTERSTATE SHIPMENT AND RECEIPT OF HUMAN ETIOLOGICAL AGENTS

All infectious agents and animal tissues/body fluids that may contain human pathogens that are imported, shipped or transported to or from OHSU should be performed in a safe manner and in compliance with all applicable regulations. This may include their transfer between OHSU campuses. The transportation of infectious agents is regulated by both state and federal agencies. Regulations regarding the ground shipment of potentially biohazardous permits are detailed in 49 CFR 171-180 and are enforced by the Department of Transportation (DOT). Materials transported by air must comply with International Air Transport Association (IATA) regulations as enforced by the Federal Aviation Administration. In addition, specific permits, such as those from the CDC or the United States Department of Agriculture (USDA), may be required. All NHP tissue that is shipped out of the country must conform with CITES regulations. Contact your campus Research Safety Program for more information on the permitting process.

Before a hazardous material is offered for transportation, all relevant persons involved in the preparation of samples for transportation must have received the appropriate training. Training is offered at OHSU and is necessary for any worker shipping biological, chemical or radioactive samples, particularly if any samples fall into the Dangerous Goods category. A fine may be levied against individuals who improperly prepare and/or ship a sample. Online training is available through Big Brain. Training records must be made available upon request to appropriate inspecting agencies. Refresher training must take place within 24 months of previous training to ensure that knowledge is current.

8.1 Transport Permits

Importation permits are required for certain infectious agents, biological materials and animals as outlined in U.S. Public Health Service, 42 CFR Part 71, Foreign Quarantine. In addition, a permit may be required through either the Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) or the CDC through its Etiologic Agent Import Permit Program (EIAPP) for importation and transportation of controlled materials, certain organisms or vectors. This includes animal and plant pathogens, certain tissue cultures and live animals. APHIS also regulates the importation, interstate movement, or environmental release of genetically engineered organisms as regulated under 7 CFR Part 340.

Packaging, shipment and transportation requirements for infectious substances, diagnostic specimens, biological products and genetically modified organisms are addressed in the Dangerous Goods Shipping training.

8.2 Hazardous Materials Transportation Guidelines

Numerous accidents occur involving the transportation of hazardous materials. These include accidents involving biological tissues and fluids. All hazardous materials must be transported in accordance with local, state and federal regulations. Biological agents should be brought to designated, trained employees for assistance with proper hazard determination, classification, packaging, labeling, marking and documentation. Only designated, trained individuals are authorized to prepare items classified as Dangerous Goods for shipment.
Some materials may not meet the definition of a Dangerous Good, but may still require special packaging and shipping procedures. Examples of materials in this category include biological samples or diagnostic specimens that are not likely to cause disease in humans or animals. A flow chart is provided on the next page as a guide to transporting hazardous materials.

**BIOLOGICAL SUBSTANCE TRANSPORTATION FLOW CHART**

- Is the sample known not to contain infectious substances?
- Have any pathogens been neutralized or inactivated?
- Are any microorganisms present non-pathogenic to humans/animals?
- Is it a dried blood spot or fecal occult blood?
- Is it an environmental sample that is not considered to pose a significant health risk?

**YES TO ALL**

- Not subject to transportation regulations unless it contains another hazardous substance (e.g., dry ice, hazardous chemical).

**NO TO ANY**

**Does it meet the definition of a Category A Substance?**

**YES**

- **Category A**
- UN 2814 infectious substance, affecting humans or UN 2900 infectious substance, affecting animals (as appropriate).
- **Packaging Instructions 602**

**NO**

**Is it a patient specimen for which there is only a minimal likelihood that pathogens are present?**

**YES**

- Exempt human specimen or Exempt animal specimen (as appropriate).
- **Triple packaging required**

**NO**

- Shipment containing dry ice must also conform to **Packaging Instructions 904**.

**8.3 Transport between OHSU Campuses**

Under no circumstances may materials be transported in a manner that requires or warrants the wearing of protective gloves or other protective clothing by the person transporting the material. No protective gloves such as latex gloves may be worn during transport.

**Personal vehicles** can be used to transport biological materials only if the samples are placed in a secondary container and this is placed into a third level of containment that should be used exclusively for sample transport and can be decontaminated by autoclaving or by chemical treatment. All containers must be labeled with a universal biohazard sign, positioned in such a way that the container remains
upright and placed as far away as possible from the driver (e.g., trunk of car). Rodents are not to be transported in personal vehicles if they are known to be infected with human pathogens. Personal vehicles can be used to transport live or dead rodents known to be free of human-related pathogens if properly labeled secondary containment that can be decontaminated by autoclaving or chemical treatment is used. Personal vehicles cannot be used to transport NHPs.

The Tram or OHSU Shuttle may be used to transport some hazardous materials between the Central and Waterfront Campus and other satellite locations. The following materials/items (Permitted Materials) may be transported by OHSU Riders on the Tram provided that, in each case, the transport complies with all applicable regulatory requirements, and with the limitations on quantity and the packaging and labeling requirements in the Tram Transport SOP.

1. Biological
   a) Blood units;
   b) Blood specimens;
   c) Tissue specimens (e.g., biopsy, frozen sections, Pathology, formalin fixed);
   d) Urine specimens.

2. Non-Biological
   a) Small amounts of chemicals or materials;
   b) Compressed oxygen for personal use; and
   c) Commercial and research pharmaceuticals and medications.

Packaging and labeling must meet all regulatory requirements. Containers of Permitted Materials must be securely closed and placed in a non-breakable secondary container such as a sealed plastic bag, which is securely closed. During transport, the secondary container must be placed in a third (outer) standardized container (“Outer Container”) approved and issued by EHRS, and the Outer Container all times during transport must be:

1) Free of all contamination;
2) Closed; and
3) Clean and with the label “Tram Transport” visible on two sides.

All legally required and appropriate labels must be placed immediately inside the Outer Container. This is accomplished by placing required labels on the secondary container. Questions concerning proper labeling of samples/products/materials should be presented to EHRS.

Trimet is not to be used to transport biohazardous materials. Trimet policy prohibits the transport of “any hazardous material, toxic chemical, combustible liquid, biological contagion or agent, radioactive substance or any other inherently dangerous substance onto a District Vehicle or other District property unless the person is a District employee or authorized personnel acting in the course of employment”.

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# 9 BIOSECURITY

Some measures are necessary to protect individuals that work in research laboratories and their visitors, and to prevent misuse of laboratory materials. All OHSU laboratories should have basic security measures in place to regulate access. Each laboratory should evaluate the need for additional security measures based upon the resources available in the laboratory and research being conducted. Additionally, proper signage should be posted alerting individuals of the potential biohazard risks in the laboratory.

## 9.1 Access

The OHSU ID Policy states that all students, employees, volunteers, contractors, and others doing business at OHSU must wear an OHSU ID badge at all times. For the security of your research and the safety of all involved, unknown individuals, especially those without a readily visible OHSU ID badge should be questioned regarding their presence in research areas.

Access to laboratories where pathogenic agents are stored or manipulated should be limited to authorized, trained and informed personnel. Any visitors should be escorted by an OHSU representative with training in proper biosafety procedures. Laboratories should have doors for access control and access should be restricted when work with biohazards is being conducted. Additionally, storage enclosures used for infectious materials need to be secured to prevent access by unauthorized persons and must be marked with the universal biohazard symbol.

Access to animal housing, use, and support areas is limited to authorized, trained, and informed personnel. Only those individuals with work-related requirements, and who have had the appropriate training, may be in animal areas unescorted. Visitors or service technicians who have not received training must be escorted by an OHSU representative trained in biosafety procedures at all times while in animal areas.

Some research requires additional security measures, such as research in animal areas, BSL3 laboratories, or work with Select Agents and Toxins. These additional measures will be covered in the training required for accessing those areas.

## 9.2 Signage

Biohazard warning labels must be affixed to containers of infectious waste, refrigerators and freezers containing tissue or body fluids, and other containers used to store, transport or ship tissue and body fluids. All labels must include the universal biohazard symbol. When appropriate, red bags or red containers incorporating the universal biohazard symbol may be substituted for labels.

Biohazard signs should be posted on the entrances to areas that contain potentially infectious materials. The entrance to any BSL2 work area where experimentally infected materials or animals are present should have a biohazard sign indicating the agent being used and names and phone numbers of responsible individuals to be contacted in an emergency should accompany the biohazard sign. BSL2 signage templates are provided in Appendix B. Animal care facilities will prepare and post signage indicating the agents in use and the minimum level of PPE that needs to be worn in animal areas.
The Occupational Health Program is provided for all employees in order to protect OHSU and its employees and to help create a safe work environment. The Occupational Health Program provides work required services free of cost, and other services may be provided for free or at a reduced rate. OHSU student employees, such as graduate students or medical students, should refer to Student Health for most services.

9.3 Select Agents and Toxins

The CDC and the USDA have determined that some biological agents and biologically derived toxins pose a significant risk to people, plants, or animals and have placed additional regulations on their use. The Public Health Security and Bioterrorism preparedness and Response Act of 2002 restricts the possession, use, handling, security and transfer of certain biological agents. The Act provides authority and responsibility to the CDC and USDA for regulating activities regarding select agents and toxins (SATs) in order to protect human and animal life. These two agencies have established separate as well as combined lists of agents that are subject to these additional regulatory requirements. A current list of SATs is found on the Select Agent website. Failure to comply with Select Agent regulatory requirements may lead to criminal and/or monetary penalties to both the individual and OHSU.

OHSU is registered with the CDC allowing individuals working in an OHSU facility to register to possess, use or transfer Select Agents. PIs may apply for registration for possession and use of Select Agents by contacting the Responsible Official (RO) for their campus. All individuals requesting access to Select Agents at an OHSU facility will need to coordinate with the RO to complete the required Bioterrorism Security Risk Assessment by the FBI. The transfer of Select Agents also requires prior written approval. PIs that work with Select Agents are required to provide to the following information to the RO upon request:

1. Names of all individuals with access to SATs,
2. Identifying information about the SATs,
3. Locations of the SATs (building, room and floor plan),
4. General research objectives related to the SATs, and
5. Any additional information requested by the RO that is necessary to fulfill Select Agent regulations.
REFERENCES


1. APPENDICES

Appendix A: Zoonotic Diseases

Every attempt is made to ensure that laboratory animals housed at OHSU are generally free of pathogens, but some animals still carry an inherit risk of being reservoirs of zoonotic organisms. The following is a table of diseases/agents and the laboratory animals in which they may be found:

<table>
<thead>
<tr>
<th></th>
<th>Rodents</th>
<th>Cats</th>
<th>Dogs</th>
<th>Ferrets</th>
<th>Sheep/Goats</th>
<th>Pigs</th>
<th>Birds</th>
<th>Reptiles/Amphibians</th>
<th>Non-human Primates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucellosis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Campylobacter</td>
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<td>Cat Scratch</td>
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<td>Erysipelas</td>
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<td>Filovirus</td>
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<tr>
<td>Herpes B Virus</td>
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<tr>
<td>Leptospirosis</td>
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<tr>
<td>Listeriosis</td>
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<td></td>
<td>Rabbits</td>
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<tr>
<td>Parasites</td>
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<tr>
<td>Pasteurellosis</td>
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<td>Psittacosis</td>
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<td>Q-Fever</td>
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<td>Salmonella</td>
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<td>SIV</td>
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<td>Tetanus</td>
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<tr>
<td>Toxoplasmosis</td>
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<tr>
<td>Tuberculosis</td>
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</tr>
</tbody>
</table>

Minimum Recommended PPE:

- Rodents (Mice, Rats, Rabbits, Guinea Pigs): eye protection, fiber facemask, gloves, body covering
- Cats: eye protection, fiber facemask, gloves, body covering.
- Dogs: eye protection, fiber facemask, gloves, body covering and shoe covers.
- Farm animals (cows, horses, pigs, sheep, etc.): eye protection, fiber facemask, gloves, body covering and shoe covers. Additional respiratory protection and PPE should be worn when working with fetal tissues or parturient sheep.
- Birds, Reptiles, and Amphibians: eye protection, fiber facemask, gloves, body covering.
• Non-Human Primates: Minimum PPE for entry to NHP areas varies and signage will be posted indicating the PPE requirements.

Disease details:

Descriptions of zoonotic diseases that can be found in laboratory animals are listed below. Non-Human Primate specific diseases are listed separately in the NHP section at the end of this appendix.

Brucellosis. This bacterial infection produces flu like symptoms and profuse sweating. Mortality is rare, but it is not unusual for patients to have some disability after recovery. Brucellosis is transmitted by direct contact with contaminated tissues or fluids.

Cat scratch disease. This disease is characterized by regional lymphadenitis that follows a skin papule at the site of the cat scratch. While the disease is self-limiting in most cases, a physician evaluation is recommended.

Campylobacteriosis. This disease is the leading cause of diarrhea in humans and animals. Symptoms include acute gastrointestinal illness that is usually self-limiting but can become quite serious. Transmission is typically oral-fecal so wearing appropriate PPE and practicing good personal hygiene habits can reduce the potential of acquiring this illness.

Erysipelas. This disease, found mainly in pigs, can be transmitted as a severe focal skin infection to humans. Symptomatic pigs should be handled with extreme caution.

Leptospirosis. This group of bacterial agents has common symptoms of sudden fever, headache, chills, sever muscle aches and other meningitis type symptoms. The illness can last from days to weeks, and untreated cases can take months to recover. Disease transmission occurs through contact of mucous membranes or broken skin with infected urine. Leptospirosis can be found in rats, mice, and ferrets in the wild.

Listeriosis. This is a serious disease that can cause meningitis and miscarriages when spread by oral-fecal contamination or by eating products from infected animals. The animals most commonly affected are sheep, cattle, swine and rabbits. A less serious problem is a rash on the hands and arms after direct contact with infectious material.

Parasites. Parasites such as larval migrans, tapeworms and sarcoptic mange are a potential risk to those handling infected animals. Infections from these agents are sometimes without symptoms.

Pasteurellosis. This disease is caused by a bacteria commonly carried in the respiratory tract and oropharynx of a large percentage of healthy cats and dogs that is spread by animal bites. Symptoms of infection include swelling and pain out of proportion to the visible wound and swollen lymph nodes with generalized infection. Onset is typically less than 24 hours after the bite occurs.

Psittacosis. This disease is found mainly in birds. All birds used in research should undergo appropriate quarantine and evaluation before being used for research or demonstration purposes.
Q fever. *Coxiella burnetii* is the causative agent of Q fever, which can be a serious disease in humans. The organism is shed abundantly from the placental membranes of sheep. Sheep used in reproductive research or other studies should be examined for possible infection. Infected individuals may be treated with antibiotics.

Rabies. While most cats and dogs used in research studies are vaccinated against rabies, large farm animals are not. When working with farm animals, pre-exposure rabies prophylaxis is encouraged.

Ringworm and other dermatomycoses. These agents cause fungal infections of the skin that are rarely serious. The fungus may survive for extended periods of time on inanimate objects. Wearing appropriate PPE and using good hand-washing practices will reduce the potential of acquiring these infections.

Salmonella. This bacterial disease is characterized by abdominal pain, fever, diarrhea and dehydration. Individuals with infection may be very ill for several days to weeks. Wearing appropriate PPE and using good hand-washing practices will reduce the potential of contracting this illness.

Tuberculosis. Many research animals are susceptible to acquiring TB infection from humans. TB is aerosol transmissible and presents a considerable risk to both human and animal populations. All persons entering animal areas require a TB test through Employee Health prior to having access granted and individuals who have access to NHP housing are monitored yearly for TB.

NHPs are extremely susceptible to TB, more so than healthy immunocompetent people. Naturally infected NHPs, or tissues/body fluids from these animals, can be potential sources for human infection. Precautions include TB testing programs in NHPs and humans, protective clothing and personal hygiene. Symptoms of an active TB infection include: weight loss, fatigue, malaise, fever/night sweats, coughing with thick sputum, possibly bloody, chest pain when breathing or coughing, or a cough that lasts longer than 2 weeks.

Tetanus. Employee health screens for a current tetanus shot, which is required for access to any animal area.

Toxoplasmosis. Cat feces are the most common source of the agent causing this disease. Other laboratory animals are not usually carriers of this agent unless they are experimentally exposed. Although this disease can be very serious in pregnant women it typically does not present unique symptoms. Pregnant women without immunity to toxoplasmosis should not be exposed to infected animals.

Non-human Primates

Appropriate PPE must be worn in all NHP housing and handling areas. Signage will be posted indicating the required minimum PPE for that area.

Special considerations for working with NHPs:
- Wear appropriate protective clothing. Work with at least one other person when handling NHPs. Minimize direct handling.
- Report any observed facial, lip or oral lesions in NHPs to a staff veterinarian.
- Immediately report all bites or scratches, contaminated needle/sharps sticks, and mucous membrane exposures involving macaques.
• Report any injury involving macaque tissues or body fluids that are associated with breaks in the skin or mucous membranes.

The following is a list of diseases/agents that are often associated with NHPs:

**Macacine herpes virus 1 (also called Herpes virus simiae, Herpes B, Monkey B or B virus).** The disease is 58-70% fatal in humans. B-virus is a member of the herpes group of viruses that occur naturally in macaques and possibly in other Old World monkeys. Infection with B-virus produces very mild disease in the macaque. Most have no obvious evidence of infection. Some macaques may have vesicles (small blisters) which progress to ulcers in the mouth, on the face, lips or genitals, and/or eye infection. These lesions spontaneously heal after a few days, but the virus typically resides permanently in the macaque and may periodically reactivate and cause ulcerative lesions. During these periods of active lesions the virus resides in the animal’s tissues or body fluids, and may be shed by the macaque to the environment. However, macaques without visible lesion symptoms may also shed the virus, so the Guidelines for Prevention of Herpesvirus simiae (B Virus) Infection in Monkey Handlers should be followed closely at all times when working with NHPs and NHP tissues/fluids. Transmission to humans occurs by exposure to contaminated macaque saliva tissue/fluids of infected macaques. Therefore, those working with macaque neural tissues or fluids are at an increase risk of exposure. The most likely routes of transmission are bites and scratches, however transmission may also occur through cuts or other breaks in the skin, or through direct contact with eyes or mucous membranes when handling infected tissues/fluids. Those at risk of contracting this disease include animal caretakers, laboratory personnel or anyone who is exposed to macaques or macaque tissues/fluids. Persons who are immunosuppressed because of medication or underlying medical conditions may be a higher risk for infection. The risk of acquiring B-virus from macaques is low if proper procedures are followed. Thousands of individuals have handled macaques and macaque tissues or body fluids since human infection with B-virus was first reported and very few cases of human infection have been described. The reasons for such an apparently low rate of transmission may include infrequent B-virus shedding by macaques, neutralizing activity in human sera against B-virus stimulated by herpes simplex virus infection and undetected asymptomatic infection. Given the potential for exposure, the number of reported human cases is very low. However, the majority of identified cases resulted in the development of encephalitis and death. Proper work practices markedly reduce the chances of infection. Symptoms of B-virus infection in humans include:

• Vesicular (small blister) skin lesions at or near the site of injury,
• Localized neurological symptoms such as pain, numbness or itching near the wound site,
• Flu-like aches and pains,
• Fever and chills,
• Headaches lasting more than 24 hours,
• Fatigue,
• Muscular incoordination,
• Shortness of breath, and
• Difficulty in swallowing.

If any symptoms characteristic of B virus occur following an injury involving a macaque, equipment contaminated with their secretions, or macaque tissues or body fluids, immediately report these to the veterinarian on call, and/or the laboratory supervisor and seek medical attention. Symptoms that occur even without overt exposure must be treated the same as a known exposure if there is a risk of infection
due to working in macaque areas. An aerosol exposure to the eye could be insidious but just as serious as one where there is a known splash to the eye.

For exposures to the skin (exposure is defined as any possibly fluid to fluid transmission), wash with soap and water continuously for a minimum of 15 minutes with a massaging motion. Examples of skin exposures could be bites, scratches (not necessarily bleeding) and needle sticks. Apply a clean sterile bandage if appropriate. If the exposure occurs in the eye or other mucous membrane, the injury site must be immediately flushed with water for a minimum of fifteen minutes. A specific post-exposure protocol for potential B-virus exposures has been developed and is posted in all areas where individuals work with macaques or macaque products.

**Enterics.** These infections are caused by various parasites and bacteria and are characterized by abdominal pain, diarrhea, fever and dehydration. Some examples of this are Shigella, giardia and cryptosporidia. Transmission is typically through the oral fecal route. While infections are typically self-limiting, severe illness may develop. A physician should evaluate the personnel with persistent dysentery symptoms.

**Filoviruses.** In humans, the diseases are marked by severe hemorrhagic fever and high death rates. However, human illness has not been associated with occupationally acquired exposures from infected macaques. Stringent procedures are taken to increase the level of worker protection during importation and quarantine of NHPs, particularly cynomolgus, rhesus macaques and African green monkeys. The OHSU NHP quarantine facility and its procedures are routinely inspected by the CDC to assure that the appropriate guidelines for handling of NHPs during transit and quarantine are being followed. Epidemiological and clinical experience with filovirus infection in humans is limited. Preliminary data suggests that the risk of illness is low, however there is a potential that must be considered. Adequate prescribed biosafety procedures should be followed for all tasks involving NHPs.

**Simian Immunodeficiency Virus (SIV).** SIV and HIV-infected non-human primates. In 1992, two workers were reported to have developed antibodies to SIV following exposures in different laboratories. One was associated with a needle stick, which occurred while the worker was manipulating a blood-contaminated needle after bleeding an SIV-infected macaque monkey. The other involved a laboratory worker who handled macaque SIV-infected blood specimens without gloves. Although no specific incident was recalled, this worker had dermatitis on the forearms and hands while working with the infected blood specimens. As of this writing neither of the two workers has developed any illness.

**Measles.** An unvaccinated NHP population is at risk for contracting measles from humans. Most individuals have had previous exposure to or vaccination against measles, so infection of the NHP population is not viewed as a significant risk at this time. Symptoms of an active measles infection include: running nose, cough, slight fever, eyes redden and become sensitive to light, fever that rises over time to a peak of 103F – 105F, red blotchy skin rash starting at the face and eventually spreading over the entire body, and/or white spots can occur inside of the mouth on the gums and the cheeks (Koplik spots). The disease is communicable by direct contact with fluids from the nose or mouth, and therefore can be aerosol transmissible and very contagious to NHP populations. Individuals with symptoms of measles should not share airspace with NHPs.

**Appendix B: BSL2 Signage**

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The universal Biohazard symbol must be affixed to incubators, freezers, refrigerators, and other equipment where viable BSL2 agents are stored. It is useful to provide a brief list of the agents being stored; for example, “lentiviral stocks; human cells; human tissue”, and up-to-date contact information.

A sign must be posted on the door to a BSL2 room and must be current. There may be situations where alerting other personnel to the presence of a BSL2 agent in use would be prudent. A sign can be posted near the work area on such an occasion. Example door signs are found on the following pages.

Stickers are available from Research Stores (Item # 142358), and in limited quantities from your campus Research Safety Program.
BSL-2 Laboratory

BIOHAZARD
Admittance to Authorized Personnel Only

Biological Agents:

Special Procedures, PPE
Or Precautions for Entry/Exit:

<table>
<thead>
<tr>
<th>Principal Investigator(s)</th>
<th>Emergency Contact</th>
<th>Protocol # (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Phone</td>
<td>Name</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Room No.: _
Date Posted: _
BSL-2 EXPERIMENT
IN PROGRESS

AUTHORIZED PERSONNEL ONLY

BIOHAZARD

Biological Agents (One or more of the following):

__________________________________________________________________
__________________________________________________________________
__________________________________________________________________
If rodents are brought into lab areas, a warning sign can be posted, which may alert personnel who have strong allergies to rodents. This is a fairly common allergy among laboratory workers, so be considerate.

HAZARD WARNING

MICE

MAY BE PRESENT IN THIS AREA
YOU MAY BE EXPOSED TO ANIMAL ALLERGENS
SUSCEPTIBLE INDIVIDUALS SHOULD TAKE PRECAUTIONS TO AVOID EXPOSURE.

For more information on occupational exposure to animal allergens contact Employee Health at 503-494-5271.
Appendix C: BSL2 Checklist

The following checklist is provided to identify items from the NIH/RAC guidelines and BMBL that BSL-2 laboratories should review annually. OHSU Biosafety Officers will assist PIs in the interpretation and implementation of these guidelines. It is useful to keep in mind that these guidelines represent a consensus view from prominent researchers, and that they can evolve as more information becomes available. PIs always have the right to petition OBA (in writing) for a relaxation of particular requirements if the petition is supported by a risk assessment. From a legal standpoint, documentation of compliance helps to establish due diligence in the event of a serious incident and follow-up investigation.
Appendix D. Chemical Disinfectants

There are many varieties and brands of commercially available chemical disinfectants. Formulations of standard disinfectants may change as companies develop “New and Improved” versions of their products. Below are tables from various sources that may help to choose which disinfectant may be most appropriate for a given situation.

The following tables provide information regarding the effectiveness, characteristics and applications of decontaminants available for use in laboratories. These tables should be used to determine the most appropriate product/system for the specific agent in use and the type of work being performed.

<table>
<thead>
<tr>
<th>Decontaminant</th>
<th>Conc. of Active Ingredient</th>
<th>Temperature (°C)</th>
<th>Relative Humidity (%)</th>
<th>Contact Time (Min)</th>
<th>Effective Against</th>
<th>Important Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoclave (15 lb/in^2)</td>
<td>Saturated steam</td>
<td>121</td>
<td>50-90</td>
<td>+ + + + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoclave (27 lb/in^2)</td>
<td>Saturated steam</td>
<td>132</td>
<td>10-20</td>
<td>+ + + + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry heat oven</td>
<td>N/A</td>
<td>160-180</td>
<td>180-240</td>
<td>+ + + + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incinerator</td>
<td>N/A</td>
<td>649-926</td>
<td>1-60+</td>
<td>+ + + + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV radiation (253.7 μm)*</td>
<td>40 μW/cm^2</td>
<td>10-30</td>
<td>+ + + ±</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>400-800 mg/l</td>
<td>35-60</td>
<td>30-60</td>
<td>+ + + + + +</td>
<td>+ +</td>
<td></td>
</tr>
<tr>
<td>Paraformaldehyde (gas)</td>
<td>0.3 g/ft^3</td>
<td>&gt;23</td>
<td>&gt;60</td>
<td>+ + + + + + +</td>
<td>+ +</td>
<td></td>
</tr>
<tr>
<td>Quaternary ammonium</td>
<td>0.1-2%</td>
<td>10-30</td>
<td>+ + +</td>
<td>+ + + + + + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>0.2-3%</td>
<td>10-30</td>
<td>+ + + ±</td>
<td>± + + + + + ±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorine compounds</td>
<td>0.01-5%</td>
<td>10-30</td>
<td>+ + + + ±</td>
<td>± + + + + ± +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodophor compounds</td>
<td>0.47%</td>
<td>10-30</td>
<td>+ + + ±</td>
<td>+ + + + + + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol (ethyl, isopropyl)</td>
<td>70-85%</td>
<td>10-30</td>
<td>+ + + ±</td>
<td>+ + + + + + +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formaldehyde (liquid)</td>
<td>7-8%</td>
<td>10-30</td>
<td>+ + + + ±</td>
<td>+ + + + + + ±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutaraldehyde</td>
<td>2%</td>
<td>10-600</td>
<td>+ + + + + + + + +</td>
<td>+ + + + + +</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ very positive response; ± less positive response. Blank denotes negative response/not applicable.

* Soil and other materials are not penetrated by UV radiation.
<table>
<thead>
<tr>
<th>Decontaminant</th>
<th>Conc. of Active Ingredient</th>
<th>Temperature (°C)</th>
<th>Relative Humidity (%)</th>
<th>Contact Time (Min)</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoclave (15 lb/in²)</td>
<td>Saturated steam</td>
<td>121</td>
<td>50-90</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>Autoclave (27 lb/in²)</td>
<td>Saturated steam</td>
<td>132</td>
<td>10-20</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>Dry heat oven</td>
<td>N/A</td>
<td>160-180</td>
<td>180-240</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Incinerator</td>
<td>N/A</td>
<td>649-926</td>
<td>1-60+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>UV radiation (253.7 μm)*</td>
<td>40 μW/cm²</td>
<td>10-30</td>
<td>±</td>
<td></td>
<td>±</td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>400-800 mg/l</td>
<td>35-60</td>
<td>30-60</td>
<td>105-240</td>
<td>±</td>
</tr>
<tr>
<td>Paraformaldehyde (gas)</td>
<td>0.3 g/ft³</td>
<td>&gt;23</td>
<td>&gt;60</td>
<td>60-180</td>
<td>+ +</td>
</tr>
<tr>
<td>Quaternary ammonium</td>
<td>0.1-2%</td>
<td>10-30</td>
<td>+</td>
<td>+</td>
<td>± +</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>0.2-3%</td>
<td>10-30</td>
<td>+ ± ± ±  ± ±</td>
<td>+ +</td>
<td></td>
</tr>
<tr>
<td>Chlorine compounds</td>
<td>0.01-5%</td>
<td>10-30</td>
<td>± + + ± ±</td>
<td>+ +</td>
<td></td>
</tr>
<tr>
<td>Iodophor compounds</td>
<td>0.47%</td>
<td>10-30</td>
<td>+ + ± + ±</td>
<td>± +</td>
<td></td>
</tr>
<tr>
<td>Alcohol (ethyl, isopropyl)</td>
<td>70-85%</td>
<td>10-30</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formaldehyde (liquid)*</td>
<td>7-8%</td>
<td>10-30</td>
<td>±</td>
<td>± ±</td>
<td></td>
</tr>
<tr>
<td>Glutaraldehyde</td>
<td>2%</td>
<td>10-600</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ very positive response; ± less positive response. Blank denotes negative response/not applicable.

* The pungent and irritating characteristics of formaldehyde preclude its use for biohazard spills.
<table>
<thead>
<tr>
<th>Agent</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Efficacy</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>Fast acting, Nonstaining</td>
<td>Flammable, drying, evaporates quickly</td>
<td>Excellent activity against gram + and gram- organisms; rapidly bactericidal against vegetative bacterial; virucidal, tuberculocidal, and fungicidal but not sporicidal</td>
<td>Intermediate level disinfectant. Used primarily for disinfecting external surfaces (e.g., stethoscopes) and as an antiseptic for disinfection of the skin</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>Sporidical at higher concentrations; by-products environmentally friendly</td>
<td>Oxidizing properties may be damaging to endoscopes; some reports of pseudo-membranous colitis associated with 3% hydrogen peroxide</td>
<td>Bactericidal, virucidal, fungicidal, sporicidal; MEC is 6% for 7.5% hydrogen peroxide and 0.85% phosphoric acid combination; no difference in germicidal effectiveness between 2% gluteraldehyde and 7.5% hydrogen peroxide; 30 min at 20°C HLD claim</td>
<td>Used for disinfection of soft contact lens, tonometers, and ventilators; 7.5% hydrogen peroxide/0.85% phosphoric acid solution acceptable for endoscope reprocessing unless incompatible with endoscopic material</td>
</tr>
<tr>
<td>Chlorine</td>
<td>Fast acting, low level of toxicity; low cost; broad spectrum activity</td>
<td>Corrosive; inactivated by organic material; unstable; efficacy decreases with increase in pH; CDC guidelines recommend that chlorine solutions be made fresh daily; Should not be used on instruments and medical devices</td>
<td>Effective against bacteria, fungi, mycobacteria, and viruses, high concentrations of chlorine required to kill mycobacterium tuberculosis; not sporicidal</td>
<td>Depending on concentration, is HLD, ILD, LLD Use 1 part bleach with 9 parts water (1:10 or 500 ppm) for disinfection of blood spills Use 1 part bleach to 99 parts water (1:100 or 5,000 ppm) for disinfection of countertops and floors</td>
</tr>
<tr>
<td>Iodophors</td>
<td>Rapid action; low toxicity and irritation; effective carrier</td>
<td>Corrosive; inactivated by organic material; staining; may burn tissue</td>
<td>Bactericidal, virucidal, mycobacteriocidal, fungicidal; not sporicidal</td>
<td>Primarily used as an antiseptic; has also been used to disinfect blood culture tubes, thermometers and hydrotherapy tanks; concentrations differ significantly between iodophor antiseptics and disinfectants; therefore antiseptic formulations are not indicated for disinfectant use</td>
</tr>
<tr>
<td>Phenolics</td>
<td>Broad spectrum</td>
<td>Leaves film on surfaces; inactivated by organic matter; may cause tissue irritation; corrosive to certain materials; has been associated with hyperbilirubinemia when used in nursery</td>
<td>Bactericidal, virucidal, fungicidal, and tuberculocidal; not sporicidal</td>
<td>Considered ILD to LLD; Used to decontaminate hospital environment (walls, floors, furnishings); should not be used on instruments and medical devices</td>
</tr>
<tr>
<td>Quaternary Ammonium Compounds</td>
<td>Good cleaning agent; less irritating to hands than some detergents</td>
<td>Not sporicidal, tuberculocidal, or virucidal against hydrophilic viruses; less microbiocidal in presence of organic matter; less microbiocidal in presence of materials such as gauze and cotton, incompatible with soap</td>
<td>Bactericidal, fungicidal; effective against lipophilic viruses</td>
<td>Considered LLD; Used for decontamination of hospital environment (walls, floors, furnishings); should not be used for disinfecting instruments and medical devices</td>
</tr>
<tr>
<td>Glutaraldehyde</td>
<td>Effective in presence of organic matter;</td>
<td>Unstable; may cause contact dermatitis</td>
<td>Bactericidal, fungicidal, virucidal,</td>
<td>HLD for medical equipment and instruments, endoscopes, RT</td>
</tr>
</tbody>
</table>

55
<table>
<thead>
<tr>
<th>Chemical</th>
<th>Description</th>
<th>Potential Carcinogen</th>
<th>Materials Compatibility</th>
<th>Compatibility Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formaldehyde</td>
<td>Broad spectrum; not affected by organic matter</td>
<td>No activation</td>
<td>Materials compatibility issues with lead, brass, copper, and zinc</td>
<td>Has been used for disinfecting hemodialyzers; consult with manufacturers for product compatibility</td>
</tr>
<tr>
<td>Paracetic Acid</td>
<td>By products not harmful; no residue; effective in presence of organic matter; sporicidal at low temperatures; rapid action</td>
<td>No activation required; mild odor</td>
<td>Stable over wide pH range of 3-9; irritating to eyes and nose; requires no activation; materials compatibility with wide range of devices</td>
<td></td>
</tr>
<tr>
<td>Orthopthaldehyde</td>
<td>Stable over wide pH range of 3-9; irritating to eyes and nose; requires no activation; materials compatibility with wide range of devices</td>
<td>Can be staining to skin and clothing</td>
<td>Excellent microbiocidal activity</td>
<td>Has FDA clearance for use as liquid HLD on flexible endoscopes</td>
</tr>
</tbody>
</table>
Appendix E: Spill Response Cue Cards

**Cut out cue cards and post in a highly visible work area**

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**SPILLS INSIDE THE BIOSAFETY CABINET**

1. Make sure the cabinet continues to operate. Wait 5 min. to allow aerosols to be pulled through the HEPA filter.
2. Decontaminate the surfaces within the cabinet wearing protective clothing. Gently cover the spill with absorbent paper towels and apply the appropriate disinfectant starting at the perimeter and working towards the center.
   * Note: Examine drain pan for contents of the spill. Disinfect if needed.
3. Discard soaked paper towels in a biohazard bag. Wipe up residual fluids. Wipe down surfaces with 70% EtOH, discarding towels in a biohazard bag.

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**SPILLS OUTSIDE THE BIOSAFETY CABINET**

**Small Spill (<10 mL, localized to small area)**

1. Alert personnel in the vicinity.
2. Check for contaminated clothing, including shoes. Decontaminate if necessary.
3. Evacuate the room. Close door. Discard potentially contaminated PPE, remove and decon any contaminated clothing. Wash hands.
4. Notify PI. Wait for 20 minutes to allow for room air exchanges to clear aerosols through room exhaust.
5. Don fresh PPE: lab coat or gown, gloves, mask, eye protection.
6. Cover spill with paper towels.
7. Soak paper towels with the appropriate disinfectant, from perimeter toward the center.
8. Allow 20 min. of contact time. Work can continue during contact time.
9. Discarded towels go in biohazard bags. Pick up sharps with tongs & place in sharps container.
10. Wipe down spill area one final time with appropriate disinfectant.
SPILLS OUTSIDE THE BSC
Major Spill (>10 mL, localized to small area)

1. Alert personnel in the vicinity.
2. Check for contaminated clothing, including shoes. Decontaminate if necessary.
4. Post warning sign: “DO NOT ENTER: Biological spill!”
5. Wait 20 min. Meanwhile, notify PI and a Biosafety Officer/Specialist (4-0655, 4-2580).
6. If assistance is needed, discuss with Biosafety Officer.
7. Don fresh PPE: lab coat or gown, gloves, mask, eye protection.
8. Re-enter the room, cover spill with paper towels.
9. Soak paper towels with appropriate disinfectant, from perimeter toward the center.
10. Allow 20 min. of contact time. Work can continue during contact time.
11. Discarded towels go in biohazard bags. Pick up sharps with tongs & place in sharps container.
12. Wipe down spill area one final time with appropriate disinfectant.
13. With PI, write up a report and submit to the Biosafety Officer.

SPILLS INSIDE AN INCUBATOR

Decontaminate water pan via autoclave.

1. Alert personnel in the vicinity.
2. Evacuate the room. Close door. Discard potentially contaminated PPE and remove any contaminated clothing. Wash hands thoroughly.
3. Notify PI.
4. Don fresh PPE: lab coat or gown, gloves, mask, eye protection.
5. Cover spill with paper towels.
6. Soak paper towels with appropriate disinfectant, from perimeter toward the center.
7. Allow 20 min. of contact time.
8. Discarded towels go in biohazard bags. Pick up sharps with tongs & place in sharps container.
9. Wipe down spill area one final time with appropriate disinfectant.
SPILLS INSIDE A CENTRIFUGE

1. Open lid of centrifuge slowly.
2. If there has been no breach of containment, spray rotor with 70% EtOH.
3. If inside of rotor is contaminated, decontaminate in the BSC. As a precautionary measure, decontaminate the centrifuge chamber.
4. If rotor buckets are damaged, close centrifuge lid.
5. Alert personnel in the vicinity. Evacuate room.
6. Wait 30 min. Meanwhile, notify PI and a Biosafety Officer/Specialist (4-0655, 4-2580).
7. If assistance is needed, discuss with Biosafety Officer.
8. Open lid slowly and add paper towels.
9. Spray walls of chamber and rotor with 70% EtOH.
10. Close centrifuge lid for 20 min. contact time.
11. Finish centrifuge clean-up as for major spill outside the BSC. Transport rotor to BSC.
12. Open and decontaminate rotor/buckets in the BSC.
13. With PI, write up a report and submit to Biosafety Officer.
Spills of Radioactive Biohazardous Material.

For small amounts of low-energy isotopes, like tritium or $^{14}\text{C}$, the radiation does not typically pose a significant hazard, so spills can be decontaminated first, following the guidelines for spills above. Radionuclides that tend to volatilize ($^{125}\text{I}$) pose a more significant hazard outside the BSC. All materials that contact the spill (paper towels, gloves, etc.) must be disposed of as radioactive waste. The area must be surveyed with a swipe test after clean-up to verify that background levels have been achieved. If radioactivity cannot be brought down to acceptable background levels, consult with Central Campus EHRS or the West Campus Research Safety Program on what to do next. For any mixed radioactive and biohazardous spill, consultation with both a Biosafety Officer and a Radiation Safety Officer may be helpful.

The immediate response is similar to any other biohazardous spill. Spills inside the BSC are “contained”, and all biologically decontaminated waste should go into Radioactive Waste. Spills outside the BSC are more problematic.

1. Stop work and ascertain the extent of the spill. Check your shoes with a survey meter. If the soles of your shoes are radioactive, remove them as you leave the room.
2. Evacuate the room, remove PPE on the way out, fold the contamination inside, and deposit it in a plastic bag or closable container.
3. Post a warning sign outside the door where the spill occurred.
4. Wash all potentially exposed skin, then monitor for radioactivity (if feasible).
5. Allow aerosols to settle for at least 30 minutes before reentering the laboratory. Assemble cleanup materials (disinfectant, forceps, paper towels, etc).
6. Put on PPE (gown, mask, gloves, shoe covers).
7. Cover the area with paper towels, and carefully pour disinfectant onto the paper towels, start at the perimeter and work toward the center. Allow at least 20 min. contact time.
8. Do Not use bleach solutions on iodinated ($^{125}\text{I}$) material: radioactive gas may be released. Instead, use an alternative disinfectant such as an iodophor or phenolic.
9. Handle any sharp objects with forceps. Wipe surrounding areas, where the spill may have splashed, with disinfectant.
10. Soak up the disinfectant and spill, and place the biologically decontaminated waste, along with all contaminated protective clothing, into an approved radiation waste container.
11. PPE must be biologically decontaminated prior to disposal as radioactive waste. Do Not autoclave the waste unless the Radiation Safety Officer approves this action. If waste cannot be autoclaved, add additional disinfectant to ensure biological decontamination of all the materials.
12. Wash hands and exposed skin areas with disinfectant; monitor personnel and spill area for residual radioactive contamination.
13. If skin contamination cannot be washed off, consult the Radiation Safety Officer.
14. Reusable items, like tube racks, pipetors, tip boxes, once decontaminated, can be cleaned as for non-biohazardous radioactive material.
15. Spray disposable items contaminated with radioactive materials with the appropriate disinfectant, allow for a 20 min contact time, and dispose of as radioactive waste.
<table>
<thead>
<tr>
<th>GENERAL WASTE</th>
<th>REGULATED MEDICAL WASTE</th>
<th>OTHER HAZARDOUS WASTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL disposable items not contaminated with infectious or otherwise hazardous waste, including:</td>
<td>ANY containers of liquid blood or any body fluid</td>
<td>Broken Glass</td>
</tr>
<tr>
<td>• ALL items containing blood or body fluids that cannot be expelled when squeezed or forcibly compressed</td>
<td>ANY items containing blood or body fluids that could be expelled when squeezed or forcibly compressed</td>
<td>Place in special broken glass containers. Seal filled containers and alert FBS for removal.</td>
</tr>
<tr>
<td>• Gloves, gowns, shoe covers</td>
<td>ALL cultures of infectious agents, including culture dishes and swabs or other devices used for transfer; inoculates and mix cultures</td>
<td>Radioactive Waste</td>
</tr>
<tr>
<td>• Paper towels</td>
<td>ANY potentially infectious materials not covered above</td>
<td>Segregate from other waste and shield as necessary. Call Environmental Health &amp; Radiation Safety (EHRS) for removal.</td>
</tr>
<tr>
<td>• Bench paper</td>
<td>ALL Biohazard Bags/Labels, new or used</td>
<td>Hazardous Chemical Waste</td>
</tr>
<tr>
<td>• Q-Tips, swabs</td>
<td>DO NOT INCLUDE:</td>
<td>Collect hazardous waste in appropriate sealed container(s). Call EHRS for removal.</td>
</tr>
<tr>
<td>• Plasticware</td>
<td>✓ garbage</td>
<td></td>
</tr>
<tr>
<td>✓ petri dishes</td>
<td>✓ other hazardous waste</td>
<td></td>
</tr>
<tr>
<td>✓ serological pipettes</td>
<td>✓ sharps</td>
<td></td>
</tr>
<tr>
<td>✓ micropipette tips</td>
<td>✓ sharps containers</td>
<td></td>
</tr>
<tr>
<td>✓ microcentrifuge tubes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>✓ plastic test tubes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Disposal Instructions**

**Regular scheduled removal by Facilities Custodial Services (FBS).**

Seal top of bag with rubber bands, or tape loosely shut. All contents must be fully enclosed and any holes must be sealed. Regular scheduled removal by FBS. Autoclaving is not necessary (but suggested for Risk Group2 agents; required for BSL2+).

Seal containers when ⅔ full. Call Kathy Croft (4-8813) for removal. Note: sharps containers should NOT be placed in red biohazard bags.

Bring to DCM for incineration.