Biosafety Manual

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Contents

Foreword

This Biosafety Manual has been adopted by the University of Wisconsin Oshkosh (UWO) to be a resource for information on policies, procedures, and guidelines to facilitate safe laboratory activities involving biological agents. Adherence to this Biosafety Manual will help eliminate or reduce the potential for exposures to individuals working in a laboratory, as well as protect the campus and surrounding community. The Institutional Biosafety Committee (IBC) has developed this manual to help ensure compliance with federal, state, and local regulations.

The UWO Biosafety Manual should not be considered the only reference for laboratory safety concerns. It is intended that the Principal Investigator (PI) will supplement this information with instruction and guidance regarding specific procedures and practices unique to the work being done in their research laboratories and classrooms. Additionally, the IBC is always available to assist with any health and safety concerns.

Contact Information and Campus Resources

If you have any questions regarding the IBC, biosafety, or this Biosafety Manual, please contact:

- **IBC Administrator**
  - Kelly Schill
    - (920) 424-3375
    - biosafety@uwosh.edu
- **IBC Chair**
  - Eric Matson
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    - matsone@uwosh.edu
- **IBC Biological Safety Officer**
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Also, the IBC website contains information related to the IBC, including IBC submission process, meetings, training, forms, and material transfer agreements.

Section 1 - Principles of Biosafety

A fundamental objective of any biosafety program is the containment of potentially harmful biological agents. The UWO biosafety manual is meant to provide information on the containment of biological agents achieved by using engineering controls, procedural controls, and personal protective equipment. A well-functioning

biosafety program will prevent the exposure of students, researchers, and community members, to biological agents used on campus.

1.1 - Risk Assessment

A risk assessment is a process used to identify the hazard characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a Laboratory Acquired Infection (LAI), and the probable consequences of such an infection. The information identified by risk assessment will provide a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs.

Risks should be identified and stated in an objective manner so that they can be well understood (e.g., possible exposure to bloodborne pathogens), and not in a subjective manner (e.g., the risk is low).

It is important to consider both agent and laboratory procedure hazards when performing a risk assessment and selecting the precautionary measures that will be used. In addition, the capability of the laboratory staff to control hazards (i.e. training, technical proficiency, and good habits of all members of the laboratory) and the operational integrity of containment equipment and facility safeguards need to be considered.

1.2 - Biosafety Levels (BSL)

The following is a general description of containment requirements as they correspond to the various biosafety levels. More specific descriptions and requirements can be found in other sections of this manual regarding various work, and also in the Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL).

> Biosafety Level 1 (BSL1)
> Containment conditions suitable for work involving well-characterized biological agents or materials that are not known to cause disease in healthy adult humans, and for which there is minimal potential hazard to laboratory personnel and the environment. The laboratory is not necessarily separated from the general traffic patterns in the building. Work is generally conducted on open bench tops using standard microbiological practices with minimal personal protective equipment (PPE) of lab coat/smock and eye protection, and gloves as needed. Special containment equipment or facility design is not required nor generally used. Laboratory personnel have specific training in the procedures to be conducted in the laboratory and are supervised by

someone with training in microbiology. Staff training includes information on the potential risks of BSL1 agents to immunocompromised individuals.

Biosafety Level 2 (BSL2)
Similar to BSL1 1 but involves work with agents of moderate potential hazard to personnel and the environment. BSL2 differs from BSL1 in that: (i) laboratory personnel have specific training in handling biological agents that are or may be potentially infectious, (ii) access to the laboratory is limited when work is being conducted, (iii) extreme precautions are taken with contaminated sharp items, and (iv) certain procedures in which infectious aerosols or splashes may be created are conducted in a BSC or other primary containment device, (v) PPE for BSL2 work usually consists of gloves, lab coat, and eye protection. Respiratory protection may be used in some circumstances where containment equipment is not feasible.

1.3 – Animal Biosafety Levels (ABSL)

The following is a general description of containment requirements as they correspond to the various animal biosafety levels. More specific descriptions and requirements can be found in other sections of this manual regarding various work, and also in the Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL).

Animal Biosafety Level 1 (ABSL1)
ABSL1 is suitable for work in animals involving well-characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment.
ABSL1 facilities are separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required as determined by appropriate risk assessment. (See Section 2, Biological Risk Assessment.)
Personnel must have specific training in animal facility procedures and must be supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

Animal Biosafety Level 2 (ABSL2)
ABSL2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. An important difference to consider when planning experiments at ABSL2, is that it differs from ABSL1 in that all manipulations of animals infected with agents needs to be conducted inside of containment (e.g., Biological Safety Cabinet).

1.4 - Risk Groups

The description of the hazards which a biological agent poses to healthy adult humans; there are no risk groups for work with animals or plants. Risk Group definitions can also be found in the Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL).

Risk Group 1: Biological agents or materials not associated with disease in healthy adult humans.

Risk Group 2: Biological agents or materials associated with human disease in healthy adult humans.

Risk Group 3: Biological agents or materials associated with serious or lethal human disease for which preventative or therapeutic interventions may be available (high individual risk but low community risk). There is no work at UWO on any of these organisms.

Risk Group 4: Biological agents or materials likely to cause serious or lethal human disease for which preventative or therapeutic interventions are not usually available (high individual risk and high community risk). There is no work at UWO on any of these organisms.

1.5 - Containment Considerations

Biosafety Levels are similar but not necessarily equal to Risk Groups. In general, start at the Biosafety Level which corresponds with the Risk Group (e.g., BSL2 for a RG2 agent) and then modify the containment as necessary after considering the factors to consider described in the next sub-section.

1.6 – Factors to Consider

Agent stability - The ability of an organism to survive environmental stress and chemical disinfection (e.g., spores are very resistant to desiccation and chemical disinfection).

Animal Study Data - The use of safety data acquired from other animals may be useful in a risk assessment if there is no safety data available from humans on the effect of biological agents. However, exercise caution when translating infectivity data between humans and other animals, as they may be differently affected after an exposure.

Concentration - The number of infectious organisms per unit volume being manipulated is directly correlated with an increase in risk of an infection after an exposure.

Infectious Dose - The number of organisms required to cause an infection. This is typically measured in the number of microorganisms required to cause an infection in 50% of a host population (ID_{50}).

Medical Surveillance - Monitoring the health of employees may be recommended in some instances to determine if engineering or procedural controls are effective (e.g., serum banking); also, post-exposure health monitoring may be required to ensure that an exposure does not result in an infection.

Pathogenicity – The ability of an organism to cause disease. This ability can vary between individuals and may depend on their immune status (e.g., vaccination, compromised immune system).

Personnel Skill/Training - Assessing the skill and training of all of the personnel (e.g., laboratorians, students, animal husbandry staff, maintenance, and custodians) who have the potential to be exposed to an agent; do all personnel with access to an agent have the proper skills and training to ensure they can safety work with/around the agent? Does the agent need to be locked to prevent access?

Prophylaxis - Availability of measures which can reduce the likelihood of an infection resulting from an exposure. If vaccines are available to protect against infections that could result from laboratory exposure, the vaccines must be offered to employees who are at risk of exposure. Non-vaccine prophylactic measures may be available for use; healthcare professionals are a good source for guidance. Prophylaxis is an example of a procedural control.

Route of Transmission - The means in which an organism typically causes an infection. This is important to consider in conjunction with the procedures which will be performed with an organism (e.g., aerosols are generated whenever force is applied to a liquid; therefore, engineering controls should be used if an organism is spread via aerosols). However, consider that an organism may be spread in a different manner as it would normally be spread in nature; consequently, the organism may be able to cause an infection via a different means than usually expected.

Toxicity - The degree in which a substance can cause illness. Toxins which act in an acute manner are typically assessed by the amount of toxin which would be a Lethal Dose to 50% of a population (LD_{50}).

Virulence - The degree of pathology caused by an organism. This degree may vary between individuals (e.g., women who are pregnant are more at risk from exposure to *Listeria monocytogenes,* which can cause fetal death).

1.7 – Infectious/Potentially Infectious Materials to Consider

The characteristics of most known infectious agents have been well identified. Information useful to risk assessment can be obtained from laboratory investigations, disease surveillance, and epidemiological studies.

Materials Containing Unknown Infectious Agents – The appropriate biosafety level must be determined with any available information. Often these are clinical or environmental specimens. Some questions that may help in this risk assessment include:

- Where was the sample acquired?
- Why is an infectious agent suspected?
- What epidemiological data are available?
- What route of transmission is indicated?
- What is the morbidity or mortality rate associated with the agent?
- What medical data are available?

The responses to these questions may identify the agent or a surrogate agent whose existing agent risk assessment can be used to determine a biosafety level. A conservative approach is recommended in the absence of data.

Materials Containing Recombinant DNA Molecules – This category of agents includes microorganisms and cells that have been genetically modified through recombinant DNA technologies. Techniques and technologies are constantly being developed to modify DNA which may add risk to experiments.

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules is the key reference in establishing an appropriate biosafety level for work involving recombinant microorganisms.

First, establish the classification of the non-modified organism, and then analyze the impact the genetic modification will have.

Some points to consider for work involving recombinant microorganisms are:

- Does the inserted gene encode a known toxin or a relatively uncharacterized toxin?
- Does the modification have the potential to alter the host range or cell tropism of the organism?
- Does the modification have the potential to increase the replication capacity of the organism?
- Does an inserted gene encode a known oncogene?
- Does an inserted gene have the potential for altering the cell cycle?
- Does DNA integrate into the host genome?

1.8 – Animal Studies

There are several hazards which are specific to working with animals. The following are descriptions of several of these hazards:

Allergens- The development of allergies is a common health risk resulting from working with research animals. As many as one third of animal care workers will develop allergies to animals in their facilities. Additionally, about one tenth of these workers will develop asthma.
Limiting exposure to animal allergens is the most effective way to control or prevent the development of allergies. The following are controls which can be used to reduce exposures to allergens:

- Handle animals inside containment devices
    - Biological Safety Cabinet
    - Down or Backdraft Table
    - Fume hood
- Rigorous and proper use of Personal Protective Equipment
    - Gloves
    - Gowns / Lab coats
    - Respirators (e.g., N95, N100, PAPR)
- Good hygiene and housekeeping
    - Maintaining clean work areas
    - Laundering reusable lab coats

Contact your supervisor if you feel that you are experiencing any allergies to research animals.

Bites and Scratches- Animal behavior is often unpredictable; to minimize the risk of an injury all researchers need to be properly trained in animal handling, restraint techniques, and any techniques particular to the species being handled. Additionally, all researchers should be trained in general first aid and be knowledgeable of whom to contact in the event of an injury.

Protocol Related Hazards- Research with animals can create new opportunities for researchers to be exposed to biological materials administered to the animals. The following hazards need to be considered when conducting a risk assessment:

- Exposure to biological materials from:
    - Bites and scratches
    - Contaminated bedding
        - Inhalation
        - Ingestion
        - Mucous membrane

Animals which are administered human source materials are typically immunocompromised because this prevents rejection of the foreign cells. When administering human source materials to these animals, any pathogens present may multiply to very high titers inside the animal and increase exposure to the pathogen during subsequent animal handling. A risk assessment must take this possibility into consideration.

Zoonosis- Infectious agents may be present in research animals. This is more likely when conducting research with animals which are caught in the wild. Exposure routes for zoonotic agents are identical to the hazards described in "Protocol Related Hazards".

Animal Experiments covered under the NIH Guidelines:
NIH Office of Science Policy published a helpful table for animal experiments covered under the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules which includes the minimum biosafety level and section of the NIH Guidelines to reference:
https://osp.od.nih.gov/wp-content/uploads/Animal_Activities_Table.pdf

1.9 – Risk Mitigation

After a comprehensive risk assessment has been conducted, the next step to take is to determine how risks will be mitigated. There are four means which can be used to mitigate the risk of exposure to biological materials, and they should be considered in the following order of preference:

1. Elimination of the Risk
2. Engineering Controls
3. Procedural Controls
4. Personal Protective Equipment

Due to the nature of biological research it may not be possible to mitigate a risk by only using one of the above measures, and often a combination will be utilized; however, always try to mitigate the risk by working down the list and analyzing what tactics can be taken for each of the types of mitigation strategies.

Elimination of the Risk
The elimination of a potential exposure is the optimal solution; however, while this will not always be possible, it should always be considered.

The following are two general examples on how to eliminate exposure risk to biologicals:

- Replace the agent with a similar agent which is less able to cause harm

    - Example: The use of an attenuated strain of a pathogen might be able to yield the same research answers as working with the pathogenic strain
- Replace a procedure with a procedure that is less able to cause harm
    - Example: Probe sonicators generate aerosols; use of a Cup Horn sonicator may achieve the same result while not generating aerosols

Engineering Controls

Use of protective equipment to prevent exposure to agent hazards or procedure hazards, for example:

- Negative Room Pressure
- Biological Safety Cabinets (BSC)
- Safety Sharps

Engineering controls are described in more detail in Section 4.4.

Procedural Controls

Use of procedures to prevent exposure to agent hazards which are formally documented and used as a part of laboratory specific training, for example:

- Deliberate positioning of equipment and agents in a manner which prevents an exposure
    - For example: Positioning a BSC in a low traffic area to ensure proper airflow into the BSC
- Block off areas where agents are being actively used to remove exposure to unnecessary laboratory personnel
    - For example: use of a demarcated area (safe zone) where one person can place an object for another person to safely pick up

Personal Protective Equipment

Personal Protective Equipment (PPE) is the last line of defense to prevent an exposure to an agent in the event of a failure of an engineering or procedural control, and PPE should never be the only means in which to mitigate and exposure risk.

PPE is described in more detail in Section 4.5.

Section 2 - Roles and Responsibilities

The biological safety program at UWO was developed to facilitate a safe and healthy environment for staff, researchers, community, and the environment. Additionally, the University is committed to institutional compliance to regulations, biosafety, and biosecurity in research and teaching activities.

2.1 – University of Wisconsin Oshkosh

UWO has created and maintains a biosafety program for employees and students who may be exposed to biological hazards during the performance of their laboratory or classroom work. The biosafety program is designed to provide information to employees and students on how to safely, and compliantly, conduct laboratory work.

To protect employees and students, and to facilitate regulatory compliance, UWO must:

- Maintain an IBC comprised of members with expertise and training to adequately review the biological work conducted on campus.
- Ensure the IBC is staffed with at least two members of the community, who are not affiliated with the University, to represent the interests of the community.
- Ensure that appropriate training is provided to personnel and students conducting work with recombinant and non-recombinant biological agents.
- Ensure that all research conducted is compliant with the NIH Guidelines.
- Implement policies for the safe conduct of work with biological agents.
- Report any significant problems, violations, or significant research related accidents or illnesses to the NIH Office of Science Policy within 30 days.

2.2 – Institutional Biosafety Committee

The IBC is responsible for the review, approval, and oversight of biological work involving recombinant or synthetic nucleic acid molecules and biological agents used in research and teaching activities. Biological agents include bacteria, viruses, fungi, protozoans, parasites, bacterial toxins, prions, genetically modified organisms; all which may be recombinant or non-recombinant. Additionally, biological agents include potentially infectious human or non-human primate derived blood, tissues, or cell lines.

The IBC is also responsible for the assessment of facilities, procedures, practices, and training of personnel and students to assure compliance with the NIH Guidelines.

The IBC has the authority to approve, require modifications to secure approval, deny approval, suspend, or terminate research activities as necessary to assure compliance with regulations and guidelines.

2.3 - Principal Investigator

The PI is a scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling biohazards.

The PI is responsible for the safe conduct of work with all biological materials in their laboratory or classroom.

The PI must:

- Accept direct responsibility for the health and safety of those under their supervision working with biological materials.
- Perform the initial risk assessment and determination of the required physical and biological containment for all biological materials.
- Supervise the safety performance of individuals conducting biological work to ensure the required safety practices and techniques are followed.
- Adhere to IBC approved emergency plans for handling accidental spills.
- Be adequately trained in good microbiological practices.
- Report any lab related accidents or illnesses to the IBC.
- Ensure all appropriate personal protective equipment is provided and used.
- Develop specific biosafety standard operating procedures for animals and biohazards used in the laboratory.
- Correct work behaviors and conditions that may result in accidents, injuries, or the release of biological materials.
- Provide all laboratory staff the protocols that describe the biological agents used in their laboratory and precautions to be taken.
- Instruct, train, and supervise laboratory personnel or students in:
    - Aseptic technique
    - Hazards associated with all biological materials used
    - Signs and symptoms of exposure to pathogens used
    - Laboratory practices and techniques required to ensure safety
    - Spill procedures
- Ensure all equipment is decontaminated before repair, maintenance, or removal from the laboratory (i.e., moved to spaces which the public can access).
- Ensure that all biological materials are decontaminated prior to disposal

Additionally, Principal Investigators are responsible for compliance with the NIH Guidelines for all work conducted with recombinant or synthetic nucleic acid molecules, including:

- Consult with the IBC to determine whether the recombinant or synthetic nucleic acid molecule work is subject to the NIH Guidelines.
- Obtain IBC approval prior to initiating or modifying any research involving the use of recombinant or synthetic nucleic acid molecules and/or biohazards. An IBC Biological Safety Protocol Application can be submitted to biosafety@uwosh.edu to initiate the review process.
- Be familiar with the NIH Guidelines as it relates to the biological materials used in the research or teaching activities.

- Teach personnel and students the NIH Guidelines applicable to their laboratory work
- Immediately report any significant problems, violations of the NIH Guidelines, or any significant accidents or illnesses to the IBC.

2.4 – Laboratory Personnel

The responsibilities of laboratory personnel include, but are not limited to, the following:

- Follow all laboratory practices, protocols, procedures, and comply with all applicable policies and guidelines.
- Participate in required training and instruction to ensure that they are knowledgeable and fully understand how to safely conduct laboratory work.
- Report any accidents, incidents, or spills to their supervisor.
- Report any unsafe behaviors or conditions to the supervisor.

Section 3 - Occupational Health, Medical Surveillance, and Individuals with Immune Compromising Conditions

3.1 - Occupational Health

For the UWO biological safety program, Occupational Health is meant to preserve the health of faculty, staff, and students on the UWO campus. Occupational Health provides a means to obtain basic medical assistance after an injury/illness related to work in a campus laboratory, or to provide vaccinations (if available) to protect against pathogens or potentially infectious materials which may present an exposure risk.

Regarding Occupational Health at UWO, there are two general groups on campus who will receive medical assistance differently: faculty/staff and students.

<u>Faculty/Staff</u>

Employees who have experienced an injury/illness related to their work, or who require a vaccine to protect against a possible exposure, should follow these steps:

<u>Non-Life-Threatening Injury/Illness</u>

1. Notify supervisor immediately after work-related injury/illness; supervisor should contact the Office of Risk and Safety within 24 hours of the injury
2. Receive medical care from a healthcare provider through personal health insurance plan
3. Work with personal physician to determine if there will be any required days away from work, or if there are any work restrictions

4. Update supervisor on the work suitability status
5. If necessary, work with Human Resources to set up a plan which enables a full return to work

Life-Threatening Injury/Illness

1. Call 911 to seek immediate emergency medical attention
2. Notify supervisor immediately after emergency is passed; supervisor should contact the Office of Risk and Safety and Human Resources within 24 hours of injury
3. Receive medical care from a healthcare provider through personal health insurance plan
4. Work with personal physician to determine if there will be any required days away from work, or if there are any work restrictions
5. Update supervisor on the work suitability status
6. If necessary, work with Human Resources to set up a plan which enables a full return to work

Obtain a Vaccine

1. Discuss risk assessment with supervisor to determine if a vaccine is appropriate
2. If vaccine is appropriate and desired, obtain vaccine through personal healthcare provider

Students

Students who have experienced an injury/illness related to their laboratory work, or who require a vaccine to protect against a possible research exposure, should follow these steps:

Non-Life-Threatening Injury/Illness

1. Notify PI/instructor immediately after laboratory related injury/illness
2. Receive medical care from Student Health Services
3. Work with Student Health Services to determine if there will be any required days away from school, or if there are any restrictions
4. Update PI/instructor on the ability to return to school

Life-Threatening Injury/Illness

1. Call 911 to seek immediate emergency medical attention
2. Notify PI/instructor immediately after emergency is passed
3. Receive medical care from Student Health Services
4. Work with Student Health Services to determine if there will be any required days away from school, or if there are any restrictions
5. Update PI/instructor on the ability to return to school

Obtain a Vaccine

1. Discuss risk assessment with supervisor to determine if a vaccine is appropriate
2. If vaccine is appropriate and desired, obtain vaccine through Student Health Services

3.2 - Medical Surveillance

For the UWO biological safety program, medical surveillance is the systematic assessment of individuals working with biological materials who may be potentially exposed to certain pathogens.

All individuals which are working with any pathogens or toxins which have a commercially available vaccine must be offered the relevant vaccine. The following are two examples of vaccines and their related biological material:

- Hepatitis B Vaccine
  - Work with primary human source materials which have not been screened for hepatitis B
- Diphtheria Vaccine
  - Work with diphtheria toxin
    - Tissue culture reagent
    - Targeted cell ablation in transgenic animals

When a risk assessment determines that medical surveillance is necessary to monitor possible exposures in the laboratory, one or both of the following may occur:

Serum Banking

Serum banking may be recommended by the IBC in consultation with a healthcare professional and entails the periodic collection and storage of serum. The purpose of banking baseline serum is to provide assistance with clinical diagnosis and/or treatment in the event of a laboratory exposure.

Antibody Titer Monitoring

A healthcare professional may recommend antibody titer monitoring in the event of a possible exposure. Blood would be drawn soon after the possible exposure to determine a baseline antibody titer against the pathogen of interest; a titer would again be analyzed several weeks later if no clinical signs develop to determine if an exposure had occurred.

3.3 – Individuals with Immune-Compromising Conditions

Individuals with immune-compromising conditions may be at increased risk for illness and more serious side effects of illness caused by infectious diseases in a laboratory setting.

There are many medical conditions that cause immune system compromise, such as:

- Infection with Human Immunodeficiency Virus (HIV)
- Treatment with corticosteroid medications or antimicrobials
- Medications used by people who have received organ transplants
- Monoclonal antibody therapy
- Long term diabetes mellitus, kidney, or liver disease
- Blood diseases
- Certain forms of cancer, leukemia, and lymphoma
- Cancer chemotherapy and radiation therapy
- Pregnancy

Individuals who have conditions that compromise or weaken the immune system should talk to their primary care physician who is familiar with their medical condition. Individuals with immune-compromising conditions may contact biosafety@uwosh.edu to request a risk assessment to help identify hazards associated with the infectious agent or materials in the laboratory. Providing the physician with a list of infectious agents present in the laboratory, frequency, and duration of contact with infectious agents, and the safety practices and equipment available can help an individual make important decisions regarding whether they wish to request an accommodation. If medical recommendations or restrictions are necessary to minimize exposure in a research or teaching laboratory, the individual's physician should provide a letter stating any restrictions.

Faculty/Staff

Employees may request workplace accommodations through the Office of Equal Opportunity, Equity & Affirmative Action. Information for requesting accommodations may be located here: https://uwosh.edu/equity/accommodations/

Students

Students may request accommodations through the Accessibility Center in the Dean of Students Office. Teaching syllabi should include the process for requesting accommodations and note that students are not required to self-disclose their condition to instructor. Information for requesting accommodations may be located here: https://uwosh.edu/deanofstudents/accessibility-center/students/apply/

Section 4 – Containment & Working in a Research Facility

The essential elements of containment used at UWO are described in this section. Additionally, a description of security, engineering controls, personal protective equipment, and techniques used for containment are described.

4.1 - Biosafety Levels

The essential elements of the biosafety levels for activities involving work with

microorganisms are summarized in this section. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community.

Biosafety Level 1 (BSL1) is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to individuals working in a laboratory and the environment. BSL1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Therefore, special containment equipment or facility design is not required, but may be used as determined by a risk assessment. Individuals working in the laboratory must have specific training in the procedures conducted in the laboratory and must be supervised by an individual proficient in microbiology or a related science.

The following standard practices, safety equipment and facility requirements apply:

Standard Microbiological Practices

- Eating, drinking, handling contact lenses, applying cosmetics, and storing food for human consumption is not permitted in laboratory areas. Food must be stored outside the laboratory in spaces designated for this purpose.
- Individuals must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- Policies for the safe handling of sharps, such as needles, scalpels, glass pipettes, and broken glassware must be developed and implemented. Whenever possible, sharps should be removed or replaced with safer alternatives.
- Used disposable needles and syringes must be placed in puncture resistant containers used for sharps disposal located at the point of use.
- Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps.
- Perform all procedures to minimize the creation of splashes and/or aerosols.
- Decontaminate work surfaces after completion of work and after any spill or splash of biological materials with an EPA approved disinfectant.
- Decontaminate all biological materials before disposal using an IBC approved method.
    - Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container with a lid. The outside of the container should be decontaminated prior to transport outside of the lab through public spaces.

Safety Equipment

- Protective laboratory coats or gowns are recommended to prevent contamination of personal clothing.
- Protective eyewear must be worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Individuals who wear contact lenses should also wear eye protection.
- Gloves must be worn to protect hands from exposure to hazardous materials. Alternatives to latex gloves should be available for use.
    - Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    - Remove gloves and wash hands when work has been completed and before leaving the laboratory.
    - Do not wash or reuse disposable gloves.

Laboratory Facilities

- Laboratories should have doors for access control.
- Laboratories must have a sink for hand washing.
- Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
- Chairs used in laboratory work must be covered with a nonporous material that can easily be cleaned and decontaminated.
- Laboratories with windows that open to the exterior should be fitted with screens.

More information on the requirements for BSL1 can be found in in the Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL).

Biosafety Level 2 (BSL2)

BSL2 builds upon BSL1 and is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL1 in that:

- Laboratory personnel have specific training in handling pathogenic agents and are supervised by individuals competent in handling infectious agents and associated procedures.
- Access to the laboratory is restricted when work is being conducted.
- Procedures in which infectious aerosols or splashes may be created are conducted in a biological safety cabinet or other physical containment equipment.

The following standard practices, safety equipment and facility requirements apply:

Standard Microbiological Practices

- Eating, drinking, handling contact lenses, applying cosmetics, and storing food for human consumption is not permitted in laboratory areas. Food must

be stored outside the laboratory in spaces designated for this purpose.

- Individuals must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
- Policies for the safe handling of sharps, such as needles, scalpels, glass pipettes, and broken glassware must be developed and implemented. Whenever possible, sharps should be removed or replaced with safer alternatives.
- Used disposable needles and syringes must be placed in puncture resistant containers used for sharps disposal located at the point of use.
- Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps.
- Perform all procedures to minimize the creation of splashes and/or aerosols.
- Decontaminate work surfaces after completion of work and after any spill or splash of biological materials with an EPA approved disinfectant.
- Decontaminate all biological materials before disposal using an IBC approved method. (See Section 7- Decontamination)
    - Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container with a lid. The outside of the container should be decontaminated prior to transport outside of the lab through public spaces.
- A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. An example Biosafety Level 2 door sign is available on the IBC forms webpage. Posted information must include:
    - The laboratory's Biosafety Level
    - Hazard(s) present
    - Name of PI and contact information
    - PPE required for entering the laboratory

Special Practices

- All persons entering the laboratory must be advised of the potential hazards and meet any specific entry requirements.
- Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
- A laboratory specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
- The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in any required microbiological practice before working with BSL2 agents.
- Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or when other potential contamination has occurred.
    - Equipment must be decontaminated before repair, maintenance, or

removal from the laboratory into public spaces.

- Incidents that may result in exposure to infectious materials must be reported to the PI. Medical evaluation, surveillance, and or treatment should be provided as needed. Records of all incidents must be maintained.
- Animals and plants not associated with the work being performed must not be permitted in the laboratory.
- All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a biological safety cabinet or other physical containment device.

Safety Equipment

- Biological safety cabinets, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
    - Procedures with a potential for generating aerosols or splashes are conducted. These may include:
        - Pipetting
        - Centrifuging
        - Grinding
        - Blending
        - Shaking
        - Sonicating
        - Opening containers of infectious materials
        - Harvesting infected tissues from animals or eggs
    - High concentrations or large volumes of infectious agents are used.
- Protective laboratory coats or gowns designated for laboratory use must be worn while working with hazardous materials and removed before leaving to non-laboratory areas.
- Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials which must be handled outside of a biological safety cabinet or containment device. Individuals who wear contact lenses should also wear eye protection.
- Gloves must be worn to protect hands from exposure to hazardous materials. Alternatives to latex gloves should be available for use.
    - Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    - Remove gloves and wash hands when work has been completed and before leaving the laboratory.
    - Do not wash or reuse disposable gloves.
    - Gloves must be disposed in a biohazard container after working at BSL2

Laboratory Facilities

- Laboratory doors to be self-closing and have locks.
- Laboratories must have a sink for hand washing and should be located near the exit door.
- Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
- Chairs used in laboratory work must be covered with a nonporous material that can easily be cleaned and decontaminated.
- Laboratory with windows that open to the exterior should be fitted with screens.
- Vacuum lines should be protected with liquid disinfectant traps.
- An eyewash station must be readily available.
    - If working with corrosive materials, access to an emergency shower must be readily available.
    - Per OSHA Standard: 1910.151(c) Where the eyes or body of any person may be exposed to injurious corrosive materials, suitable facilities for quick drenching or flushing of the eyes and body shall be provided within the work area for immediate emergency use.

More information on the requirements for BSL2 can be found in in the Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL).

4.2 – Animal Biosafety Levels

The essential elements of the animal biosafety levels for activities involving work with experimentally infected animals, or maintenance of laboratory animals that may naturally harbor zoonotic infectious agents, are summarized in this section. Laboratory animal facilities are a special type of laboratory and can present unique challenges.

In the animal room, the activities of the animals themselves can present hazards which are not found in microbiology laboratories. Animals may generate aerosols, they may bite and scratch, behave unpredictably, and they may be infected with a zoonotic agent.

Animal Biosafety Levels are meant to work alongside Biosafety Levels and are determined by a risk assessment. Like Biosafety Levels, they are in ascending order, by degree of protection provided to personnel, the environment, and the community.

Animal Biosafety Level 1 (ABSL1)
The following standard practices, safety equipment and facility requirements apply:

Standard Microbiological Practices

- A safety manual specific to the animal facility is prepared or adopted in

consultation with the animal facility director. The safety manual must be available and accessible. Personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.

- A sign incorporating safety information must be posted at the entrance to the areas where infectious material and/or animals are housed or manipulated. Posted information must include:
    - The Animal Biosafety Level
    - Hazard(s) present
    - Name of PI and contact information
    - PPE required for entering the laboratory
- Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the facility.
- Eating, drinking, handling contact lenses, applying cosmetics, and storing food for human consumption is not permitted in laboratory areas. Food must be stored outside the laboratory in spaces designated for this purpose.
- Perform all procedures to minimize the creation of splashes and/or aerosols.
- Policies for the safe handling of sharps, such as needles, scalpels, glass pipettes, and broken glassware must be developed and implemented. Whenever possible, sharps should be removed or replaced with safer alternatives.
- Decontaminate all biological materials before disposal using an IBC approved method. (See Section 7- Decontamination).
- All wastes from the animal room (including animals tissues, carcasses and bedding) are transported from the animal room in closed, leak-proof, and non-breakable containers.
- Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps.
- Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
- Gloves are worn to prevent skin contact with contaminated, infectious and hazardous materials, and when handling animals.
- Individuals must wash their hands after removing gloves, and before leaving areas where infectious materials and/or animals are housed or are manipulated.
- Used disposable needles and syringes must be placed in puncture resistant containers used for sharps disposal located at the point of use.

Special Practices

- None required.

Safety Equipment

- Protective laboratory coats or gowns designated for laboratory use must be worn while working with hazardous materials and removed before leaving to

non-animal facility areas.

- Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Individuals who wear contact lenses should also wear eye protection.
- Persons must wash their hands after handling animals and before leaving areas where infectious materials and/or animals are housed or manipulated.
- Gloves must be worn to protect hands from exposure to hazardous materials. Alternatives to latex gloves should be available for use.
    - Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    - Remove gloves and wash hands when work has been completed and before leaving the animal facility.
    - Do not wash or reuse disposable gloves.

Animal Facilities

- The animal facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.
- The animal facility must have a sink for hand washing.
- Emergency eyewash and shower are readily available; location is determined by a risk assessment.
    - If working with corrosive materials, access to an emergency shower must be readily available.
    - Per OSHA Standard: 1910.151(c) Where the eyes or body of any person may be exposed to injurious corrosive materials, suitable facilities for quick drenching or flushing of the eyes and body shall be provided within the work area for immediate emergency use.

More information on the requirements for ABSL1 can be found in in the Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL).

Animal Biosafety Level 2 (ABSL2)
ABSL2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL1. ABSL2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

ABSL2 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in the animal facility procedures, the handling of infected animals, and the manipulation of pathogenic agents; 3) personnel must be

supervised by individuals with adequate knowledge of potential hazards, microbiological agents, animal manipulations and husbandry procedures; and 4) BSC's or other physical containment equipment is used when procedures involve the manipulation of infectious materials, or where aerosols or splashes may be created.

These additional standard, special practices, safety equipment, and facility requirements apply to ABSL2:

Standard Microbiological Practices

- A sign incorporating the universal biohazard symbol must be posted at the entrance to the areas where infectious material and/or animals are housed or manipulated. Posted information must include:
    - The Animal Biosafety Level
    - Hazard(s) present
    - Name of PI and contact information
    - PPE required for entering the laboratory

Special Practices

- Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and offered (at no cost) appropriate immunizations for agents handled or potentially present, before entry into animal rooms.
- Procedures involving high potential for generating aerosols should be conducted within a biological safety cabinet or other physical containment device. When a procedure cannot be performed within a biosafety cabinet, a combination of personal protective equipment and other containment devices must be used.
- Restraint devices and practices that reduce the risk of exposure during animal manipulations should be used whenever possible.
- Equipment, cages, and racks should be handled in a manner that minimizes contamination of other areas.
- Equipment must be decontaminated before repair, maintenance, or removal from the areas where infectious materials and/or animals are housed or are manipulated.
- Incidents that may result in exposure to infectious materials must be reported to the PI. Medical evaluation, surveillance, and or treatment should be provided as needed. Records of all incidents must be maintained.

Safety Equipment

- Biological safety cabinets, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
    - Procedures with a potential for generating aerosols or splashes are conducted. These may include:

- Necropsy of infected animals
- Harvesting of tissues or fluids from infected animals or eggs
- Intranasal inoculation of animals

Animal Facilities

- A sink for hand washing is located at the exit of the areas, and segregated areas, where infectious materials and/or animals are housed or are manipulated.
- The supervisor or PI must ensure that animal care, laboratory, and support personnel receive appropriate training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (e.g., physical hazards, splashes, aerosol production).

More information on the requirements for ABSL2 can be found in in the Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL).

4.3 – Arthropod Containment Levels

The essential elements of the containment levels for activities involving work with arthropods are summarized in this section. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community.

Arthropod Containment Level 1 (ACL1)

ACL1 is suitable for work with arthropods that are either non-infectious or infected with an agent that is non-infectious (e.g., a Risk Group 1 agent or and agent that has been attenuated to be non-infectious).

Standard Practices

- A sign is posted at the entrance to the areas where arthropods are housed or manipulated. Posted information must include:
  - The Arthropod Containment Level
  - Hazard(s) present
  - Name of PI and contact information
  - PPE required for entering the laboratory
- A system is implemented which enables the monitoring and detection of escaped arthropods.
- A system is implemented which prevents the accidental escape of arthropods via the waste stream.
- Housing of arthropods is designed in a manner which minimizes the possibility of human contact or arthropod escape.
- Conditions which facilitate unintended growth/incubation of arthropods are eliminated.

- Containers holding arthropods are designed to prevent escape for all life cycle stages.
- Methods are used (appropriate for all life stages) to ensure arthropods are killed prior to disposal.
- Containers holding arthropods are labeled to indicate relevant information (e.g., species, strain).
- Precautions are created which minimize the escape of arthropods on personnel leaving the facility.
- Used disposable needles and syringes must be placed in puncture resistant containers used for sharps disposal located at the point of use.
- All procedures are designed to prevent the escape of arthropods
- Access to the arthropod facility is limited. Only those persons required for program or support purposes are authorized to enter the facility.

Special Practices:

- Animals which are used to propagate, or feed arthropods, must be housed in a manner which minimizes the possibility of arthropod escape.
- Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and offered appropriate immunizations for agents handled or potentially present, before entry into animal rooms.

Safety Equipment

- Gloves must be worn to protect hands from exposure to arthropods. Alternatives to latex gloves should be available for use.
    - Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
    - Remove gloves and wash hands when work has been completed and before leaving the arthropod facility.
    - Do not wash or reuse disposable gloves.
- Protective laboratory coats or gowns designated for laboratory use must be worn while working with arthropods and removed before leaving to non-arthropod facility areas.

Arthropod Facilities

- The arthropod facility is separated from areas that are open to unrestricted personnel traffic within the building. External facility doors are self-closing and self-locking.
- The animal facility must have a sink for hand washing.
- Emergency eyewash and shower are readily available; location is determined by a risk assessment.
    - If working with corrosive materials, access to an emergency shower must be readily available.

- Per OSHA Standard: 1910.151(c) Where the eyes or body of any person may be exposed to injurious corrosive materials, suitable facilities for quick drenching or flushing of the eyes and body shall be provided within the work area for immediate emergency use.

Arthropod Containment Level 2 (ACL2)
ACL2 is suitable for work with arthropods that are:

- Non-native to Wisconsin
- Infected with Risk Group 2 agents
- Genetically modified
    - Where the modification does not increase viability

ACL2 builds upon the practices, containment equipment, and facility requirements of ACL1.

These additional practices, safety equipment, and facility requirements apply to ACL2:

Standard Practices

- Persons must wash their hands after handling arthropods and before leaving areas where arthropods are housed or manipulated.
- All doors, cabinets, and drawers are tight-fitting closures and are not propped open when not in use.
- Cages are autoclaved or disinfected with an appropriate chemical after housing arthropods.
- Cages are shatter-proof and designed to minimize the possibility of escape when adding or removing arthropods.
- Methods are used (appropriate for all life stages) to ensure all arthropods are killed prior to disposal (i.e., arthropods which are non-native, infected with a microorganism, or genetically modified).
- IBC applications for work in the facility are accessible to all facility personnel.

Special Practices:

- Arthropods are not to be manipulated or killed using hands.
- The escape of arthropods must be reported to the IBC.

Safety Equipment

- Additional PPE (above what is required for ACL1) will be required if deemed necessary.
- Clothing will be worn which minimizes exposed skin.

Arthropod Facilities

- The arthropod facility is separated from areas that are open to unrestricted personnel traffic within the building by two doors. External facility doors are self-closing and self-locking.
- Air curtains are used to prevent the escape of flying arthropods.
- Vacuum lines within the facility must have a filter located at the wall to prevent arthropods from escaping the facility into the vacuum system.
- Floor drains are designed to prevent the escape of arthropods.
- Penetrations of walls, ceilings, and floors are sealed.
- Electrical or plumbing conduits running along walls should be minimized.
- Exhaust air is filtered to prevent escape of arthropods.
- The facility has a hand-washing sink with hot water and plumbing designed to prevent arthropod escape.
- The facility is inspected annually for compliance to ACL2 requirements.

4.4 – Plant Biosafety Levels

The essential elements of the containment levels for activities involving work with plants are summarized in this section. The principle of plant containment is to avoid the unintentional release of recombinant plants, non-native plants, or plant pathogens. The containment principles are based upon the recognition that the organisms used pose no health threat to humans (unless they have been modified for that purpose), and that the containment conditions are meant to reduce the likelihood of the spread of a plant pathogen or introduction of a non-native or recombinant plant to the local ecosystem.

Plant Biosafety Level 1 (BSL1-P)

BSL1-P is recommended for all experiments with transgenic plants and associated agents that have no or limited threat potential. For example: transgenic plants that are not noxious weeds or agents that have no recognized potential for rapid dissemination. Examples of agents worked with at BSL1-P include *Agrobacterium tumefaciens* and *Rhizobium spp*.

Standard Practices

- A sign is posted at the greenhouse entrance. Posted information must include:
    - The Plant Biosafety Level
    - Plants in use
    - Hazard(s) present (e.g., herbicides, pesticides, biohazards)
    - Name of PI and contact information
    - Any special requirements for using the area
- Access to the greenhouse facility is limited. Only those persons required for program or support purposes are authorized to enter the facility.
- Prior to entering the greenhouse, personnel shall be required to read and

follow all greenhouse standard operating procedures.

- A record shall be kept of experiments currently in progress in the greenhouse facility.
- Experimental organisms shall be rendered biologically inactive (devitalized) by appropriate methods prior to disposal outside of the greenhouse facility.
- A system is implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms.
- Arthropods and other and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.
- Experiments involving non-recombinant native plants may be conducted concurrently with experiments that require BSL1-P, provided that all work is conducted in accordance with BSL1-P greenhouse practices.

Greenhouse Facilities

- The term “greenhouse” refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of translucent material to allow passage of sunlight for plant growth.
- The term “greenhouse facility” includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.
- The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.
- Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation. The use of screens is recommended.

<u>Plant Biosafety Level 2</u> (BSL2-P)

BSL2-P is recommended for transgenic plants that are noxious weeds, plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent, plants associated with transgenic non-exotic microbes that has a recognized potential for serious detrimental impact on managed or natural ecosystems, or plant pathogens that have a recognized potential for serious detrimental impact on managed or natural ecosystems. Examples of agents worked with at BSL-2P include: *Meliodogyne incognita* (root-knot nematode), *Pepino mosaic virus* (PepMV), and *Pseudomonas syringae*.

BSL2-P builds upon the practices and facility requirements of BSL1-P.

These additional practices and facility requirements apply to BSL2-P:

Standard Practices

- A record shall be kept of experimental plants, microorganism, or small animals that are brought into or removed from the greenhouse facility.
- The Principal Investigator shall report any greenhouse accident involving the inadvertent release of spill of microorganisms to the IBC.
- If part of the greenhouse is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.
- Experiments requiring containment lower than BSL2-P may be conducted concurrently with experiments that require BSL2-P provided that all work is conducted in accordance with BSL2-P greenhouse practices.
- If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.
- Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.
- A greenhouses practices manual shall be prepared and adopted. This manual shall:
    - Advise personnel of the potential consequences if such practices are not followed.
    - Outline contingency plans to be implemented in the event of the unintentional release of organisms.

Greenhouse Facilities

- The arthropod facility is separated from areas that are open to unrestricted personnel traffic within the building by two doors. External facility doors are self-closing and self-locking.
- A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil.
- An autoclave shall be available for the treatment of contaminated greenhouse materials.
- If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.
- BSL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the above

requirements.

4.5 – Security

Laboratory security is an important part of an effective safety program. Follow the steps to ensure a secure working environment in your laboratory:

- Keep laboratory doors closed and locked when unoccupied.
- Keep stocks of organisms and hazardous chemicals locked when the laboratory is occupied.
- Keep an accurate record of laboratory materials (e.g., cultures, chemicals, stocks, growth media).
- Notify the UWO Police if materials are damaged or missing from a laboratory.
- When research is completed for the day, ensure that biological materials and chemicals have been stored properly and securely.
- Decontaminate materials and work surfaces after completing work.
- After completing work for the day: turn off equipment, vacuum lines, biological safety cabinets, and close fume hood and biological safety cabinet sashes.
- Ask strangers to identify themselves, and ask them to exit the lab laboratory if they are not authorized to be there.
- Discuss all security related concerns with your supervisor and fellow laboratorians.

4.6 – Engineering Controls

Use of protective equipment to prevent exposure to agent hazards or procedure hazards, for example:

- Negative Room Pressure
- Biological Safety Cabinets (BSC)
- Safety Sharps

<u>Negative Room Pressure</u>

The air pressure inside of a laboratory needs to be negative as compared to the adjoining hallway. This pressure differential aids in the prevention of any aerosols, vapors, or fumes from exiting the laboratory.

Keeping laboratory doors closed helps maintain the pressure differential.

Contact the IBC if you have any concerns about a laboratory being under negative pressure.

<u>Biological Safety Cabinets</u>

The Biological Safety Cabinet (BSC) was developed to provide personnel, environmental, and sample protection during the manipulation of infectious microorganisms; additionally, they are used to protect non-infectious samples from

contamination. A description detailed description of how to use a BSC can be found in Section 5.1.

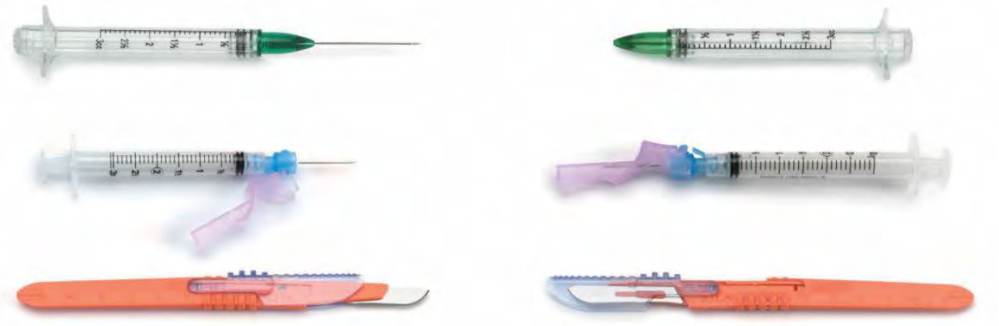
Safety Sharps

The safety engineered sharp is created in to reduce the risk of an injury by use of a mechanism which creates a barrier between the sharp and the worker, blunting the sharp, or encapsulation of the sharp after use. Safety sharps should always be used whenever possible.

The following tactics should be used to reduce the risk of an injury whenever any type of sharp is used:

- Eliminate the need for a sharp (substitute with a different tool)
- Isolate the sharp during use so that it does not pose a hazard
- Provide a means to isolate or encase the sharp after use
- Have a rigid sharps container available at the point of use for easy disposal
- Never directly pass an exposed sharp between workers
    - One worker should place the sharp in a demarcated area (safe zone) so it can be safely picked up by a second worker
- Sharps should never be recapped after use

The following are some examples of safety sharps which can be safely covered after use:



Section 4.5 - Personal Protective Equipment

Personal Protective Equipment (PPE) is the last line of defense to prevent an exposure to an agent in the event of a failure of an engineering or procedural control.

The following is a list of common PPE which are used:

- Laboratory Coats
- Eye Protection
    - Safety Glasses
    - Goggles
    - Face Shield
- Hand Protection
    - Disposable Gloves
    - Thermo/Cryo-Protection Gloves
- Respirator
    - N-95
    - Half-Face Mask
    - Powered Air Purifying Respirator (PAPR)

<u>Laboratory Coats</u>

When worn in the laboratory setting, laboratory coats protect against accidental spills. In the event that a lab coat has biological material spilled on it, the laboratory coat should be considered contaminated and be laundered. Non-disposable laboratory coats used for teaching laboratories should be laundered at least once/semester. In the event that a lab coat has biological material spilled on it, the laboratory coat should be considered contaminated and laundered.

Laundering Laboratory Coats:

Laboratory coats should be washed using approved laundry facilities on campus. When handling contaminated laboratory coats during the initial washing process, a laboratory coat and gloves should be worn for the first cycle. Load up to 6 laboratory coats in the washer and distribute evenly so the load is balanced.

The following procedure is followed for laundering laboratory coats contaminated with blood (per OSHA standard):

- 1st Cycle
    - do not add soap during the first blood removing wash cycle
    - set wash temp to cold
    - set load size to extra large
    - set dial to soak, pull dial to begin cycle
- 2nd Cycle, leave the same load in the washer
    - add a scoop of soap to the load
    - temp = change to hot
    - load size = medium
    - set dial to Normal Regular Wash

- 3rd Cycle, leave same load in the washer; this will be an extra rinse cycle
    - do not add soap during the third extra rinse wash cycle
    - wash temp = change to warm
    - load size = medium
    - set dial to Normal Permanent Press
- Once washer stops transfer laboratory coats to dryer
    - set dryer to Permanent Press, select the "more dry" setting
    - press start
- Remove laboratory coats from the dryer and place the freshly washed coats at the back of their respective sections to encourage a rotation of coat usage.

Eye Protection

Several types of eye protection are used to offer protection against a variety of potential exposure settings where workers may need to protect their eyes.

The type of eye protection used should be chosen for specific work situations and will depend on the possible types of exposure, other PPE used, and personal vision needs. Eye protection must be comfortable and allow for sufficient peripheral vision and must be adjustable to ensure a secure fit. It may be necessary to provide several different types, styles, and sizes of eye protection to ensure that all laboratories have access to appropriate eye protection for their personal needs and fit.

Selection of protective eyewear for a given task should be made from an evaluation of each activity which will be performed. This hazard assessment requires a clear understanding of the following:

- Work tasks and opportunities for exposure
- Potential routes of exposure
- Nature and extent of worker contact

Common eye protection devices are as follows:

- Safety Glasses- Should be worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear safety glasses. Options of various sizes and fits should be available for workers to facilitate better protection and comfortable wear.
- Goggles- Provide the most reliable eye protection from splashes, sprays and respiratory droplets. Options for various sizes and fits should be available for workers to ensure that the goggles provide proper protection and are comfortable to wear.
- Face Shields- Face shields are commonly used and can offer protection to other facial areas. To provide better face and eye protection from splashes and sprays, a face shield should have crown and chin protection and wrap

around the face to the point of the ear, which reduces the likelihood that a splash could go around the edge of the shield and reach the eyes.

Hand Protection

Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment.

Hands should always be washed after removal of hand protection and prior to leaving the laboratory.

Common types of hand protection are as follows:

- Disposable Gloves- Containers of disposable gloves should be present in all areas where biological materials are used.

  The following guidelines should be followed when using disposable gloves:
  - Do not reuse gloves
  - Change gloves when contaminated or integrity is compromised
  - Provide alternatives to latex (due to allergy concerns)
    - Nitrile gloves are a common alternative
  - Choose proper glove size for good fit
    - Too big: difficult to hold objects; looseness offers decreased protection
    - Too small: more likely to rip; uncomfortable squeezing of hand
  - Avoid wearing disposable gloves in common areas
    - Gloves may be contaminated

  The following is an appropriate procedure to use when removing disposable gloves:
  - Grab the palm of your right glove with your left hand
  - Pull towards your fingertips
    - The glove will turn inside out
  - Hold onto the empty glove with your left hand
  - Put two right-hand fingers in the top of your left glove
    - Avoid contacting your skin
  - Pull toward your fingertips until you have pulled the glove inside out and off of your hand
    - The right glove will be inside the left glove
  - Dispose of the gloves in an appropriate waste container

- Thermo/Cryo-Resistant Gloves- Laboratories often involve manipulations of various items which are much hotter or colder than room temperature. These gloves offer protection from extreme heat or cold and allow a worker to move these items and to hold a hot item for short durations without suffering an

injury.

The fit of these gloves should be appropriate for the task; for example, gloves should be long enough to protect the forearm when used while adding or removing items from an autoclave or freezer, but a glove that only covers the hand may be appropriate for handing items on a lab bench.

- Thermo-resistant Gloves - These gloves are designed to allow handling of materials which are extremely hot.
  - These gloves offer moderate cryo-protection; however, these gloves should NOT be used to for adding or removing items from liquid nitrogen because they do not offer any protection against liquid exposure.
- Cryo-Resistant Gloves – These gloves are designed to allow handling of materials which are extremely cold.
  - These gloves are waterproof and can therefore be used to handle material stored in freezers and liquid nitrogen

Below are some common examples of gloves which offer protection above the wrist:

Thermo Gloves Cryo Gloves

Only use gloves which are designed for the task or activity for which they will be used.

Respiratory Protection

A respirator is a personal protective device that is worn on the face, covers at least the nose and mouth, and is used to reduce the wearer's risk of inhaling infectious particles.

Respirators should only be used when engineering control systems are not feasible; therefore, respirators should be used as a last line of defense.

A medical screening and fit test are required prior to initiating any work which requires a respirator. The medical screening is conducted by a healthcare

professional and the fit test is conducted by a properly trained individual.

Common types of respiratory protection are as follows:

- N95- This is a disposable mask which is intended for single use only. It will filter out 95% of particles 0.3um in size.
- Half-Face Mask- These masks reusable masks which cover the nose and mouth. They are equipped with two detachable HEPA filter cartridges which can filter out 99.99% of particles 0.3um in size. This respirator may be preferable to an N95 due to its reusable nature; the cost is approximately the same a dozen N95 respirators. In a normal laboratory environment, the HEPA filter cartridges will last for several years.
- Powered Air Purifying Respirator (PAPR)- These respirators offer the highest level of protection against aerosols. This respirator has a hood with a face shield and works by blowing HEPA filtered air over the users face from a motor which is worn on a belt. Because this respirator supplies the air, and does not require the wearer to exert effort to inhale air across a filter, the user only needs to be trained on proper usage and a fit test is not required.

Here are some examples of respirators:

N95 Half-Face Mask PAPR

Important Note: Surgical Masks are not designed to offer protection against aerosols, and only offer moderate protection against exposure to splashes; these masks do not form a complete seal around the nose and mouth. These masks were designed to protect others from exposure to contamination from the wearer.

The filters used in modern respirators are considered fibrous in nature — constructed from flat, nonwoven mats of fine fibers. Fiber diameter, porosity (the ratio of open space to fibers) and filter thickness all play a role in how well a filter collects particles. The N95 and HEPA filters are rated against their ability to filter out particles which are 0.3um in diameter, which is the least efficient particle size to filter; these filters become more efficient as the size of the particle moves away from 0.3um (larger *or* smaller).

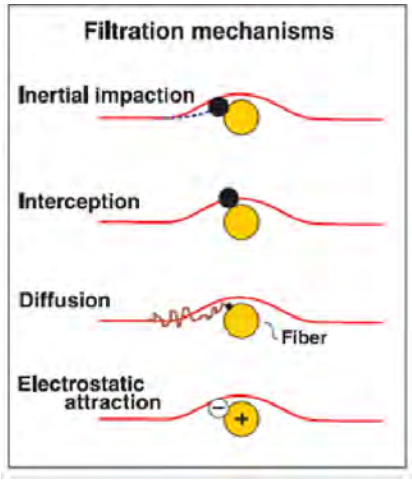
Perhaps the most misunderstood aspect of filter performance is that filters DO NOT act as sieves; therefore, they do not filter the air via the same mechanism in which a 0.22um filter might be used to filter sterilize a liquid.

There are four collection mechanisms which operate to capture particles:

- Inertial Impaction- Particles having too much inertia due to size or mass cannot follow the airstream as it is diverted around a filter fiber. This mechanism is responsible for collecting particles ~1um or larger.
- Interception- As particles pass close to a filter fiber, they may be intercepted by the fiber. This mechanism is responsible for collecting particles larger than ~0.3um.
- Diffusion- Small Particles are constantly bombarded by air molecules, which causes them to deviate from the airstream and come into contact with a filter fiber. This mechanism is responsible for collecting particles smaller than ~0.3um.
- Electrostatic Attraction- Filters are constructed from charged fibers. Oppositely charged particles are attracted to a charged fiber. This collection mechanism does not favor a particular particle size and enhances particle collection without increasing breathing resistance.

In all cases, once a particle contacts a filter fiber, it is removed from the airstream and is strongly held by molecular attractive forces. It is very difficult for such particles to be removed once they are collected.

General overview of the four filter collection mechanisms:



Section 5 – Laboratory Equipment Use

Many different types of equipment are commonly used in laboratories where work is conducted with biological agents. Always follow the manufacturer's instructions

when operating laboratory equipment.

This section contains descriptions of how to safely use equipment common to most laboratories:

- Biological Safety Cabinet (BSC)
- Autoclave
- Centrifuge
- Bunsen Burners
- Incinerator

Section 5.1 - Biological Safety Cabinet

The Biological Safety Cabinet (BSC) was developed to provide personnel, environmental, and sample protection during the manipulation of infectious microorganisms; additionally, they are used to protect non-infectious samples from contamination.

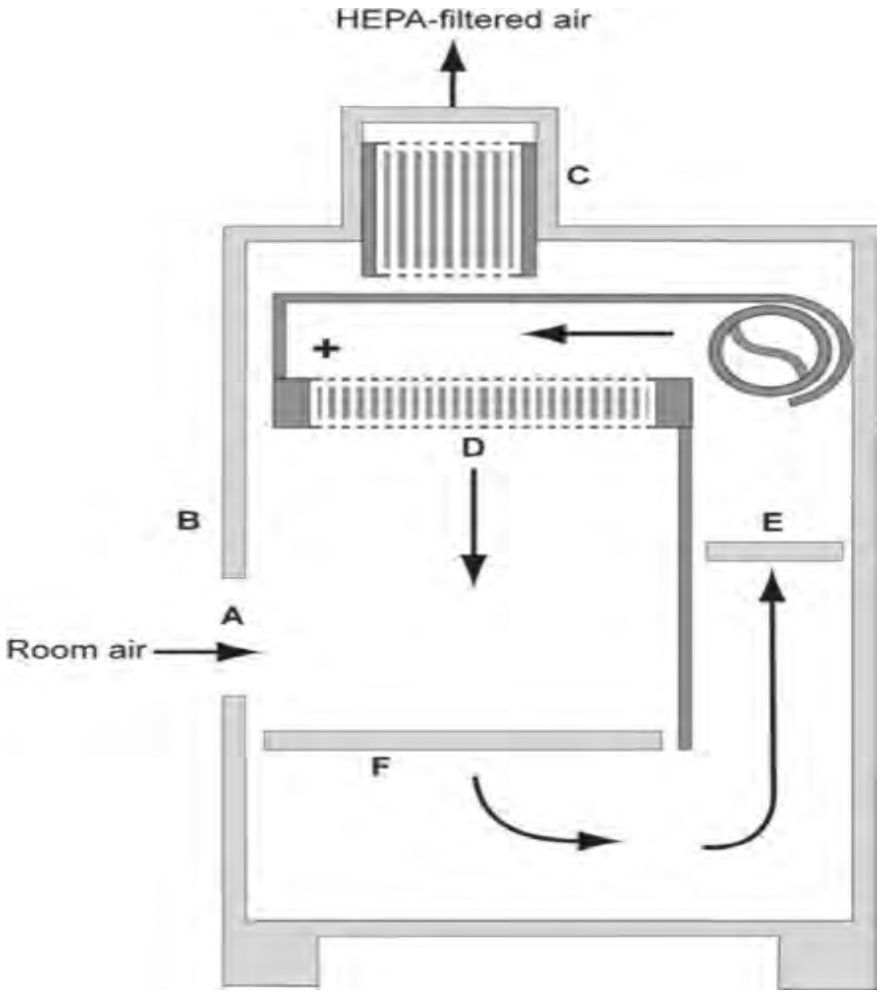
The following are factors to consider for ensuring proper BSC function:

- Location- The ideal location for a BSC is away from doors and traffic areas. This is because the air curtain, which provides all the protection to the worker and sample, is fragile and is only moving at approximately 1mph; therefore, even small fluctuations in air movement near the BSC can disrupt the curtain and prevent the BSC from functioning properly.
- Service- The BSC must be certified at least annually by a qualified technician to ensure that it is functioning properly. Additionally, a BSC must certified when any of the following occur:
    - Change of location
    - Change of airflow in the room (e.g., HVAC rebalancing)
    - Addition of equipment which cause new airflow patterns in room
    - Pressure across the HEPA filter is greater than ~0.25" of water
        - This suggests the HEPA may need to be replaced
    - When there is doubt that the BSC is functioning properly (e.g., contamination problems, strange noises
- Decontamination- BSCs that have been used for infectious, or potentially infectious materials, must be fully decontaminated when any of the following occur:
    - Significant change of location
        - A change in location to a different floor or building
        - Contact the IBC for guidance in determining if a decontamination is required
- Turn on BSC >5 min prior to use- It is important to let the BSC run for at least 5 min prior to use for the following reasons:
    - Ensure that proper airflow is established within the BSC
    - Allow the HEPA filters to become electrostatically charged

        - Facilitates the capture of particles smaller than 0.3um
- Do not use open flames- Open flames (e.g., Bunsen burner) disrupt airflow inside the BSC. Also, flaming of a loop generates aerosols which could escape through the air curtain. A ceramic incinerator would be preferable if it is necessary to work with a reusable loop inside the BSC.
    - Any use of an open flame needs to be approved by the IBC
- Training of Personnel- A properly functioning BSC will not offer protection if personnel are not trained of proper use. In addition to the other items discussed in this section, the following elements should be included in specific laboratory training:
    - Protect the integrity of the air curtain
        - Do not waive arms from side-to-side through the air curtain
        - Arm movement in and out of the BSC should be direct and perpendicular to the grill
        - Avoid quick motions in and out, or across the face of the grill
        - Do not cross arms while working
        - Keep materials at least 4" from the front grill
        - Do not rest arms on the front grill
        - Do not cover any portion of front grill (e.g., with pipets, paper towels, vortex unit)
    - Protect the proper airflow of the BSC
        - Do not block the back wall
        - Leave at least 2" of space between items
        - Do not overfill the BSC with equipment or with items which are not needed for work being conducted
    - Decontaminate the BSC before initiating work and after work is completed
    - Do not use flammable chemicals inside the BSC
        - Only small quantities may be used if vapor concentration will not approach the lower explosion limit for the chemical
            - Consult with the Chemical Hygiene Officer for help in determining if a desired amount of chemical would be safe to use inside a BSC
- Ultraviolet Lamps- Ultraviolet Lamps are not considered necessary for use in a BSC. However, the following steps should be performed if they are to be used:
    - Clean lamps weekly to remove any dust and dirt that may block the germicidal effectiveness of the ultraviolet light
    - Periodic testing of the ultraviolet lamp
        - Energy output of a lamp decreases over time; therefore, the germicidal activity of the lamp will also decrease. Note: this decrease will not be visible to the naked eye.
            - The radiation output should be periodically tested, and

to be effective measurements in the direct center of the BSC should not be less than 40 microwatts per square centimeter at a wavelength of 254nm.

- Overview of BSC Function- The most common type of BSC used is a Class 2 Type A2 BSC; below is a diagram of this type BSC with a description of how it operates:



Front opening; room air is pulled in, and then downward, at a rate of 100 linear feet per minute (~1mph). This is the air curtain which provides protection to both the user and the sample.

(a) Adjustable sash; this can be raised or lowered as needed; however, when the BSC is certified and is marked to where it can function properly at the highest sash level; BSC should not be used when raised higher than this mark.

(b) Exhaust HEPA filter; ~30% of the air than enters the cabinet is exhausted to the room via this HEPA filter.

(c) Supply HEPA filter; ~70% of the air that enters the cabinet is reused after passing through this HEPA filter.

(d) Positive pressure common plenum; this plenum provides the air which will be exhausted or recirculated in the BSC. To prevent a leak of contaminated air into the room, this plenum is constructed to that it is surrounded by the negative pressure plenum.

(e) Negative pressure plenum; air is pulled into this plenum and is composed of air from the room and from the supply HEPA filter.

Understanding how to properly maintain and use a BSC will enable protection to both the worker and sample. The Operating a Biosafety Cabinet Laboratory Sign should be posted near the BSC.

Section 5.2 – Autoclave

Autoclaves are used in laboratories to sterilize glassware, instruments, solutions, and biohazardous waste.

The hazards associated with autoclave use include heat, steam, and pressure. It is important to familiarize yourself with these hazards and know how to protect yourself before operating autoclave. Do not operate an autoclave until you have been trained.

Sterilization will only result when conditions of time, temperature, pressure, and humidity have been met. Incorrect selection of time of exhaust cycle may damage the autoclave, cause liquid to boil over, or break bottles. Following these recommendations will minimize the chance of injury or damage to the autoclave:

- Wear Personal Protective Equipment
  - Lab Coat
  - Safety Glasses or Face Shield

    - Thermo-Resistant Gloves (long enough to offer forearm protection)
- Prepare the Autoclave
    - Inspect the door gasket for any cracks or bulges
    - Clean the drain screen of debris (if necessary)
    - If any problems are found, contact a person qualified for autoclave repair
    - Turn the autoclave on, and allow time for the jacket to reach sufficient temperature and pressure
- Prepare Items to be Sterilized
    - Liquids:
        - Leave caps loose (or cover with foil)
            - Allows steam penetration
            - Prevents explosion at end of cycle
    - Dry Items:
        - Loosely tape or tie
            - Allows steam penetration
    - NOTE: Not all materials are suitable for autoclaving:
        - Do NOT autoclave flammable, reactive, corrosive, toxic, or radioactive materials
            - Autoclaving bleach generates toxic chlorine gas
        - Check that materials made of plastics are compatible with the autoclave
            - Some plastic materials will melt in autoclave
    - Place all items in an autoclave bin to prevent spillage of items inside the autoclave
- Load the Autoclave
    - Ensure that the jacket has reached sufficient pressure to start a cycle
    - Place loaded autoclave bin onto autoclave rack
    - Do not overload the autoclave
        - Overloaded autoclaves may not sterilize materials
    - Allow sufficient space between items to ensure steam contact
- Operate the Autoclave
    - Close and lock the door
        - Ensure the door is secure before starting a cycle
    - Select the appropriate cycle autoclave
        - Solid waste: minimally at 121°C / 30 minutes / 2ATM
    - Record the autoclave run on a log sheet
    - Check on the autoclave mid-cycle to verify the proper sterilization temperature has been reached (121°C)
    - Do not open the autoclave door during a cycle
        - If necessary, abort the cycle and wait until the chamber depressurizes
- Unload the Autoclave

- Only open the autoclave after the chamber has depressurized
- Wear the above-mentioned PPE
- Slowly open the door and allow steam to slowly escape
  - Keep your face away from the door
- Allow items to sit in autoclave for at least 10 minutes before removing
- Carefully remove items and place in safe area to cool
- Liquids may be superheated and may explosively boil spontaneously

Autoclaves should be tested to ensure they are functioning properly:

- Temperature Validation: Autoclave tape should be used with each load placed in autoclave. Autoclave tape works by changing color after exposure to temperatures commonly used in sterilization processes, typically 121°C in a steam autoclave.
- Monthly Efficacy Monitoring: Autoclave efficacy monitoring should be conducted and documented on a monthly basis using *Geobacillus stearothermophilus* ProSpore Ampoules (ATCC 7953). These ampoules will be autoclaved and then viability will be checked per manufacturer's instructions as described below:
  - Description: Mesa ProSpore is intended for the use in the monitoring of saturated steam sterilization cycles at 121°C. Each ProSpore ampoule contains a spore suspension of *Geobacillus stearothermophillus* (#7953) within a growth medium also containing Bromocresol Purple to function as a pH indicator. The acid production associated with growth causes a change in color from purple to or toward yellow.
    1) Refrigerate at 2-8 °C upon receipt until use.
    2) Ampoules should be purple and undamaged prior to use. Do not use after expiration date.
    3) Since ProSpore contains live cultures, ampoules should be handled with care.
    4) ProSpore is not intended for flash sterilization processes.
    5) This is a single use product. Use of a unit in multiple cycles will invalidate the results and could potentially result in the release of non-sterile product.
  - Instructions for Use:
    1) Exposure: Place one or more ProSpore ampoule in the most difficult location to sterilize, usually near the drain or suspended in a volume of liquid. Run cycle.
    2) Caution: After sterilization, handle ampoules with care. Contents of the ampoule are hot and under pressure. Failure to allow sufficient cooling time (10-15 minutes) may result of bursting of the ampoule.
    3) Incubation: Place the processed ampoule in a vertical position

in an incubator at 55-60°C (131-140°F). Mark a control ampoule as such and incubate along with processed ampoule to ensure spore viability. Incubation time of 48 hours recommended.

4) Monitoring: Examine the ProSpore ampoules daily during incubation. Record observations. All positive ampoules should be recorded and disposed of immediately.
5) Interpretation:
    - Control: The control ampoule should exhibit a color change to or toward yellow and/or turbidity. If the control ampoule does not show signs of growth, consider the test invalid.
    - Test: A failed sterilization cycle is indicated by turbidity and/or a change in color to or toward yellow. A test ampoule that retains its purple color indicates an adequate sterilization cycle.
6) Disposal: Sterilize all positive and expired units prior to disposal.

Section 5.3 – Centrifuge

Centrifuges operate at high speeds and have great potential for injuring users if not operating properly. The majority of centrifuge accidents result from user error. To avoid injury, laboratorians should follow the manufacturer's operating instructions for each make and model of centrifuge used.

Follow these steps for safe operation of centrifuges:

Before Centrifugation:

- Work Surface must be level and firm
- Ensure that centrifuge rotors, buckets, and tubes are dry.
- Ensure that the spindle is clean.
- Use matched sets of tubes, buckets, and other equipment.
- Inspect tubes for cracks or flaws before using
- Avoid overfilling of tubes
    - In fixed angle rotors, centrifugal force may drive the solution up the side of the tube or container wall.
- Tightly cap tubes prior to centrifugation or vortexing
- Always use centrifuge safety cups to contain potential spills and prevent exposure to aerosols.
    - Centrifuge safety cups are required to be used when centrifuging biological materials which require BSL2 containment.
- Fixed angle rotors: Ensure that the rotor is properly seated on the driveshaft.
- Swing Bucket Rotors: Ensure that the bucket is properly seated on the rotor.

- Balance centrifuge. Each opposing tube must be of equal weight. A scale or balance can be used to ensure that the weights match. The tubes should be filled using liquids with precisely the same density; this is more important when using a fixed angle rotor at high speed.
- Check all O-rings on the centrifuge rotor to make sure they are sealing properly.
- Secure the rotor lid before closing the centrifuge
- Check the speed settings on the centrifuge to avoid speeds that exceed safe levels for the rotor and the tubes used to hold samples.

<u>During Centrifugation:</u>

- Never open the centrifuge lid during operation.
- Make sure that the centrifuge is operating normally before leaving the area.
    - To ensure the load is balanced, and that the centrifuge is operating correctly, wait for a few minutes after centrifuge has reached operating speed before leaving.
- Do not tip or bump centrifuge
- Turn off and unplug centrifuge immediately if you notice any unusual noises or shaking

<u>After Centrifugation:</u>

- Wear a face shield
- Make sure rotor has come to a complete stop before opening
    - With infectious materials, wait 10 minutes after rotor has stopped moving before opening lid. Or open in a fume hood or biosafety cabinet.
- If a hazardous spill occurs, wait at least 30 minutes before opening lid then clean the centrifuge and rotor using 10% bleach with a 30 minute contact time (or other EPA approved disinfectant)
- When centrifuging at BSL2 containment, only open the sealed centrifuge rotor, or sealed centrifuge buckets, inside of a BSC or a fume hood.
    - If a BSC or fume hood is not available, wait at least 10 minutes before opening the centrifuge rotor or centrifuge buckets.
- The centrifuge and rotor should be cleaned whenever there is a spill, and at least once a week. Store removable rotors upside down so condensation can run off and the metal can be dry.
- Centrifuge rotors should have an expiration date on them and be replaced every 10 years

Section 5.4 – Bunsen Burners and Incinerator Equipment Use

The use of Bunsen burners originated in the mid-nineteenth century as a general piece of laboratory equipment for heating and sterilization. However, we now limit the use of open flames in the laboratory: we use hotplates for heating and benchtop incinerators are used for sterilization to avoid the generation of aerosols

when used to flame loops for manipulation of microbes.

Additionally, while a biological safety cabinet may protect against the generation of aerosols, use of a Bunsen inside a biological safety cabinet is not recommended because the heat generated can disrupt proper airflow.

IBC approval must be granted for the use of Bunsen burners for flaming loops when manipulating microbes or for use inside a biological safety cabinet.

Section 6 – Shipping and Transport of Biological Materials

Close attention must be given when shipping or transporting biological materials. Specific procedures must be followed to prevent the exposure of people or the environment to biological materials.

Importantly, the shipping of biological materials is highly regulated by the Department of Transportation (DOT) and the International Air Transport Association (IATA) as well as other regulatory bodies depending on the material to be transported. Proper containment and safety procedures must be followed whether transporting biological materials on campus or off-campus.

Section 6.1 – Intra-Campus Transport

The transport of biological materials outside of a laboratory, and through public access areas, requires that the materials to be safely packaged in a manner that meets or exceeds the following procedure:

- Biological material placed in sealed, leak proof 1° container
- 1° container should be placed in 2° container to contain accidental leaks
- Absorbent materials placed between 1° and 2° container
    - In sufficient quantity to absorb contents of 1° container(s)
- Place name and contact information on the outer container
- Place biohazard sticker on outermost container when biohazardous materials are transported
- Decontaminate the outermost container prior to transport
- Use a wheeled cart to transport packages between buildings, floors, and other areas outside of the lab
- If transporting packages in a university vehicle, secure the 2° container of materials to prevent tipping
- Individuals transporting biohazardous agents should be knowledgeable about the procedure for handling spills.
- Please note that biohazardous materials should <u>not</u> be transported in personal vehicles. Please use a state vehicle instead for safety and liability reasons.

Deviations from this procedure must be approved by the IBC.

Section 6.2 – Off-Campus Shipping

Many biological materials fall within the category of dangerous goods for the purpose of shipping. Federal or state permits may be required for shipping some biological materials. Material transfer agreements may also be necessary in some circumstances if restrictions on the use of the material is limited. All individuals involved in the transport of hazardous materials off-campus (including the preparation for transport) must be trained and certified in shipping regulated materials.

Training Requirements

Any individual who is involved in the shipment of regulated biological materials at UW Oshkosh is required to undergo shipping certification every two years. Shipping certification is available through the following sources: CITI Program BSS-Shipping Regulated Biological Materials, Wisconsin Safety Council, UW Madison BioHazMat Training, FedEx, or other DOT/IATA compliant shipping training.

Classes of dangerous goods regulated in transportation include:

- Class 6.2: Infectious Substances
    - IATA Dangerous Goods Regulations define infectious substances as organisms which are known or expected to contain pathogens such as bacteria, viruses, rickettsia, parasites, fungi, or other agents which can cause disease in humans or animals.
        - Category A- capable of causing permanent disability, life-threatening, or fatal disease in otherwise healthy humans or animals
            - Infectious Substance affecting humans UN2814
            - Infectious Substance affecting animals UN2900
        - Category B- infectious substances that do not meet the criteria for Category A
            - Biological Substance, Category B UN3373
                - Includes Diagnostic Specimens, Clinical Specimens
- Class 9: Miscellaneous Hazardous Materials
    - Genetically modified organisms/microorganisms (GMO)
        - Biological material where the genetic material has been modified in a manner which does not occur naturally. *Note: GMO does not matter if it is an infectious substance—it would be classified as Class 6.2*
    - Exempt Substances-Non Hazardous Materials
        - Biological materials which are not known or suspected to be infectious to humans or animals or genetically modified (if

shipped outside US)
        - Exempt Human Substance
        - Exempt Animal Substance
        - Biological Product
    - May be shipped with dry ice as long as the dry ice is properly declared
- Dry Ice
    - Solid state carbon dioxide shipped alone or used as a preservative freezing agent with a biological shipment
        - Low level hazard; packaging and labeling is secondary to higher hazard materials in the package such as infectious substances

Shipping of biological materials off-campus requires that the materials be packaged and labeled in a specific manner. For additional information, please reference the University of Wisconsin Oshkosh Biohazard Materials Shipping Guide.

Biological materials are commonly shipped with dry ice or "liquid nitrogen dry shippers", and although neither dry ice nor nitrogen are biological materials, they are considered hazardous material in transportation and are subject to DOT and IATA shipping regulations.

Section 7 – Decontamination

Decontamination is a process or treatment that renders a material safe to handle. A decontamination procedure can vary from washing hands with soap and water to autoclaving.

There are three categories of decontamination:

- Sterilization – Physical or chemical process to destroy all infectious agents
- Disinfection – Physical or chemical process which reduces the number of infectious agents to a safe level on an inanimate object
- Antisepsis – Physical or chemical process which reduces the number of infectious agents to a safe level on living tissue

All materials and equipment contaminated with infectious, or potentially infectious, agents should be decontaminated:

- Upon completion of procedures with these agents
- In the event of a spill
- Prior to being washed, stored, or discarded
- At least daily

There are two common means of decontamination of contaminated materials on

campus:

- Exposure to approved chemical disinfectants
- Autoclaving

Section 7.1 – Decontamination of Liquids

Liquids are most commonly decontaminated with the use of chemical disinfectants. Chemical disinfectants are regulated by the Environmental Protection Agency (EPA), and their use must be approved by the EPA for use on each specific biological material. The Environmental Protection Agency maintains an online list of EPA registered disinfectants.

The most common chemical disinfectant used is sodium hypochlorite, which is the active ingredient in bleach. This an excellent disinfectant because it can be used to decontaminate most infectious agents and is readily available as household bleach (~5.5% sodium hypochlorite).

The following is a common procedure which can be used to decontaminate a liquid culture:

- Add bleach to the liquid culture to be decontaminated
    - 10% final concentration of bleach
- Let stand for at least 30 minutes
- Discard down a laboratory sink

Section 7.2 – Decontamination of Solids

Solids (e.g., surfaces, equipment) which have come in contact with infectious, or potentially infectious, materials are commonly decontaminated by one of the following procedures:

- Chemical decontamination with bleach
    - Use 10% solution of bleach (prepared fresh at least weekly)
    - Expose solid to bleach solution
    - Let stand for at least 30 minutes
    - Wipe with water to remove bleach residue

- Autoclave
    - Expose solid to a 30 minute autoclave cycle at 121°C

Autoclaving is the preferred method to decontaminate solids because it sterilizes the material and should be used whenever possible.

Section 8 – Waste Management Plan

This plan provides guidance on the proper disposal of all biological materials used

or generated in UWO research and teaching laboratories, and is based on federal standards and guidelines established by the Centers for Disease Control (CDC), the National Institutes of Health (NIH), and Wisconsin Department of Natural Resources (DNR) infectious waste rules regarding disposal of biological waste.

Section 8.1 – Purpose of Waste Management Plan

This plan is meant to ensure that all biological materials are disposed of in a manner consistent with how similar infectious (or potentially infectious) materials would be properly disposed; regardless if the material has any potential to cause an infection. This ensures that all infectious, potentially infectious, or recombinant materials are properly disposed. Acceptable methods for the treatment of contaminated materials include decontamination using chemicals (e.g., 10% bleach) and steam (i.e., autoclave). The use of alternative disposal procedures need to be approved by the Institutional Biosafety Committee (IBC).

Section 8.2 – Responsibilities

It is the responsibility of UWO Principal Investigators (PIs) conducting or supervising laboratory work involving the use of biological materials to train personnel and/or students in the proper disposal procedures applicable to the type of work conducted in their laboratory.

Section 8.3 – Definitions

The following are definitions of various materials used in biological research or teaching laboratories:

Animal Source Material: any material derived from an animal (e.g., whole animals, organs, tissues, and primary or established cell lines), that are recombinant (transgenic) or non-recombinant.

Biological Material: anything biological in nature including: animals, biological toxins, human source material, infectious proteins, microorganisms, nucleic acid, and cell culture; recombinant or non-recombinant.

Biological Toxin: all protein toxins which are generated *in vivo* or *in vitro* as a result of a biological process

Cell Culture: any cells which are grown in culture that are recombinant or non-recombinant.

Contaminated: the presence or the reasonably anticipated presence of blood, infectious, or potentially infectious material on equipment or surface.

Human Source Material: any material derived from a human (e.g., organs, tissues, and primary or established cell lines) that are recombinant or non-recombinant.

Infectious or Potentially Infectious Materials: all materials which are capable of, or have the potential to, cause an infection in an immunocompetent individual.

Infectious Proteins: all proteins known or thought to cause infectious diseases (e.g., prions).

Microorganism: all viruses, bacteria, and fungi (pathogenic or non-pathogenic) that are recombinant or non-recombinant.

Nucleic Acid: DNA or RNA.

Section 8.4 – Decontamination Procedures

Acceptable disposal practices for the various biological materials used at UWO are listed below. The use of any disposal procedure not detailed in this plan will require the approval of the IBC prior to implementation.

Biological Toxins – All biological toxins shall be disposed of in a manner appropriate to the specific toxin, and which has been approved by the IBC.

Cell Culture – Cells which are grown in culture, shall be disposed of by one of the following methods:

- Expose cells to a 10% final concentration bleach solution for at least 30 minutes prior to disposal in the sanitary sewer (e.g., lab sink)
- Autoclave cells for at least 30 minutes prior to disposal in the regular trash, or (if appropriate) disposal in the sanitary sewer (e.g., lab sink)

Human Source Material – Material derived from humans shall be disposed of in a method appropriate to the type of material:

- Primary Cells – Cells generated from human tissue shall be disposed of using one of the following methods:
    - Expose cells to a 10% final concentration bleach solution for at least 30 minutes prior to disposal in the sanitary sewer (e.g., lab sink)
    - Autoclave for at least 30 minutes prior to disposal in the regular trash, or (if appropriate) disposal in the sanitary sewer (e.g., lab sink)
- Other Potentially Infectious Human Materials (OPIM) – Materials shall be disposed of using one of the following methods:
    - Expose solids/liquids to a 10% final concentration bleach solution for at least 30 minutes prior to disposal in the sanitary sewer (e.g., lab sink)
    - Autoclave solids/liquids for at least 30 minutes prior to disposal in the regular trash, or (if appropriate) disposal in the sanitary sewer (e.g., lab sink)

- In a manner appropriate to the specific material and which has been approved by the IBC

Infectious Proteins – All infectious proteins (e.g., prions) shall be disposed of in a manner appropriate to the infectious protein, which has been approved by the IBC, and according to the method specified in the IBC protocol.

Microorganisms – All microorganisms shall be disposed of using one of the following methods:

- Expose microorganisms to a 10% final concentration bleach solution for at least 30 minutes, prior to being disposed of in the sanitary sewer (e.g., lab sink)
- Autoclave microorganisms for at least 30 minutes prior to being disposed of in the regular trash or, if appropriate, disposed of in the sanitary sewer (e.g., lab sink)
- An alternate method which has been approved of by the IBC

Nucleic Acid – All DNA or RNA used in research shall be disposed of using one of the following methods:

- Expose DNA/RNA to a 10% final concentration bleach solution for at least 30 minutes prior to disposal in the sanitary sewer (e.g., lab sink)
- Autoclave DNA/RNA for at least 30 minutes prior to disposal in the regular trash or sanitary sewer (e.g., lab sink)
- An alternate method which has been approved of by the IBC

Sharps & Non-Sharps – Materials used in research shall be disposed of using one of the following methods:

- Regulated Sharps (e.g., needles, syringes, lancets, blades) should be placed in hard-walled, puncture-proof, leak-proof, biohazard-labeled sharps containers located at the point of use. Do not overfill (i.e. limit filling to ¾ full). When sharps containers are ¾ full, close and autoclave for at least 30 minutes.
- Non-Sharps – Materials (e.g., pipet tips, centrifuge tubes, unbroken glass pipettes, plastic pipettes, durable glass or plastic vacutainers and petri dishes) which have come in contact with biological materials require disposal using one of the following methods:
  - Expose non-sharps to a 10% final concentration bleach solution for at least 30 minutes prior to disposal in the regular trash (place glass in designated disposal box)
  - Autoclave non-sharps for at least 30 minutes prior to disposal in the regular trash (place glass in designated disposal box)

Section 9 – Emergency Planning and Exposure Control Plan

Rigid adherence to proper safety practices will greatly reduce the likelihood of a laboratory incident. However, it is important to have proper plans designed to guide us in the event of emergencies which are easiest to predict. This section provides guidance on how to respond to spills or exposures which may occur.

Section 9.1 Spill Response

The IBC has prepared the following recommended procedures for a) responding to a biological spill outside of containment, b) decontaminating surfaces which may have been exposed to biological materials, and c) disposal of biological waste.

A. Spill Response: The following is the recommended procedure for decontaminating a spill outside of containment:

1) Notify supervisor and lab mates of spill and close off area to traffic; for large spills of >100ml, leave room for 30 minutes to allow for aerosols to settle.
2) Remove all contaminated clothing and place in biohazard bag for autoclaving, wash any exposed skin with soap and water, and wash exposed eyes for 15 minutes in eyewash.
3) Contact Local Medical Service (Mercy Medical or Aurora) if an exposure requires emergency medical attention (Call 911).
4) Contact University Police (424-1212) if necessary to request assistance limiting access to spill.
5) Put on any required PPE (do not attempt cleanup if adequate PPE is not available).
6) Cover the spill with absorbent material (e.g., paper towel).
7) Prepare fresh bleach solution (at least 10%).
8) Gently pour bleach solution on spill (starting from outside of spill).
9) Add more paper towels as necessary to contain liquid.
10) Allow 30 minutes contact time for bleach to decontaminate spill.
11) Clean-up any broken glass with dust pan or forceps and dispose in glass waste container; any glass not yet decontaminated can be placed in a container (e.g., beaker) for decontamination with a 10% bleach solution.
12) Clean up paper towels and dispose in a biological waste container.

Section 9.2 Exposure Control Plan

The Exposure Control Plan (ECP) assists our university in implementing and ensuring compliance with the OSHA Bloodborne Pathogens Standard (CFR 1910.1030) in an effort to protect UW Oshkosh employees. The UW Oshkosh Exposure Control Plan is reviewed and maintained by the EH&S Committee. A copy of the plan may be located on the IBC Forms Page.