Biosafety Program

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I. Introduction

Definition of Biohazardous Materials

Biohazardous materials are those materials that may cause harm to humans, domestic or wild animals or plants. Some examples include, recombinant DNA, transgenic animal or plants; human, animal or plant pathogens, biological toxins; human blood and certain human body fluids; and human or primate cell cultures.

Purpose

The Purpose of the Biosafety Program at the University of Northern Iowa is to provide assistance in protecting faculty, staff and students from being exposed to biohazardous materials and to prevent the release of such materials that may cause harm to humans, animals, plants or to the environment and to protect the integrity of experimental materials.

The Biosafety Program seeks to fulfill these goals by providing guidance for the Biological Safety Committee, managing the Blood-borne Pathogen Exposure Control Plan, and conducting exposure assessments for the Occupational Health Program (Consult with Arrowhead Clinic).

This manual outlines appropriate practices, university policies and regulatory requirements for working safely with biohazardous materials. Additionally, EH&S staff are available to assist researchers in ensuring proper practices and facilities are selected for biocontainment, biohazardous waste are properly disposed of, all regulatory guidelines that apply to research projects are strictly followed, and providing assistance in obtaining necessary regulatory permits.

University Biosafety Program

When using biohazardous materials, laboratory personnel must comply with the NIH guidelines, BMBL recommendations, and the American Biological Safety Association (ABSA) best practices as well as federal, state and local regulations.

Administrative Responsibilities

Laboratory Personnel are expected to follow all applicable safe practices and procedures presented in this manual and all other manuals and documents that may apply to safe practices within the laboratory. Personnel who may be pregnant, immuno-compromised or have other related health issues should consult with the Safety Data Sheet (SDS) for all hazardous chemicals, radioactive and pathogenic organisms to determine if any risks may exist. It is also important for these individuals to consult with their supervisor, Occupational Health (Arrowhead Clinic) or their personal physician to determine if potential risk may exist and how best to manage those risks.

Principal investigators, instructors and supervisors ensure personnel under their jurisdiction (including those doing collaborative research) are properly trained and strictly adhere to all the policies and guidelines presented in this manual.
Institutional Biosafety Committee serves as the institutional biosafety committee in all matters involving recombinant DNA studies, as required by the National Institute of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules. The IBC is required to oversee any use of human, animal or plant pathogens or biological toxins and to set policies that comply with federal, state, and local regulations and recommendations. The IBC has the authority to require operational changes in the event of noncompliance with required conditions.

Departments involved with the use of biohazardous materials in a laboratory facility are responsible for the application and implementation of the Biosafety Program within the laboratories under their administrative control.

Environmental Health and Safety has the responsibility of developing and maintaining the Biosafety Program. The office will also assist Principal Investigators with risk assessment upon request and will provide assistance with major spills and environmental releases to the environment.

The University Safety Officer has the responsibility for development and implementation of the environmental, health and safety programs at the University of Northern Iowa. The University Safety Officer or designee will oversee the development, application, and implementation of all safety programs designed to protect the health and safety of all personnel on the campus of the University of Northern Iowa.

Office of Research and Sponsored Programs
A unit of the Office of the Executive Vice President and Provost, provides administrative services and leadership for the University of Northern Iowa's research services and research compliance programs. The research compliance program is to assist the Provost in maintaining a research environment at UNI that fosters honesty, integrity, and a sense of community.

The University of Northern Iowa is responsible for assuring the health and safety of its employees and compliance with all related requirements of local, state and federal regulations. The University encourages employees to promote positive attitudes regarding safety by incorporating safety into their work practices and to cooperate fully with the implementation of all environmental, health and safety programs.
II. Regulations and Guidelines

The following federal and international regulations apply to work performed with potentially biohazardous materials.

Animals, Animal Pathogens and Related Products

U.S. Department of Agriculture (USDA) Regulations for animals and animal products (9CFR 001-199)

- Import/transport permits are issued by the Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS) branch.
- Quick reference and applications for import and interstate transport permits available at the USDA Import-Export Directory for USDA-APHIS Veterinary Services.

USDA Agricultural Bioterrorism Protection Act of 2002; Possession, Use and Transfer of Biological Agents and Toxins (9CFR121)

- Registration program for possession and transfer of pathogens of biological toxins defined as USDA VS Select Agents.

Biosafety Cabinets


- Information on the NSF Biohazard Cabinetry Program, which sets the criteria for standard methods by which biosafety cabinets are tested in order to be certified.

Human Blood, other Potentially Infectious Human Body Fluids or Tissues, and Human Cell Lines

- Explained in the Departmental Bloodborne Pathogens Manual

Human Pathogens and Biological Toxins

Biosafety in Microbiological and Biomedical Laboratories (BMBL).

- Guidelines for human pathogen use published by the Centers for Disease Control and Prevention (CDC) and the National Institutes of Health (NIH).

U.S. Public Health Service (USPHS) Foreign (42 CFR 71) and Etiological Agents, Hosts, and Vectors, (part 71.54) Regulations.

- CDC Importation Permits for Etiologic Agents.

CDC Possession, Use and Transfer of Select Agents and Toxins. (42CFR 72 and 73 and 42 CFR 1003)
- Registration program for possession and transfer of pathogens or biological toxins defined as Department of Health and Human Services (DHHS) Select Agents.
- Infectious Agent MSDS from Health Canada’s Laboratory Centre for Disease Control
- Quick safety references for pathogenic microorganisms in and MSDS format from Health Canada’s Laboratory Centre for Disease Control

**Plants, Plant Pests, Plant Pathogens, Noxious Weeds and Soil**

USDA introduction of Genetically Engineered Organisms (GM)) Regulations (7 CFR 340.0-340.9)

- Biotechnology transport, introduction and import permits issued by the APHIS Biotechnology Regulatory Services Branch. Use this link for information on labeling and packaging of GMO material.
- Field Testing of Genetically Modified Plants

USDA Agricultural Bioterrorism Protection Act of 2002; Possession, Use and Transfer of Biological Agents and Toxins (7 CFR 3310)

- Registration program for possession and transfer of pathogens or biological toxins defined as USDA PPQ Select Agents.

**Recombinant DNA**

NIH Guidelines for Research Involving Recombinant DNA Molecules

- Federal Requirements for all Recombinant DNA molecules
- Guidelines for Institutional Biosafety Committees.

**Biohazardous Waste Disposal**

US Environmental Protection Agency (EPA) Hospital/Medical/Infectious Waste Incinerators Regulations ((40 CFR 62)

- Emissions requirements for hospital, medical and infectious waste incinerators

University of Northern Iowa Sharps and Biohazardous Waste Policy.
III. Responsibilities of the Institutional Biosafety Committee (IBC)

The following teaching or research projects must be approved by the IBC:

- Recombinant DNA, which includes the use of transgenic animals or plants.
- Human, animal, or plant pathogens (examples are bacteria, viruses, fungi, prions or parasites.
- Biological toxins (ex. Tetanus toxin or aflatoxin)

Submitting an application for a project to the IBC

*The PI must complete an IBC Project Review Form and submit it to the IBC Coordinator.* (Office of the Dean of Research, UNI)

If the project involves animal use, the PI completes only the Protocol Review Form-USE of Animals in Research and includes the applicable IBC sections. This form must be completed and reviewed by the IBC committee prior to approval of research taking place.

Review process

The IBC will model after NIH IBC standards (*NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES, including :*

**The purpose of the IBC is:**

- To ensure adequate containment of potentially hazardous biological agents,
- To add a level of expert review and monitoring of potentially hazardous experiments
- To inform the public about experimental plans that have a potential to be hazardous
- To provide a means of communication among researchers and healthcare providers about potentially hazardous protocols

IBC review will be invoked:

- Prior to initiation of the research
- At regular intervals during the activity
- When a change of protocol occurs
- When new technologies are introduced

**More specifically**, the IBC will operate in accordance with NIH: Section III-D. Experiments that Require Institutional Biosafety Committee Approval Before Initiation. Prior to the initiation of an experiment that falls into this category, the Principal Investigator must submit
a registration document to the Institutional Biosafety Committee which contains the following information:

(i) the source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the NIH Guidelines. For experiments in this category, the registration document shall be dated, signed by the Principal Investigator, and filed with the Institutional Biosafety Committee. The Institutional Biosafety Committee shall review and approve all experiments in this category prior to their initiation. Requests to decrease the level of containment specified for experiments in this category will be considered by NIH (see Section IV-C-1-b-(2)-(c), Minor Actions).

Experiments NOT requiring the IBC include, for example:

• When using less than half of a virus genome
• When the host is E.coli K12 with non-conjugation proficient plasmids or phage
• If the insert does not encode a toxic protein
• When the culture has less than 10 liters
• When cloning small fragments or other innocuous components

The IBC will review all experimental protocols, containment, disposal and training protocols for lab personnel. Particular focus will be on compliance with NIH regulations for biosafety in NIH Section III-D. The IBC will consist of at least five members with expertise in recombinant DNA, of which there are:

- Two or more local members are not affiliated with institution
- One expert in plant biology
- One expert in animal containment principles
- A Biological Safety Officer only if BL3, 4 or large-scale production is used (NOT APPLICABLE to UNI)

More specifically, the IBC (in Accordance with NIH-Section IV-B-2-a-(1) will collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research and to identify any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the Institutional Biosafety Committee) and who represent the interest of the surrounding community with respect to health and protection of the environment (e.g., officials of state or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community).

The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P, Physical and Biological Containment for Recombinant DNA Research Involving Plants,
require prior approval by the Institutional Biosafety Committee. The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q, Physical and Biological Containment for Recombinant DNA Research Involving Animals, require Institutional Biosafety Committee prior approval. When the institution conducts recombinant DNA research at BL3, BL4, or Large Scale (greater than 10 liters), a Biological Safety Officer is mandatory and shall be a member of the Institutional Biosafety Committee (see Section IV-B-3, Biological Safety Officer). When the institution participates in or sponsors recombinant DNA research involving human research participants, the institution must ensure that: (i) the Institutional Biosafety Committee has adequate expertise and training (using ad hoc consultants as deemed necessary); (ii) all aspects of Appendix M have been appropriately addressed by the Principal Investigator; (iii) no research participant shall be enrolled (see definition of enrollment in Section I-E-7) in a human gene transfer experiment until the RAC review process has been completed (see Appendix M-I-B, RAC Review Requirements); and (iv) final IBC approval is granted only after the RAC review process has been completed (see Appendix M-I-B, RAC Review Requirements). Institutional Biosafety Committee approval must be obtained from the institution at which recombinant DNA material will be administered to human research participants (rather than the site involved in manufacturing gene transfer products).

**Authorization**

The PI must receive the authorization form, specifying any special conditions under which authorization is granted. If approval is denied, a written notification will be forwarded to the PI. The notification will explain the decision and will identify possible modification to the project that would allow the project to proceed.

**Renewal**

Projects must be reviewed for Biosafety compliance annually. Reauthorization forms will be sent to the PI at least 45 days prior to the authorization anniversary date. Every 5 years a full application must be submitted to the IBC.

If the renewal is not submitted by anniversary date the project will be considered terminated and administratively closed and according to federal regulations and departmental policy, all work on the project must cease.

Take into consideration and provide alternative procedures for faculty (PIs) who have been off campus, on sabbatical, etc.

A new application must be submitted and approved before work may proceed.
Change in Procedure

If a change is planned for the project prior to the authorization approval anniversary date, changes in the scope of the research that would not be covered in the original approved application, personnel changes or change in location, the PI must notify the IBC. IBC approval must be obtained before implementation of any modifications. Requests for modification changes can be submitted to the IBC committee (Get descriptions of modification submittals) The IBC will determine whether the changes to the project are major enough as to warrant a new application being required. Approval will not change the date which annual renewal of the project must take place.

IV. Medical Surveillance

A description of the medical surveillance process may be found in the University Chemical Hygiene Plan under the heading of Medical Emergencies in the Laboratory.

Exposure to human blood, tissues, cell lines and other potentially infectious materials (OPIM) as defined by the OSHA Bloodborne Pathogen Standard (29 CFR 1910.1030, requires medical surveillance and annual Bloodborne Pathogen Exposure Control Training. The Department maintains a written Bloodborne Pathogen Exposure Control Plan.

Hazard Inventory

Principal investigators must complete a Hazard Inventory prior to working with identified and regulated hazardous materials or conditions. Information from the inventory form will be used by EH&S after consulting with Arrowhead Occupational Clinic to determine if vaccination to determine if vaccination is necessary, if a pre-exposure serum sample must be taken, or if other medical surveillance is necessary.

Vaccinations and Testing

Personnel working with human pathogens must be offered the choice of receiving a vaccine, if it is available, and informed of the risks associated with the vaccine. Personnel that work with human blood, tissues, cell lines or OPIM must be offered the Hepatitis B vaccination. High-risk personnel, such as health care workers must also be offered a titer test 2 months after the final Hepatitis B vaccine dose. Personnel whose job duties may potentially expose them to tuberculosis must be offered routine testing to monitor exposure. Vaccinations and tuberculosis testing will be administered by Arrowhead and billed to the PI or department, as applicable.

Affected personnel choosing not to receive a vaccination must complete the Decline to Vaccinate portion of the Consent or Decline of Vaccination Form. The department supervisor must ensure that the completed and signed decline form is placed in the individual’s department personnel file.
Information about specific vaccines and exposure tests commonly given to personnel can be viewed from the CDC web site.

- Hepatitis A
- Hepatitis B
- Influenza, inactivated vaccine
- Influenza, live intranasal vaccine
- Rabies
- Tetanus/Diphtheria
- Tuberculosis testing

Exposure to Biohazardous Materials

Prior to working with human pathogens, blood tissues and cell lines or OPIM, all applicable safety information, such as the Material Safety Data Sheet (MSDS) for a specific pathogen, must be reviewed. Human pathogen SDS’s can be obtained at Health Canada’s Laboratory Center for Disease Control. Familiarity with exposure routes, symptoms and treatment methods will provide better preparation for the possibility of exposure to the human pathogens, blood, tissues and cell lines or OPIM being used.

V. Principles of Biosafety

The fundamental principle the University Biosafety Program is containment of harmful biological agents. The fundamentals of containment include the microbiological practices, safety equipment, and facility safeguards that protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory.

The most important part of containment is a strict adherence to standard microbiological practices and technique. Students and faculty who work with hazardous biological and chemical material must be aware of the potential hazards, and must be properly trained and be proficient in the practices and techniques required for handling such material safely. The professor in charge of the laboratory is responsible for providing this training and will ensure that all safety practices and procedures are enforced.

Every laboratory, handling potentially hazardous biological agents, should develop or adopt a biosafety or operations manual identifying the types of hazards that may be encountered and the practices and procedures to eliminate or minimize these hazards. Personnel should be aware of special hazards and be expected to read and follow the required practices and procedures.

Facility design and engineering controls, safety equipment and management practices must augment laboratory personnel, safety practices and techniques.
Safety Equipment includes Biological Safety Cabinets (BSC’s), enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The BSC is the main device used to contain infectious droplets and aerosols generated by varying types of microbiological procedures. There are three main types of Biological Safety Cabinets. These are Class I, Class II and Class III. Open-fronted Class I and Class II BSC’s are primary barriers that offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques. Class II biological safety cabinets also provide protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet.

Hazardous chemicals should never be used in a BSC for the HEPA filter which removes chemicals from the air does not remove chemical fumes. The possible exception of BSC’s being used in for hazardous chemicals would be a Class II Type B2 which is also a UL-classified fume hood. The CDC booklet, Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, provides more detailed information on the different types of BSC’s.

Open flames, such as Bunsen burners, should never be used in a BSC. This will disrupt the airflow, placing the worker and material being handled at risk of possible contamination. Open flames are extremely dangerous around flammable materials such as ethanol often found in a BSC. Sterile instruments and electric incinerators provide an excellent alternative to Bunsen burners.

It is imperative that the work area of a BSC be thoroughly cleaned and disinfected after each use with a chemical disinfectant such as iodophor. Allow sufficient disinfection time for the chosen disinfectant used. Do not use bleach for it is known to corrode steel and 70% isopropyl alcohol dries evaporates to quickly for it to be effective. Ultraviolet or UV lights are not recommended in a BSC due to their ineffectiveness and safety risk. UV light loses its effectiveness over time loses its effectiveness and has little power to penetrate through even the smallest dust particle so UV light in a BSC is useful only if a secondary disinfectant is used to keep the area clean between uses. When used the UV light should be turned off between uses for exposure over long periods of time will cause burns. New UV units have safeguards to prevent exposure but older units may not have this protection.

BSCs used for containing biological hazards should be recertified annually through a contracted qualified servicing company. Testing is done according to the internationally accepted standards of NSF International. A label should be tagged on each BSC to indicate the date it was certified.

BSCs not to be used for biological hazards must be tagged as “NOT FOR USE WITH BIO-HAZARDS.”
Area maintenance can perform minor adjustments to BSC airflow but filter changes should be performed by a qualified servicing company.

Personal protective equipment or PPE is most commonly used when handling hazardous biological or chemical material. Gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles are the most common types of personal protective equipment. PPE can be used in conjunction with BSC’s and other devices that contain the agents, animals or materials being handled. In cases where it may not be feasible to work in a BSC, personal protective equipment may be the primary barrier between personnel and the infectious material. Some examples of this include animal necropsy, animal studies, agent production activities and activities relating to maintenance, service or support of the laboratory facility.

Training and Education

Anyone planning to work with biohazardous material must be properly trained prior to handling biohazardous material. It is recommended that the lab supervisor or PI train conduct training annually so as to ensure continued safety. It is also their responsibility to pass on any changes in safety information to their respective students or employees. Training should include:

- A discussion of the Biohazard Safety manual and how it applies to specific work areas
- Explanations of health hazards and signs and symptoms of exposure to biohazardous materials used in specific work areas
- Description of actions personnel can take to protect themselves from exposure such as special work practices, use of safety equipment, vaccinations, emergency procedures.

Signs and Labeling

Anyone entering areas where biohazardous materials are used must be aware of potential hazards. The required signage for hazardous area where biological agents are being used is:

Red door signs indicating human biohazards must be posted at the entrance of rooms where microorganisms or biological toxins known to cause disease in humans are used. This includes microorganisms classified as Biosafety Level 2 (BSL-2) or greater and human blood, tissues, cell lines or other potentially infectious material (OPIM). Red or orange biohazard labels must be placed on containers and storage units (refrigerators, freezers, incubators, waste containers, etc.) used for microorganisms or biological toxins that cause disease in humans, or human blood, tissues, cell lines or OPIM. Contaminated equipment and biohazardous waste must be labeled in a similar manner.

Yellow door signs indicating animal biohazards must be posted at the entrance of rooms where strict animal pathogens are used.
Dark green door signs indicating plant biohazards must be posted at the entrance of rooms where strict plant pathogens or pests are used, or where certain GMO plants are grown or processed.

Where multiple biohazards are present, human hazards generally take precedence over animal and plant hazards when choosing which sign to use.

**Storage**

Hazardous biological materials stored within the lab shall be inventoried annually. One copy should be stored with the lab safety plan and another should be sent to the Environmental Health and Safety Office in order for the University to comply with Federal regulations regarding biological agents and toxins.

**Autoclaves**

Autoclaves shall be used properly so as to effectively sterilize their contents. When used for microbiological media preparation various time and temperature settings are required for sterilization. Individual trials should be run in order to verify adequate sterilization.

Autoclaving biohazardous waste must take into account the volume of the waste and the ability of the steam to penetrate the load. The minimum cycle time for autoclaving biohazardous waste is 45 minutes at 121°C (250 °F). The following elements all contribute to autoclave effectiveness.

- **Temperature:** Unless specifically instructed by media manufacturer’s directions, autoclave chamber temperature shall be at least 121°C.
- **Time:** Autoclave cycle time will vary according to the contents of the autoclave. If media is to be prepared, then the manufacturer’s instructions should be followed. Adequate autoclaving time for biohazardous waste is a minimum of 45 minutes, measured after the temperature of the material being sterilized reaches 121°C and 15 PSI pressure. The tighter the autoclave is packed, the longer it will take to reach 121°C in the center of the load. It is important to assure that the material you are autoclaving is properly inactivated.
- **Contact:** Steam saturation of the load is essential for effective decontamination. Air pockets or insufficient steam supply will prevent adequate contact. To ensure adequate steam contact, leave autoclave bags partially open during autoclaving to allow steam to penetrate into the bag. Add a small amount of water inside the bag to help ensure heat transfers to the items being decontaminated (water should not be added if it will cause biohazardous materials to splash out of the bag.)
• Containers: Only leak proof containers should be used for items to be autoclaved. Where possible, non-glass containers should be considered for use in the autoclave. Plastics such as polypropylene, polypropylene copolymer or fluoropolymer products can be repeatedly autoclaved. Non-borosilicate glass bottles should be place in a tray of water to help prevent heat shock. Plastic bags should be place in a secondary container in the autoclave in case liquids leak out. Plastic or stainless steel containers are considered as appropriate secondary containers. Make sure plastic bags and pans are autoclavable, to avoid having to clean up melted plastic.

• Indicators: Tape indicators can only verify that the autoclave has reached normal operating temperatures for decontamination. Most chemical indicators change color after being exposed to 121°C but cannot measure the length of time spent at 121°C. Biological indicators (e.g. Geobacillus stearothermophilus spore strips or spore suspension) and certain chemical indicators (e.g. Sterigage) verify that the autoclave reached adequate temperature for a long enough time to kill microorganisms.

• Use autoclave tape on all bags of biohazardous waste. Before autoclaving bags of biohazardous waste, place an “X” with autoclave indicator tape over the biohazard symbol. Autoclave tape can also be used to indicate if media or equipment has been autoclaved.

• Once a month, a biological indicator should be used to verify that the autoclave is working correctly (e.g., Geobacillus stearothermophilus spore strips or spore suspension). Bury the indicator in the center of the load to validate adequate steam penetration. Document the biological indicator results in a log book.

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**Autoclave Safety**

Autoclaves use saturated steam under high pressure to achieve sterilizing temperatures. Proper use is important to ensure the safety of the operator. Injuries can be prevented by observing the following rules when using the autoclave:

• Wear heat resistant gloves, eye protection, closed-toe shoes, and a lab coat, especially when unloading the autoclave.

• Prevent steam burns and shattered glassware by making sure that the pressure in the autoclave chamber is zero before opening the door at the end of a cycle. Slowly open the autoclave door and allow any residual steam to escape gradually.

• Allow items to cool for at least 10 minutes before removing them from the autoclave. Be careful with glass containers that contain liquids. Superheating is a condition that occurs often in autoclaves. Superheating occurs when liquids are at a temperature above their normal boiling point but do not appear to be boiling. In situations where personnel are in a hurry removing flasks or bottles from the autoclave, these superheated containers can explode.
- Never put sealed containers in an autoclave. They can explode. Large bottles with narrow necks may boil over violently if filled to full of liquid.
- Never put solvents, volatile or corrosive chemicals (e.g., phenol chloroform, bleach, formalin, fixed tissues, etc.) or radioactive materials in an autoclave. Call Environmental Health and Safety at 3-3445 if you have questions about proper disposal of these materials.

Security

A certain level of security should be practiced for all laboratories based on the assessment of risk and regulatory requirements. Supervisors and/or PIs should conduct a risk assessment to determine appropriate security measures. Some prime examples of security measures include locked buildings, laboratories, and storage units, limiting distribution of access keys, proximity cards or key codes.

Laboratory Practice and Techniques

Workplace-acquired infections are rare. In order for infection and disease to occur, an adequate number of organisms to cause disease (infectious dose) must be present and a route of entry into the body must be available. It is important to know how infectious organisms are transmitted and what their infectious dose is in order to help evaluate the risk and avoid infection. It is important to obtain as much information about the organism prior to the commencement of work. Good places to start are Infectious Agent SDS sheets and the current edition of Biosafety in Microbiological and Biomedical Laboratories.

Infectious agents are transmitted through one of the following routes:
- Sharps injuries (needle sticks, cuts with contaminated broken glass, etc., also known as parenteral exposure).
- Inhalation of aerosols (microscopic solid or liquid particles small enough to remain dispersed and suspended in air for long periods and are approximately 5 micrometers or less in diameter).
- Ingestion (oral-fecal routes of contamination are common sources of infection. Hand washing is important).
- Mucous membrane exposure (including the eyes, inside of the mouth and nose, and the genitals)

Using common work practices that block routes of exposure can prevent workplace infection. Good microbiological techniques must always be used the laboratory

- Wearing appropriate personal protective equipment (PPE) blocks potential routes.
• Eating, drinking, chewing tobacco, applying cosmetics or storing food in laboratories is strictly prohibited. Potentially contaminated hands should be kept away from the mouth, eyes and non-intact skin.
• Hands must be washed frequently, even after wearing gloves, and scrubbed vigorously with soap and water for a full 30 seconds (as long as it takes to sing the Happy Birthday Song). The physical removal of organisms from the skin is as important as using disinfectant.

Work surfaces and equipment must be decontaminated immediately after using biohazardous materials.

Pipetting

Common risks of pipetting include creation of aerosols and splashing. Micropipettors have also been known to create aerosols.

• Never pipette by mouth. Use only mechanical pipetting aids.
• If possible pipette only in biosafety cabinet.
• Use cotton-plugged pipettes and cotton-plugged micropipette tips where possible.
• Use “to deliver” pipettes instead of pipettes requiring blowout when using biohazardous material.
• In order to prevent splashing, biohazardous material should be dispensed from a pipette or micropipettor by allowing it to run down the receiving container wall.
• Pipettes should be placed horizontally in a pan filled with enough liquid disinfectant to completely cover them after they are used. Ensure adequate disinfection time is allowed to take place prior to disposal.
• Micropipette tips should be place in a puncture resistant container.
• All waste and/or disinfecting containers must be kept inside the cabinet while they are being used.

Centrifugation

The greatest risk with centrifugation is the creation of aerosols.

• Do not overfill centrifuge tubes. This will help to prevent leaks. The outside of the tubes should be wiped with a disinfectant after it is properly filled and sealed.
• A safety centrifuge cup is recommended for it is a primary barrier which is an enclosed container designed to prevent aerosols from being released during centrifugation. In order to minimize aerosol hazards, containment controls such as BSC’s or centrifuge cups must be used when handling infectious agents.
• Sealed tubes, O-ring sealed rotors or O-ring sealed safety buckets must be used. To avoid spills from broken tubes, the tubes, lids, O-rings, buckets, and rotors should be inspected for damage before each use.
• Ensure rotor is balanced before centrifugation
• Open rotors and centrifuge tubes must be opened inside a safety cabinet. If a biosafety cabinet available allow 10 minutes settling time before opening

Proper Use of Needles, Syringes, and other Sharps

The greatest risks are accidental injections and the creation of aerosols when using sharps.

• Use needles and syringes only when there is no reasonable substitute. Safety needles and syringes must be used in these instances.
• Keep sharps away from the fingers as much as possible. Sharps must never be bent, sheared or recapped. Needles should never be removed from syringes after use. If a contaminated needle must be recapped or removed from a syringe a mechanical device such as a forceps must be used.
• Air bubbles should be minimized when filling a syringe.
• A pad moistened with disinfectant must be placed over the tip of the needle when expelling air. Work must be performed in a biosafety cabinet where possible.
• Appropriated sharps container must be kept close to the work area to avoid walking around the contaminated sharps. Care must be taken not to overfill sharps containers. They are considered full when they are 2/3 full. The University of Northern Iowa Laboratory Sharps and Biohazardous Waste Procedures details proper disposal methods.

Animal Handling

Spreading infectious between animal populations or between animals and humans can be prevented by following some basic guidelines.

• Use footpaths where available when entering or exiting a room where animals are housed.
• Animal room doors must be closed unless entering or exiting.
• Disposable gloves must be worn when handling animals, bedding or soiled cages.
• Disposable or washable outer garments (including lab coats, gowns, coveralls) should be worn to protect personal clothing when working with animals.
• Eating, drinking, applying cosmetics, and handling contact lenses in animal or procedure rooms are strictly forbidden.
• Avoid hand contact with nose, eyes and mouth while handling animals.
• Wash hands with soap and water immediately after handling animals or handling equipment prior to leaving the animal facility.
• Use extreme caution when handling needles or other sharp equipment used with animals. Ensure needles remain capped until it is ready for use then promptly dispose of in the
proper container (never recap needles) of according to University of Northern Iowa Laboratory Sharps and Biohazardous Waste Procedures.

- Handle only those animals for which proper animal handling techniques have been provided.
- Any bites or wounds should be promptly washed with soap and water and appropriate medical attention given. Accidents and injuries occurring at work or in the course of employment must be reported to the individual’s supervisor even if no medical attention is sought.
  - The supervisor is responsible for completing a First Report of Injury Form and submitting it to the Human Resources office within 24 hours of being notified.
- Unauthorized personnel are prohibited from entering the animal facility.

### Cell and Tissue Culture

Most cell and tissue cultures contain viruses. All cell lines should be considered potentially infectious. Most cell and tissue cultures can be safely manipulated using BSL-2 practices and containment.

- All primary and permanent human or other primate cell lines or tissue cultures must be handled using BSL-2 practices and containment.
- Any personnel handling human cell tissue cultures must participate in the Bloodborne Exposure Control program.
- If any cells or tissue cultures are known or suspected to contain a specific pathogen or oncogenic virus, appropriate biosafety practices for handling that virus must be used when working with cell or tissue culture.
- BSL-1 practices, procedures and containment may be used for cell lines that meet all of the following criteria:
  - Not be of human or other primate origin
  - Be confirmed not to contain human or other primate pathogens, including viruses, pathogenic bacteria, mycoplasma or fungi.
  - Be well established.

### VI. Biosafety Levels

Four Biosafety Levels or BSLs are described below. These are a combination of laboratory practices and techniques, safety equipment and laboratory facilities. Each combination is specifically appropriate for the operations performed, the document or suspected routes of transmission of the infectious agents, and the laboratory function or activity.

#### Biosafety Level 1

Biosafety Level 1 practices, safety equipment and facility design are appropriate for undergraduate and secondary educational training and teaching laboratories and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. Some
examples of these microorganisms are Bacillus subtilis, Nigeria gruberi, infectious canine hepatitis virus, and exempt organisms under the NIH guidelines

BSL-1 is basic level of containment relying on standard microbiological practices with no special primary or secondary barriers recommended other than a sink and hand washing.

The following standards practices, safety equipment and facility requirements must be followed at the University of Northern Iowa when working at the University of Northern Iowa.

**Standard Microbiological Practices**

- The laboratory supervisor must enforce policies that control access to the laboratories.
- Persons must wash their hands after working with potentially hazardous materials and prior to leaving the laboratory.
- No eating, drinking, handling contact lenses, applying cosmetics and storing food for human consumption must be permitted in laboratory areas. Food must be stored only in designated cabinets or refrigerators outside the laboratory.
- Only mechanical pipetting is permitted. Mouth pipetting must never be performed.
- Policies for the safe handling of sharps such as needles, scalpels, pipettes and broken glassware should be strictly followed. Laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharp injuries wherever possible. Precautions should be followed when working with sharps, especially the following:
  - Ensure needles and other sharps are managed accordingly. Do not bend needles, shear them, break them, recap needles removed from syringes or manipulate them by hand prior to disposal.
  - Dispose of needles according to University of Northern Iowa Sharps and Biohazardous Waste Procedure.
  - Non-disposable needles must be placed in hard walled container for transport to processing area. Dispose of according to university sharps disposal program.
  - Do not handle broken glass directly. Remove only by brush and dustpan, tongs and forceps. Use plastic wherever possible.
- Follow all protocols and procedures to prevent or minimize the creation of splashes and/or aerosols.
- Decontaminate all work surfaces after the work is completed and following any spills or splashes of potentially infectious material with the appropriate disinfectant.
- Ensure all cultures, stocks and other potentially infectious material has been decontaminated prior to disposal according to established methods. When the material is being transported the following methods should be used:
  - If the material is being decontaminated outside the laboratory where the work was taking place, it must be placed in a durable, leak proof container and properly secured for transport.
If the material is to be decontaminated at another facility, it must be packed according to all local, state and federal regulations.

- A posting of the universal biohazard symbol must be posted at each entrance to the laboratory when infectious agents are present. The posting may include the name of the agent(s) in use, and the name and phone number where the laboratory supervisor or other responsible personnel may be reached. Agent information should be posted according to the university posting criteria.

- Adherence to a strict pest management program.

- The PI or laboratory supervisor must provide the proper training for all laboratory personnel regarding their duties, precautions to prevent exposure and procedures to follow in case of exposure. Personnel are required to receive annual updates and whenever protocols or procedures are changed. Personal health status may affect their ability of fight infection and ability to receive immunizations or prophylactic interventions. All laboratory employees and students especially women of child bearing age should receive information regarding immune competence and conditions in which they may be predisposed to infection. Personnel with these conditions are encouraged to identify their condition to their healthcare provider for appropriate counseling and guidance.

Special Practices

There are no special practices required at this level.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

- Special containment devices and equipment such as BSC’s are not required at this level.
- Protective laboratory coats, gowns or uniforms should be worn to prevent the contamination of clothing.
- Wear protective eyewear when conducting procedures where there is a possible splash hazard of microorganisms or other hazardous material. Protective eyewear should also be worn over contacts.
- Appropriate gloves should be worn to protect hands from exposure to hazardous material. It is important that the type of glove selected is based on the material being manipulated. An alternative should be available to those individuals allergic to latex. Hands should be washed after removing gloves prior to leaving the laboratory. In addition personnel working in BSL-1 laboratories should:
  - Change gloves when gloves become contaminated or the integrity of the glove is compromised.
  - Remove gloves and wash hands when working with hazardous material has been completed and prior to leaving the laboratory.
  - Disposable gloves must never be re-used. Dispose of used gloves as contaminated waste. It is important to follow hand washing protocols.
Laboratory Facilities (Secondary barriers)

- Laboratories should have doors to ensure access controls
- Laboratories must have at least one sink for hand washing.
- The laboratory should be designed so that it can be easily cleaned. Carpets and rugs are not appropriate for a laboratory setting.
- Laboratory furniture must be capable of handling anticipated loads and uses. Spaces between benches, cabinets, and equipment should be easily accessible for cleaning.
  - Benchtops must be impervious to water and should be resistant to heat, organic solvents, acids, alkalis and other chemicals.
  - Chairs used for laboratory work must be covered with a nonporous material that is easily cleaned and decontaminated with an appropriate disinfectant
- Laboratory windows that open to the exterior should be fitted with screens.

Biosafety Level 2

Biosafety Level 2 are applicable to clinical, diagnostic, teaching and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. Using good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provide the potential for producing splashes or aerosols is low. Hepatitis B virus, HIV, the Salmonella, and Toxoplasma re representative of microorganisms assigned to this containment level. BSL-2 is appropriate when work is done with human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Personnel who are working with human derived materials should consult with the OSHA Blood-borne Pathogen Standard.)

The main hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or infestations of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not often transmissible via the aerosol route, procedures with aerosol or high splash potential that may increase the risk to such personnel must be performed in a primary containment equipment or in a BSC or safety centrifuge cups. PPE should be used as needed such as gloves, splash shields, gowns and face protection.,

Secondary barriers, such as hand washing sinks and waste decontamination facilities, must be available to reduce environmental contamination.

Biosafety Level 2 builds on the standard microbiological practices of BSL-1. BSL-2 is used for work involving agents posing moderate hazards to personnel and the environment. The difference between BSL-2 and BSL-1 is that 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious
agents and their respective procedures; 2) access to the laboratory is restricted while work is being performed; and 3) all procedures where infectious aerosols or splashes may occur will be conducted in BSC’s or in other physical containment equipment.

The following standard and special practices, safety equipment and facility requirements apply to BSL-2.

**Standard Microbiological Practices**

Follow BSL-1 practices and procedures.

**Special Practices**

- All persons entering the laboratory must be advised of potential hazards prior to entry and meet all specific entry/exit requirements.
- Laboratory personnel must be provided medical surveillance as appropriate, and offered available immunizations for agents handled of potentially present in the laboratory.
- Consider the need for collection and storage of serum samples for personnel who are at risk.
- A laboratory-specific biosafety manual must be prepared and adopted as policy. The manual must be available and readily accessible.
- The laboratory supervisor must ensure laboratory personnel demonstrate proficiency in standard and special microbiological practices before allowed to work with BSL-2 agents.
- Potentially infectious materials must be placed in durable, leak proof container during collection, handling, processing, storage or transport within a facility.
- Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
- Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material
- Equipment must be decontaminated prior to repair, maintenance, or removal from the laboratory.
- Incidents that may result in exposure to infectious material must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatments should be provided and appropriate records maintained.
- Animal and plants not associated with the work being performed are not permitted in the laboratory.
- All procedures involving manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment device.
Safety Equipment (Primary barriers and Personal Protective Equipment)

- Biosafety Cabinets must be properly maintained, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
  - Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonication, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
  - High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
- Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas, e.g., cafeteria, library and administrative offices). Dispose of protective clothing appropriately or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
- 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 when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses should also wear eye protection.
- Glove selection at the BSL-2 level is the same as that at BSL-1
- Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

Laboratory Facilities (Secondary Barriers)

- Laboratories should have doors to ensure controlled access.
- Laboratories must have at least one sink for hand washing.
- The laboratory should be designed so that it can be easily cleaned. Carpets and rugs are not appropriate for a laboratory setting.
- Laboratory furniture must be capable of handling anticipated loads and uses. Spaces between benches, cabinets, and equipment should be easily accessible for cleaning.
  - Benchtops must be impervious to water and should be resistant to heat, organic solvents, acids, alkalis and other chemicals.
  - Chairs used for laboratory work must be covered with a nonporous material that is easily cleaned and decontaminated with an appropriate disinfectant
• Laboratory windows that open to the exterior at this level are not recommended. However if a laboratory does have windows that open to the exterior, screens must be installed.
• BSC’s must be installed so fluctuations of the room air supply and exhaust do not interfere with proper operations. BSC’s should be located away from doors, windows that can be opened, heavily traveled areas, and other possible airflow disruptions.
• Vacuum lines should be protected with liquid disinfectant traps.
• Eyewash stations must be readily available.
• There are no specific requirements for the ventilation systems, but when new facilities are being planned planners should consider mechanical ventilation systems that provide an inward flow of air without recirculation to the spaces outside the laboratory.
• HEPA filtered exhaust air from Class BSC can be safely re-circulated back into the laboratory if the cabinet is tested and certified annually and operated according manufacturer’s recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
• A means of decontaminating laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration or other approved decontamination procedures).

Biosafety Level 3

Biosafety Level 3 are applicable to clinical, diagnostic, teaching, research or production facilities where work is performed on indigenous or exotic agents with a potential for respiratory transmission and may be the cause a serious infection which may in fact be lethal. Some examples are Mycobacterium tuberculosis, St. Louis Encephalitis, and Coxiella burnetii. Materials requiring BSL-3 facilities and practices are not currently used at the University of Northern Iowa.

Biosafety Level 4

Biosafety Level 4 are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted as an aerosol for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to BSL-4 agents should be handled at this level. When sufficient data are obtained work with these agents may continue at this level or lower. Examples of BSL-4 viruses include Marburg or Congo-Crimean hemorrhagic fever.

Materials requiring BSL-4 facilities and practices are not used at the University of Northern Iowa.
Biohazard Spill Kit

Laboratories where biohazardous materials are in use must have appropriate equipment and supplies available to manage spills and accidents involving use of biohazardous materials. It is important an eyewash, emergency shower, handwashing facilities and supplies are located either within or immediately adjacent to the lab. A complete Biohazard Spill Kit is available. The spill kit should include:

- Laboratory biohazard spill clean-up protocol. (This should be included in the Laboratory Safety Plan.)
- Nitrile disposable gloves (8 mil) (ensure gloves are checked for holes or deterioration; gloves should be replaced every 2 years)
- Lab coat(s) or gowns.
- Goggles or safety glasses with side shields.
- Face masks
- Disposable shoe covers (booties)
- Absorbent material, such as paper towels, granular absorbent material, etc. (a disposable or cleanable scoop will be needed for granular absorbent)
- All-purpose disinfectant, such as normal household bleach (freshly diluted 1:10), iodophor (e.g., Wescodyne) or quarternary ammonia preparation (e.g., EndBac II)
- Autoclavable bucket for diluting disinfectant (can be used as kit storage when bucket is not in use.)
- Something disposable or which can be easily disinfected such as tongs, forceps, manila folders, etc. that could easily be used for picking up broken glass, other types of contaminated sharps or absorbent.
- Biohazard sharps waste containers(s)
- Autoclavable biohazard waste bags.
- Biohazard spill warning signs.

All non-disposable items shall either be autoclavable or compatible with the disinfectant to be used.

Spill/Release Kit

The following protocol is generic and is intended for use with microorganisms classified as BSL-2 or lower. The right protocol for any situation depends on the type of specific biohazardous material used, quantity spilled, and the location of the spill. Spill plans should be located within the Laboratory Safety Plan and shall include, but not limited to, procedures, training, and names and contact information of trained personnel from the laboratory or department.
Biohazard Spill Clean-Up Protocol

1. Spills outside the laboratory
   - Evacuate the immediate area for at least 30 minutes to allow any potential aerosols to settle. If outdoors, personnel should remain upwind from the spill, if possible.
   - EH&S and UNI Public Safety are available to assist in evacuation perimeter control. Laboratory personnel should secure the site while someone else is sent for help.

2. Biohazardous spill within the laboratory
   - Outside of a BSC: the laboratory must be evacuated for at least 30 minutes to allow any potential aerosols to settle. It is the responsibility of the last person out to ensure that all doors have been closed.
   - Within a centrifuge; the centrifuge should be closed as soon as the spill is noticed. Wait 30 minutes to allow aerosol to settle before opening to clean and disinfect.
   - Within a BSC: the BSC must remain running.

3. Everyone not needed for the spill cleanup must be cautioned to stay away from the spill area until cleanup has occurred. Signs may be posted if necessary.

4. Any clothing that may have been contaminated must be removed and placed in a biohazard waste bag for decontamination.

5. Hands and any other contaminated skin must be washed thoroughly with soap and water.

6. Appropriate PPE must be worn. At minimum, nitrile gloves, eye protection and a lab coat must be worn. A face shield or mask (splash protection) is advised for spills greater than approximately 10 ml outside a BSC or any spill inside a centrifuge. If there is a potential for aerosolization of the spilled material, use a respirator (see the University Respiratory Protection Manual).

7. Any sharp contaminated objects must be removed from the spill area using mechanical means, never with hands.

8. Disinfectant must be poured carefully around the edges of the spill, with care taken to avoid splashing. Paper towels can be used to absorb as much of the spilled material as possible. Working from the outside of the spill toward the center avoids spreading the contamination. Place discarded paper towels in a biohazard bag for disposal.
   - Note: Alcohol is not recommended as disinfectant for large spills, especially inside a BSC, because large amounts of alcohol pose an explosion hazard and small amounts evaporate too quickly to ensure disinfection.

9. If the spill is inside a centrifuge, the rotor and its contents should be moved to a BSC, if possible. The external surfaces should be decontaminated prior to moving to the BSC.

10. If the spill is inside a BSC, the spill tray underneath the work area and the trough below the air intake grill must be cleaned as well. These are likely to be contaminated when the
spill is large. The cabinet should be left running for at least 10 minutes before resuming use.

11. After initial clean up, the spill area must be flooded with disinfectant and left to soak for at least 15 minutes or according to manufacturer’s instruction. Adequate contact time is important to ensure complete decontamination.

12. Disinfectant can be absorbed with paper towels.
   A final wipe-down should be done with clean paper towels soaked with disinfectant.
   Laboratory personnel should be sure to disinfect any equipment, walls or other areas likely to have been splashed by the spill.

13. If radioactive material is involved in the spill, also wash the surface with detergent according to radioactive spill guidelines.

14. All contaminated waste be disposed of properly

15. Hands must be washed thoroughly with soap and water.