EASTERN
WASHINGTON UNIVERSITY

# EWU Biosafety Manual

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# Emergency Contact Information

**PI/Lab Manager:** _____

**Daytime contact:** _____ **After hours contact:** _____

## Emergency

| | |
|---|---|
| **EWU Police Department** | **509-539-7676** |
| **Cheney Fire Department** | **509-498-9291** |
| **After-hours or weekends** | **911** |

## Medical

| | |
|---|---|
| Cheney Medical Center | 509-235-6151 |
| Sacred Heart Medical Center | 509-474-3131 |
| Deaconess Medical Center | 509-473-5800 |
| Providence Occupational Medicine - Airway Heights | 509-474-2650 |
| - Spokane | 509-474-5858 |
| - Spokane Valley | 509-944-8907 |

## Environmental Health and Safety

| | | | |
|---|---|---|---|
| Chad Johnson, Manager, Radiation Safety | 509-359-6455 | Cell | 509-359-5768 |
| Kathy Kees, Hazardous Waste Coordinator | 509-359-2788 | Cell | 509-220-7049 |
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## Electrical Shop Supervisor

| | | |
|---|---|---|
| Steve Williams | swilliams@ewu.edu | 509-359-4686 |

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|---|---|
| **Work Order Desk** | **509-359-2245** |
| **Hazardous Material Incident** | **509-359-6496** |
| **Poison Center** | **800-222-1222** |
| **National Emergency Response** | **800-424-8802** |
| **Washington Department of Ecology** | **509-329-3400** |

# 1. Introduction

## 1.1 Definitions

**Biohazards** are biological substances that pose a threat to the health of living organisms. They include infectious and 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, 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.

> Synthetic DNA segments that are not expressed in vivo as a biologically active polynucleotide or polypeptide products are exempt from the NIH Guidelines.

## 1.2 Purpose

This manual provides laboratory work practices and procedures that are necessary to ensure that EWU students and employees are protected from health hazards associated with biological agents used in laboratories. 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 EWU. 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 specific health and safety protocols to meet those requirements.

This manual contains a small amount of information regarding the use of animals in research; it should not be used in place of the information from the Institutional Animal Care and Use Committee (IACUC) or from animal facilities.

## 1.3 Roles and Responsibilities

Ensuring biosafety at EWU is a cooperative effort involving everyone at the university. The Biosafety Manual applies to all Principal Investigators (PIs), Project Directors (PDs), supervisors, laboratory employees, and students engaged in research involving potential biohazards.

### 1.3.1 Principle Investigators, Project Directors, and Supervisor

PIs, PDs, and supervisors (referred to as PIs from here) 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.

PIs are responsible for:

- Performing hazard assessments for their research project(s) and using the information to select appropriate laboratory practices, controls, and personal protective equipment to minimize the risk.
    - Providing personal protective equipment and safety equipment necessary for students and employees to perform their tasks safely.

- Ensuring all laboratory members have appropriate training. They may provide and document trainings themselves or request trainings from Environmental Health & Safety (EH&S).
    - Training documentation should be forwarded to EH&S annually for retention.
- Ensuring that their protocols have been appropriately documented, any approvals needed have been obtained, and that the protocols are reviewed and updated annually.

### 1.3.2 Employees and Students

Individuals that work with biohazardous materials have a responsibility to follow the guidelines in this manual and the protocols in their laboratories.

Employees and students are responsible for:

- Attending required safety trainings.
- Consulting with their PI or EH&S if they have questions regarding risks associated with their assigned tasks.
- Following all safety procedures while working.
- Staying alert, while performing their duties, for previously unrecognized hazards.
- Bringing any concerns and suggestions to their PI.

### 1.3.3 Environmental Health & Safety

EH&S is responsible for inspecting laboratories annually to ensure they are being maintained in a manner that will provide a safe working environment for students and employees.

EH&S provides training for general laboratory safety and Bloodborne Pathogen Awareness. EH&S is available to provide specialized trainings, and to help PIs develop trainings specific to their lab environment and work.

### 1.3.4 Institutional Biosafety Committee

EWU policy 302-07 established the Institutional Biosafety Committee (IBC) and authorized it to ensure research involving biohazardous materials is performed safely.

The IBC is responsible for:

- Reviewing proposed research using rDNA, SNA, and/or biohazardous materials and issuing approval or disapproval of proposed research.
    - Modifications may be requested where necessary to allow approval.
- Performing risk assessments and setting containment levels for experiments as specified in the NIH Guidelines (see section 1.4 below).
- Reporting violations or significant research related accidents and illnesses to the NIH Office of Science Policy.
- Investigating complaints or concerns and making recommendations.
- Ensuring individuals involved in approved research have appropriate training.
- Adopting emergency plans for approved research.

## 1.4 Regulations and Guidelines

**National Institute of Health (NIH): *Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines).** These guidelines address the safe conduct of research that involves construction and handling of recombinant DNA molecules and organisms containing them.

**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.

Washington State Department of Labor & Industries (L&I) has specific regulations involving *Occupational Exposure to Bloodborne Pathogens* WAC 296-823, and labeling requirements to *Protect employees from biological agents* WAC 296-800-10045. *Safety Standards for Core Rules* WAC 296-800 has many workplace safety requirements outlined in it, including requirements for PPE WAC 296-800-160. There are also requirements relating to *Hazardous Chemicals in Laboratories in* WAC 296-828.

PIs should be familiar with the BMLB and any regulations that are relevant to their research. Any PIs with research involving recombinant DNA must be familiar with, and adhere to, the NIH Guidelines. PIs with questions should contact EH&S or the IBC for assistance.

# 2. Training

According to state and federal laws, all researchers at EWU must be adequately trained to understand the nature of hazards found in their work area and procedures for safe handling them. Training must occur when new laboratory members arrive and when new hazards are introduced. Refresher training should be offered periodically and may be required by law for personnel who do not follow required procedures.

## 2.1 General Trainings

EH&S or PIs can provide Laboratory Safety Training which is mandatory for everyone working in labs.

EH&S provides Bloodborne Pathogen Training (BBP), which is mandatory for anyone who could be exposed to, or works with, human fluids and tissues. BBP training is required annually according to WAC 296-823.

- PIs who have completed BBP training can provide training to their students.

Anyone participating in research involving biological hazards must complete a Collaborative Institutional Training Initiative (CITI) training course online. Please follow this link to begin the registration process and select Eastern Washington University as your affiliated institution. Once registered, you can then select your curriculum. Options to select multiple courses exist, but at a minimum, the following classes need to be completed based on the types of research being conducted:

- PIs, staff, and graduate students must take *Training for Investigators, Staff and Students Handling Biohazards.*
- Undergraduate students must take *Biohazards and Biosafety for Students.*
- Researchers and students working with animals must take *Animal Biosafety.*
- EWU Biosafety Committee members must take *Institutional Biosafety Committee Member Course.*
- Members of the IACUC committee must take *Essentials for IACUC Members.*

Additional CITI training may be required for certain research. For example, specific training classes are needed for researchers and students working with fish or amphibians. Please consult with your PI or EH&S for further information on recommended courses based on the type of research being performed.

### 2.2 Laboratory-Specific Training

It is the responsibility of PIs to ensure that all research personnel under their direction are trained to work safely. Training should include safe microbiological practices in accordance with NIH guidelines and BMBL, safety precautions for lab specific protocols, and the appropriate response to spills or accidents. This responsibility may be delegated at the discretion of the PI, but the PI will be held responsible for laboratory-acquired infections where the root cause was lack of training.

All laboratory-specific trainings must be documented with the names of the trainees, the trainer, and the date. Training records should be sent annually to EH&S where they will be maintained. The PI must also keep a copy of the safety training content, such as a lesson outline or summary and a copy of the applicable standard operating procedure, to demonstrate the scope of the training.

### 2.3 Animal Handling

All personnel who work with animals will need to complete trainings required by IACUC. Training on animal use in research is conducted online through the CITI. Check with the IACUC chair or your faculty advisor regarding department and/or institutional requirements for animal care and use training.

## 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.

### 3.1 Risk Assessment

A risk assessment is a process of identifying potential hazards and determining the probable result of exposure or release of each hazard. The probability of exposure to the hazard and the result and severity of exposure should be considered in the risk assessment. Risk assessments for biohazards should include potential adverse effect on human health or the environment associated with exposure to or release of a pathogen or genetically modified organism (GMO).

Risk assessments must be conducted as part of the planning phase for new research projects; the risk assessment is used to determine the appropriate work precautions and PPE needed to protect people and the environment. The risk assessment should be included in the Standard Operating Procedures for the lab and sent with other submission documents to the IBC for projects requiring their approval.

Risk assessments should be carried out on a case-by-case basis (each new organism or project needs its own risk assessment) and should be based on known scientific facts. The biological risk assessment is a six-step process; each new step takes into account the results of the previous steps.

1. Hazard Identification – Identify characteristics of the organism and project which could cause adverse effects on human health or the environment, the nature of these effects, and the pathways of exposure.

2. Hazard Characterization – Evaluate the potential consequences of each identified hazard.
3. Exposure Characterization – Evaluate the likelihood of exposure.
4. Risk Characterization – Evaluate the risks associated with each step of a procedure and the overall risk of the process as a whole.

## 3.2 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). There are no BSL3 or BSL4 facilities at EWU.

**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 E. coli K12 strains, yeast S. cerevisiae and S. pombe, insect Sf9 cells, and helper-free adeno-associated viral (AAV) vectors.

**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.

**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: Mycobacterium tuberculosis (TB), Coxiella burnetii, and West Nile Virus.

**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.

Below is a table with information about BSL1 and BSL2, these are the two levels of biosafety being used at EWU. An extensive list of agents assigned to BSL levels 1-4 is provided in Appendix B of the NIH Guidelines.

| BSL | Agents | Practices | Safety Equipment | Facilities |
|---|---|---|---|---|
| 1 | Not known to consistently cause disease in healthy human adults | Standard Microbiological Practices | PPE:<br>• Lab coats; gloves; face protection as needed | Laboratory bench, furniture with non-porous surfaces, and sink |
| 2 | • Agents associated with human disease<br>• Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure | BLS1 practice plus:<br>• Limited access<br>• Biohazard warning signs<br>• Biosafety Manual defining any needed waste decontamination or medical surveillance policies | Primary Barriers:<br>• Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splash or aerosols of infectious materials<br>PPE:<br>• Lab coats; gloves; face protection as needed | BSL1 plus:<br>• Autoclave available |

**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.
- Mouth pipetting is prohibited; mechanical pipetting 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.

## 3.3 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 5µm 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 daily and 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.

***Pipetting*** can introduce aerosols and splashes. Micropipettes 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, Lyophilization, 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 in a biosafety cabinet.

## 3.4 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.

### 3.4.1 Biosafety Cabinets

A 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 so BSCs are not suitable for use as fume hoods. Likewise, chemical hoods are not suitable for use as a BSC.

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, sonicating, 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.

BSCs must be certified annually. Any BSC with out of date certification must not be used until it has been recertified. Any BSC that fails certification must not be used until it has been repaired and recertified. Use of a BSC without up-to-date certification could jeopardize the health of students and employees, as well as the environment.

### 3.4.2 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 barrier between the worker and a sample containing an infectious agent.

***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. Arrangements should be made within your department to insure proper laundering procedures are followed.
- It is recommended that the end of lab coat sleeves be tucked into gloves prior to lab work.

***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, the PI or lab supervisor must choose gloves appropriate to the tasks. Contact EH&S for help with proper glove selection.
- Alternatives to latex gloves should be available.
- Gloves should be changed whenever they become contaminated, their integrity becomes compromised, or whenever 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 must 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, contact EH&S if your work requires a respirator.

### 3.5 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. Carpeting and upholstered furniture are strictly prohibited in research facilities.

## 4. Exposure Sources

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[2]. Many of these instances were associated with research or clinical work focused on a specific infectious agent. BMBL (6th ed.) and Pike[2] 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 yearly. 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. *Occupational Exposure to Bloodborne Pathogens* *WAC 296-823* requires that all workplaces with potential occupational exposures to human tissues or body fluids perform an Exposure Determination. Occupational exposures is defined 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
- Tissues

- Synovial fluid (around joints)
- Pericardial fluid (around the heart)
- Organs
- Semen
- Saliva from dental procedures
- Vaginal secretions
- Cerebrospinal fluid (CSF)
- Amniotic fluid (around a fetus)

OPMI also includes: HIV-containing cells or tissue cultures, organ cultures, HIV- or HBV-containing culture medium or solutions; blood, organs, or other tissues from experimental animals infected with HIV or HBV; blood, organs, or other tissues from experimental animals infected with any other bloodborne pathogen.

**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. There are cures available for most HCV strains but the cost is quite high, most insurance companies will only cover the cost of the cure after extensive liver damage.

**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.

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.

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, especially 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 animals to avoid accidental 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 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 EWU representative trained in biosafety procedures at all times while in animal areas.

## 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[3]. 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[4]. 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. 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[5]. 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

***Hepatitis B Vaccination.*** In compliance with *Occupational Exposure to Bloodborne Pathogens* *WAC 296-823-13005*, EWU will make the HBV vaccine and vaccination series available to all EWU 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 Hepatitis B Vaccination Declination.

***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 Providence Occupational Medicine. EWU 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.

For exposures arising from work with live animals, report the incident to the supervisor and the attending veterinarian. Human tissue related incidents should be reported to the PI or supervisor as a potential Bloodborne Pathogen exposure.

All incidents must be reported on EWU Incident Report form. The purpose of Incident Reporting is to expedite and optimize post-exposure treatment as well as to alert EWU management to conditions that could lead to further injuries or illnesses. The Incident Report assists the safety committee in reviewing the incident for suggestions of risk reduction strategies. Information about Incident Reporting can be found on the EH&S website, here.

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 Health Care Professionals

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

1. A copy of Post-exposure Requirements (to Bloodborne Pathogens) WAC 296-823-160.

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, EWU will document that consent was not given. 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.

Records (from Occupational Exposure to Bloodborne Pathogens) WAC 296-823-170 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 Environmental Health and Safety Department is responsible for keeping records on human tissue/body fluid exposures. This record consists of the following:

1. The name and employee 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 EWU 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.

EWU 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. EWU will maintain these records for the duration of employment plus 30 years.

# 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 or 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.

### 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% ethanol 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 ethanol to 100% does not help because the increased concentration of ethanol decreases the time before evaporation. 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 stain absorbent material, such as a lab coat.

**Phenolics**-based disinfectants, like **Amphyl**, 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.

### 6.1.2 Autoclave Use and Requirements

EWU 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 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 or container 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/container 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. Autoclaves must be certified annually.

## 6.2 Spills and Spill Kits

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.

EWU requires all laboratory workers to know the location of the nearest spill kit. Employees and students must be trained in spill response procedures when they start working in the laboratory and the protocol should be reviewed periodically. Certification of trainings must be kept by the PI or supervisor and included in the annual training record sent to EH&S. The location of a laboratory's spill kit should be found in the Chemical Hygiene Plan for that laboratory.

### 6.2.1 BSL1 Spills

These spills should be relatively easy to deal with. Generally biological spills in a BSL1 environment can be dealt with using the following steps.

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.

### 6.2.2 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, and biohazards with toxic chemicals. In addition, labs working with biological toxins must have Standard Operating Procedures for decontamination of those agents.

Templates for, and examples of, SOPs can be found on the EH&S website.

### 6.2.3 Incident Response and Reporting

Spills of biohazardous materials may or may not involve injury or overt exposure. Spills of infectious agents can present scenarios in which there is a possibility of exposure via aerosols or inadvertent contact during spill decontamination. Anyone cleaning up biohazardous spills should be aware of the symptoms of exposure to the agent. Any symptoms consistent with an infectious agent that was recently spilled should be reported. For detailed information on follow-up after an injury or overt exposure, refer to 5.2 Post-Exposure Evaluation and Follow-up.

EWU requires that any spill be documented with an Incident Report, these reports will be used to identify areas where protocols may need to be altered to protect students and employees. The Incident Reports are also necessary for reporting to state and federal agencies.

Additional reporting requirements are imposed by NIH/OBA for incidents involving recombinant DNA or BSL2 infectious agents. The IBC and EH&S work together to report spills, accidents, and exposures as required by NIH/OBA. **Please note that incidents involving documented exposure to a BSL2 agent MUST BE REPORTED TO the IBC and EH&S IMMEDIATELY.** This is mandated by the NIH.

## 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:

1. 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.
2. 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.
3. 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.
4. 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.

5. 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.

## 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 and labeled with the contents. Bags used to hold biohazardous waste must be either red or orange in color, autoclavable, and be marked with the universal biohazard symbol. The EWU procedure for dealing with biological waste can be found in the Exposure Control Plan on the EH&S website.

Additional guidance for dealing with anatomy lab wastes can be found here: Anatomy Lab Waste Disposal.

**Liquid waste** containing biohazardous material should be decontaminated with bleach or another effective disinfectant or autoclaved (see 6.1.2 Autoclave Use and Requirements for more details) prior to disposal (exceptions are 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 needs to be disposed of through EH&S. **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 before they are ¾ full and a new sharps container provided. Syringes used without needles are still considered “sharps”, and must be disposed of in sharps containers. Most biohazard sharps on campus are autoclaved prior to disposal. Contact the departmental autoclave operator for autoclaving. The autoclave operator will contact EH&S for final disposal in these cases. If the sharps container will not be autoclaved, contact EH&S for pickup of full containers. More information about EWU sharps disposal policy can be found in the Sharps procedure on the EH&S website.

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

# 8. Biosecurity

Some measures are necessary to protect individuals that work in research laboratories and their visitors, and to prevent misuse of laboratory materials. All EWU 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.

## 8.1 Access

Access to laboratories where pathogenic agents are stored or manipulated should be limited to authorized, trained and informed personnel. Personnel with training in proper biosafety procedures should escort any visitors. Laboratory 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. Personnel with training in biosafety procedures will escort any visitors or service technicians in animal areas.

## 8.2 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 must have a Hazard Door Sign. Rooms where biohazard work is temporary should post a Biohazard Work in Progress Door Sign during biohazard work. Animal care facilities should prepare and post signage indicating the agents in use and the minimum level of PPE that needs to be worn in animal areas.

# References

1. Biosafety in the Laboratory: Prudent Practices for the Handling and Disposal of Infectious Materials. 1989. National Research Council. National Academy Press, Washington DC

2. Pike, R.M. 1979. Laboratory-associated Infections: Incidence, Fatalities, Causes, and Prevention. Ann. Rev. Microbiol. 33: 41-66.

3. Hollander, A. et al., 1996. Cat and Dog Allergy and Total IgE Risk Factors for Laboratory Animal Allergy. J. Allergy Clin Immunol. 98:545-54.

4. Agrup, G. et al. 1986. Allergy to Laboratory Animals in Laboratory Technicians and Animal Keepers. B. J. Ind. Med. 43: 192-98.

5. Eggleston, P.A., et al., 1989. Task-related Variation in Airborne Concentrations of Laboratory Animal Allergens: Studies with Rat n l. J Allergy Clin Immunol. 84:347-52.