NORTH DAKOTA STATE UNIVERSITY BIOSAFETY MANUAL

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Abstract

The NDSU Biosafety Manual shall be used as a useful resource for investigators to provide the necessary information to protect themselves, the community, and the environment from the potential hazards associated with biohazardous agents and materials used in research.

University Police and Safety

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I. PURPOSE AND MISSION

The Mission of the University Police and Safety Office (UPSO) at North Dakota State University is:

> *To provide professional services and resources to help the campus be a safe and secure place to live, learn, work and visit, while being prepared to respond to the emergency services needs of the campus community.*

To assist in accomplishing this Mission, the Environmental Health and Safety Unit (EHS) of the UPSO has created this Biosafety Manual as a useful resource for investigators to provide the necessary information to protect themselves, the community, and the environment from the potential hazards associated with biohazardous agents and materials used in research.

The goal of this Biosafety Manual was to provide the aforementioned information that has been tailored for the needs of NDSU researchers by assembling salient information from the following resources:

- The Centers for Disease Control and Prevention Biosafety in Microbiological and Biomedical Laboratories, 6th Edition, 2020. (This document is referred to as the BMBL throughout this Manual.)
- NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), Office of Biotechnology Activities, 2019. (May be referred to as "The NIH Guidelines" in this Manual).
- The Centers for Disease Control and Prevention Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008
- The Biosafety Manuals of the University of Florida and The University of Washington were also used as references.

Discussing the hazards associated with chemical, radiation, and nanomaterial research at NDSU is beyond the scope of the Manual. Please refer to the links provided for online safety manuals as resources, or contact the Safety Office (701-231-7759) for advice on working with these materials.

For the rest of the manual, the terms "shall", "will", or "must" indicate biosafety requirements that are considered mandatory. Terms such as "recommend", "should", or "may" are practices that are recommended for adoption and are considered as good or standard biosafety laboratory practices.

II. DEFINITIONS

For the purposes of this manual, the definition of biohazardous agents will include:

1. Infectious agents including viruses, bacteria, rickettsia, fungi, protozoa, parasites, and prions.
2. Human blood, tissues, bodily fluids, and cell cultures (primary or immortalized).
3. Recombinant or synthetic DNA molecules, including organisms and viruses containing recombinant or synthetic DNA molecules and vectors.

   Recombinant and synthetic DNA molecules include molecules that are chemically or otherwise modified analogs of nucleotides, or both. According to the NIH Guidelines the definition of recombinant or synthetic DNA molecules include the following:

   (a) molecules that are constructed by joining nucleic acid molecules and that can replicate in a living cell (i.e., recombinant nucleic acids);

   (b) nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules (i.e., synthetic nucleic acids);

   (c) molecules that result from the replication of those described in (a) or (b).
4. Biologically active toxins.
5. Plants or animals (or tissues/part of plants or animals) that contain items 1-4 above.
6. All wastes generated from items 1-5 above, if they do or are reasonably considered to contain the biohazardous agent.

III. ROLES AND RESPONSIBILITIES

A. THE PRINCIPAL INVESTIGATOR

The Principal Investigator of a laboratory holds the primary and ultimate responsibility for the safe operation of his or her lab. The PI must become familiar with all policies, procedures, regulations, and laws that apply to the research conducted by members of their laboratory, and provide training such that the people they supervise are aware of these as well.

The PI must be trained in good microbiological technique and must ensure that all laboratory members receive proper training before being allowed to conduct work in the lab. Responsibility for the supervision of lab workers, and enforcing the usage of proper biosafety work practices and techniques at all times rests with the PI.

The PI is responsible for the submission of all protocols falling under the purview of the Institutional Biosafety Committee (IBC) for review at the appropriate times, and keeping the protocols updated. Please refer to the IBC policy and guidelines for a current description of what research and teaching activities fall under the IBC purview.

When there is a spill or release of biohazardous agents, an accident, or an exposure to a biohazardous agent in their lab, the PI is responsible for timely reporting of such incidents to the Environmental Health and Safety (EHS) Office, the IBC, and the Greenhouse Manager (if working in the Greenhouse). Reporting requirements to the NIH exist if the exposure/release involves recombinant or nucleic acids. Please see the NIH Guidelines for a more thorough explanation of reporting requirements, or ask for clarification from the IBC or the NDSU EHS Office.

B. LABORATORY MANAGERS

When delegated by the PI, Laboratory Managers who have a supervisory role are responsible for ensuring that all University and laboratory biosafety practices and protocols are followed. Lab managers are responsible for identifying any unsafe lab practices or workers and reporting them to the PI.

C. STUDENTS AND EMPLOYEES

All students and employees who work with biohazardous agents and materials must read this manual and understand the contents. All individual lab workers must be committed to working safely and following all laboratory biosafety practices. This commitment not only protects the individual, but also protects co-workers, lab neighbors, the campus community, and the environment. If there is an accident, or a spill in the lab, the student or employee must report the details as soon as possible to their PI or lab supervisor and seek medical attention if necessary.

D. DEANS, DIRECTORS, CHAIRPERSONS

These men and women are responsible for all of the students, employees, and visitors in the spaces under their control. They must be aware of what research is happening in these spaces, the risks associated with that research, and the control methods proposed by the PIs.

E. INSTITUTIONAL BIOSAFETY COMMITTEE

The IBC reviews and oversees projects at NDSU that involve recombinant or synthetic nucleic acids, infectious agents, and human blood, bodily fluids and tissues (IBC Guidelines). The IBC works together with the EHS Office to ensure compliance with the NIH Guidelines and other

biosafety regulations. The IBC also recommends training for researchers working on protocols falling under their purview.

F. ENVIRONMENTAL HEALTH & SAFETY OFFICE

The Environmental Health and Safety Office within the University Police and Safety Office provides expertise in developing the NDSU Biosafety Program and supports BSL-1, BSL-2 laboratories, and ABSL-1, ABSL-2, BL1-P, BL2-P, and BL3-P spaces. They develop, implement, and update biosafety policies and procedures that are necessary for an effective, compliant, and efficient biosafety program. The EHS Office plays an important role in providing technical support to the IBC, and PIs on campus as well as working closely with researchers to provide necessary training on biosafety procedures and proper use of containment equipment.

The Environmental Health and Safety Office is responsible for conducting periodic laboratory inspections of all areas on campus involved in research utilizing biohazardous agents to assure appropriate safety controls, containment, and compliance. The results of these laboratory inspections are shared with the IBC as part of the protocol approval process for all labs and spaces operating at BSL-2, ABSL-2, and BL2-P and above.

The EHS Office develops and provides educational materials and training. When accidents, exposures, or spills happen in the lab, the EHS Office responds to, investigates, and follows up with suggestions and strategies for a resolution to prevent reoccurrences.

IV. RISK ASSESSMENT

Prior to beginning work or a procedure with any biohazardous agent, a risk assessment needs to be performed which considers both agent and procedure hazards. When submitting a new protocol for review to the IBC, the PI performs the initial risk assessment for the project. This process is critical to determining what work and containment practices need to be employed in order to safely conduct the work required with the biohazardous agent(s).

The CDC's BMBL defines Risk Assessment as: "the process that enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can prevent laboratory-associated infections (LAI)."

An extension of this definition is important for plant or agricultural pathogens because of the significance of performing an appropriate risk assessment in order to prevent a release of infectious agents or genetically modified plants/plant associated pathogens or pests into the environment where they may exact a serious detrimental impact on natural or managed ecosystems, or other important agricultural entities.

The NIH Guidelines defines Risk Assessment as a multi-step process:

- Subjective process which requires a comprehensive approach
- Investigator makes initial risk assessment based on agent Risk Group (RG) assignment
- Protocol requirements are considered
- RG assignment and protocol requirements are considered together in order to set the appropriate containment level

A. RISK GROUPS

The principal hazardous characteristics of an agent are: 1) its capability to infect and cause disease in a susceptible host; 2) its virulence as measured by the severity of disease; 3) and the availability of preventive measures and effective treatments for the disease.

There are 4 Risk Groups for human/animal pathogens described below that use these agent characteristics for general group characterization:

Table is from the BMBL 5th ed., page 10

Table 1: Classification of Infectious Microorganisms by Risk Group

Risk Group Classification	NIH Guidelines for Research involving Recombinant DNA Molecules 2002[2]	World Health Organization Laboratory Biosafety Manual 3rd Edition 2004[1]
Risk Group 1	Agents not associated with disease in healthy adult humans.	(No or low individual and community risk) A microorganism unlikely to cause human or animal disease.
Risk Group 2	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are *often* available.	(Moderate individual risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.
Risk Group 3	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).	(High individual risk; low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
Risk Group 4	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).	(High individual and community risk) A pathogen that usually causes serious human or animal disease and can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.[3]

For veterinary and economically important agricultural pathogens, the following risk groups described in the table below apply:

Biosafety Matrix for Livestock Pathogens

Risk Group	Definition	BSL recommendation for Animals Large / Small
1	Unlikely to spread; may cause mild disease; low risk; no official control program	1 or 2 / 1
2	Limited spread; produces moderate disease; moderate risk; possible control programs	2 or 3 / 2
3	Can spread readily; causes serious disease; high risk; control programs in place; treatment and prevention available	3 or 3-Ag / 3
4	Can spread rapidly; causes severe disease; maximum risk- exotic; strict control programs in place; treatment and prevention not effective	3-Ag / 3-Ag

From Emerging Diseases of Animals, Chapter 2: Biosafety Classification of Livestock and Poultry Animal Pathogens, 2000, J. Scott Rusk

B. AGENT HAZARDS

Besides considering Risk Group assignment, the following agent hazards are deliberated when doing a formal risk assessment:

1. Probable routes of transmission:
    - What exposures to the agent could lead to an infection?
        - direct skin, eye or mucosal membrane exposure.
        - parenteral inoculation by a syringe needle or other contaminated sharp, or by bites from infected animals and arthropod vectors.
        - ingestion of liquid suspension of an infectious agent, or by contaminated hand to mouth exposure.
        - inhalation of infectious aerosols- if aerosol exposure is a probable route of infection, careful measures need to be taken to prevent the generation of aerosols in the laboratory (see pages 10-11).

2. Infectious dose: the lower the infectious dose, the higher the risk; a low infectious dose is compounded if the route of infection is via aerosol.
3. Stability in the environment (biological decay): if the agent is very stable in the environment, lab disinfection protocols need to take this into consideration.
4. Host range:
    - Are humans a natural host?
        - If working with a plant or animal pathogen, is the natural host found in the environment where the research is happening? If yes, then measures to prevent transmission or release into the environment are required.
5. Endemic nature:
    - Important for agricultural and plant pathogens- if working with exotic pathogens, or agricultural pathogens not found in the geographical area, risk to the environment is high requiring strict containment measures.
6. Laboratory Associated Infections (LAIs):
    - Reports of LAIs are a clear indicator of hazard, though the absence of a report does not indicate minimal risk.

A special note about conducting a risk assessment on genetically modified organisms. Researchers may have to consider the risk assessment for the wild-type organism as the baseline. Therefore, the initial protocol risk assessment may be incomplete, and may have to be adjusted during the project as new data are acquired.

Genetic alterations have the capacity to alter virulence, such as:

- Change tropism, cellular and host (example: viral receptor modification)
- Alter susceptibility to available treatments (example: antibiotic or herbicide resistance)
- Merge characteristics of two organisms (example: gain of function experiments)

C. PROCEDURE HAZARDS

After all agent hazards have been factored into the risk assessment, the next step in conducting a risk assessment is to consider the procedures applied to the biohazardous agent(s) in the project.

Some common procedure hazards to consider in a project risk assessment:

- Splash Hazards such as pouring or transferring liquids,
- Working with animals (bites, scratches, exposure to zoonotics and aerosols),
- Sharps (needles, dissecting tools, razor blades, and lack of good sharps practices),
- Agent concentration and volumes worked with in the procedure,

- Complexity of the proposed procedures:
  - How many manipulations are included?
  - Is transportation of the agent required?
  - Injections (inclusions of sharps) versus just culturing?
  - Are other hazards involved in the experiment(s) (for example hazardous chemicals, radiation, etc.)? Additional hazards can divide focus, and possibly be a source for error.

D. AEROSOLS

The creation of aerosols is a procedure hazard that warrants special consideration. From the CDC's BMBL: "Small-particle aerosols have respirable size particles that may contain one or several microorganisms. These small particles stay airborne and easily disperse throughout the laboratory. When inhaled, the human lung will retain those particles. Larger particle droplets rapidly fall out of the air, contaminating gloves, the immediate work area, and the mucous membranes of unprotected workers. A procedure's potential to release microorganisms into the air as aerosols and droplets is the most important operational risk factor that supports the need for containment equipment and facility safeguards."

Aerosols can be ubiquitous in laboratory procedures such as:

- Forceful pipetting
- Necropsy
- Blending
- Changing of animal cages/bedding
- Removing contaminated gloves quickly/improperly
- Dropping of culture dishes
- Centrifuging using non-aerosol tight centrifuges cups or rotor lids
- Sonication
- Homogenizing
- Electroporation
- Vortexing
- Intranasal inoculations of animals
- Flaming inoculation loops
- Inserting hot inoculation loops into a culture
- Streaking plates
- Freeze drying samples
- Opening lyophilized cultures, plates, ampoules, tubes, bottles
- Vacuum aspirating

Aerosols are very small (≤5μm), usually go undetected, and are extremely pervasive, placing the laboratory worker carrying out the procedure and other persons in the laboratory at risk of exposure and possibly infection. There is general agreement among those who have investigated laboratory associated infections that an aerosol is the probable source of many infections, particularly in cases involving workers whose only known risk factor was that they worked with an agent, or in an area where that work was done. If you are working with an agent that can be spread via infectious aerosols, this requires rigorous containment controls.

If you have identified the generation of aerosols as a procedure hazard in your protocol, methods that can be utilized to prevent aerosol generation in the lab include:

- Working inside a biosafety cabinet provides the best protection from exposure to aerosols.
- Centrifuging using aerosol tight caps/lids on the buckets, rotors, or tubes; if not available, open containers in the biosafety cabinet after allowing to settle.
- Allowing settling time before opening containers after centrifuging, transporting, blending, homogenizing, etc.; required settling time depends on the sample type and agent however a minimum of 10 minutes is most often recommended.
- Do not use glass containers when blending, sonicating, homogenizing, etc. to prevent breakage and dispersal of infectious agents.
- If possible, when blending or sonicating place a towel moistened with disinfectant over the top of the device.
- Wear airway (N-95 respirator*, not a surgical mask) and mucous membrane protection to prevent exposure when working outside the biosafety cabinet

*N-95 respirators, like all respirators, require medical clearance and fit testing. Please read about NDSU's Respirator Protection Program, or contact the Safety Office (701-231-7759) for more information.

V. CONTAINMENT LEVELS

A. BIOSAFETY LEVELS (BSL)

From the CDC's BMBL 6th Edition, page 24:

> *"A fundamental objective of any biosafety program is the containment of potentially hazardous biological agents and toxins. The term containment describes a combination of primary and secondary barriers, facility practices and procedures, and other safety equipment, including personal protective equipment (PPE), for managing the risks associated with handling and storing hazardous biological agents and toxins in a laboratory environment. The purpose of containment is to reduce the risk of exposure to staff and the unintentional release of hazardous biological agents or toxins into the surrounding community and environment. Final determination on the combination of containment measures required to address the relevant biosafety risk present at a facility should be based on a comprehensive biosafety risk assessment."*

A biosafety level (BSL) consists of a combination of laboratory practices and techniques, safety equipment, and laboratory facilities which allow safe handling of a particular organism. The PI prepares the initial risk assessment for a project, which may be reassessed by the IBC during protocol review. The essential elements of BSL-1 and BSL-2 for activities involving biohazardous agents are directly derived from the CDC's BMBL and are summarized below. We encourage anyone considering a project that requires BSL-3 containment to contact the EHS Office well before work is planned to discuss these requirements. **Work at Biosafety Level 4 is not permitted/possible at NDSU.**

ABSL, and BL-P requirements will be discussed later in this manual.

The biosafety levels are designated in ascending order, by degree of protection provided. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

Standard Microbiological Practices to be followed at <u>both BSL-1 and BSL-2</u>:

Laboratories under all biosafety levels are required to adhere to the following Standard Microbiological Practices:

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.

2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption are not permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.

5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

a. Precautions, including those listed below, must always be taken with sharp items.

i. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.

ii. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

iii. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

iv. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.

6. Procedures should be performed carefully to minimize splashes and/or aerosols.

7. Work surfaces must be decontaminated with appropriate disinfectant after completion of work and after any spill or splash of potentially infectious material.

8. All cultures, stocks, and other potentially infectious materials must be decontaminated before disposal. Depending on where the decontamination will be performed, the following methods should be used prior to transport:

i. Must be placed in a durable, leak proof container and secured for transport.

ii. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.

9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents that pose an infectivity threat to humans or animals are present. The sign will include the name and phone number of the PI or other responsible personnel, the agents being used in the space, and required entry/exit procedures.

10. An effective integrated pest management program is required.

11. The PI/ laboratory supervisor must ensure and document that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur.

12. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals with these conditions are encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

BIOSAFETY LEVEL 1 – (BSL-1)

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immune-competent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by an appropriate risk assessment.

Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by an individual with training in microbiology or a closely related science.

The following standard practices, safety equipment, and facility requirements apply to all laboratory personnel.

LABORATORY BIOSAFETY LEVEL 1 CRITERIA – BSL-1

1. Special Practices:
   a. None required.

2. Safety Equipment (Primary Barriers and Personal Protective Equipment)
   a. Special containment devices or equipment, such as biosafety cabinets (BSCs), are not generally required.
   b. Laboratory coats, gowns, or uniforms are recommended to prevent contamination of personnel.
   c. Protective eyewear 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 eye protection.
   d. Gloves must be worn to protect hands from exposure to hazardous materials.
   e. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available.
   f. Wash hands prior to leaving the laboratory.
      i) In addition, BSL-1 workers should:
         (1) Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
         (2) Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
         (3) Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.

3. Laboratory Facilities (Secondary Barriers)
   a. Laboratories should have doors for access control.
   b. Laboratories must have a sink for hand washing.
   c. The laboratory should be designed so that it can be easily cleaned. Carpets and rugs in laboratories are not allowed.
   d. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
   e. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
   f. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
   g. Laboratories windows that open to the exterior should be fitted with screens.

BIOSAFETY LEVEL 2 – (BSL-2)

All standard microbiological procedures apply to BSL-2. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted (e.g., doors are closed); and 3) all procedures in which infectious aerosols or splashes may be created are conducted in biosafety cabinets (BSCs) or other physical containment equipment.

LABORATORY BIOSAFETY LEVEL 2 CRITERIA – BSL-2

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

1. Special Practices
    a. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements. This information must be included on the door signage.
    b. Laboratory personnel are offered appropriate immunizations for agents handled or potentially present in the laboratory.
    c. The University and lab specific biosafety manual must be prepared, available, updated annually, and accessible.
    d. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
    e. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
    f. Laboratory equipment must be routinely decontaminated, as well as after spills, splashes, or other potential contamination.
    g. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
    h. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
    i. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory PI/supervisor and the EHS Office.
    j. Animals and plants not associated with the work being performed are not permitted in the laboratory.
    k. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a Biological Safety Cabinet (BSC) or other physical containment device.

2. Safety Equipment (Primary Barriers and Personal Protective Equipment)
    a. Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:
        i) Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
        ii) High concentrations or large volumes (greater than 10 L as per NIH rDNA Guidelines) of infectious agents are used.
        iii) Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
    b. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials.
    c. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately. Laboratory clothing will not be taken home to be laundered. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for splashes or sprays of infectious or other hazardous materials when the microorganisms are 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 in laboratories should also wear eye protection.
    d. Gloves must be worn to protect hands from exposure to hazardous materials.
        i) Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available.
        ii) Gloves must not be worn outside the laboratory
        iii) In addition, BSL-2 laboratory workers should:
            (a) Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
            (b) Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
            (c) Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.
    e. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

3. Laboratory Facilities (Secondary Barriers)
    a. Same as BSL-1, plus the following:
        i) Laboratory doors should be self-closing and have locks in accordance with the institutional policies.

ii) Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
iii) BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
iv) Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps are required.
v) An eyewash station must be readily available.
vi) There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
vii) The Biological Safety Cabinet (BSC) will be tested and certified annually, or after relocation and/ or repair, and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified before each use.
viii) A method for decontaminating all laboratory wastes should be available and records maintained in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

B. ANIMAL BIOSAFETY LEVELS (ABSL)

Animal biosafety levels (ABSL) are also derived directly from the CDC's BMBL, and provide containment recommendations for both experimentally infected animals and those that may naturally harbor zoonotic infectious agents. The animal room can present unique hazards not found in a standard laboratory. Animals can generate aerosols, they may introduce agents through bites or scratches, and their wastes present another source of infectious or zoonotic agents. The co-application of biosafety levels, and animal biosafety levels are determined by protocol-driven risk assessment and, as a general principle, the BSL recommended for working with the infectious agent *in vivo* and *in vitro* are similar.

**Large animal ABSL-2, as well as ABSL-3, ABSL-4 and BSL-3Ag work is currently not possible at NDSU due to the lack of appropriate animal containment facilities. Therefore, these ABSL descriptions apply to rodent/small animal work on campus.

The following practices apply to <u>both ABSL-1 and ABSL-2</u>**:**

STANDARD MICROBIOLOGICAL PRACTICES:

1. The animal facility director establishes and enforces policies, procedures, and protocols for institutional policies and emergencies. Each institute must assure that worker safety and health concerns are addressed as part of the animal protocol review. Prior to beginning a study animal protocols must also be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC).
2. A safety manual specific to the animal facility is prepared or adopted in consultation with the animal facility director and appropriate safety professionals. 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.
3. The supervisor must ensure that animal care, laboratory and support personnel receive appropriate training regarding their duties, animal husbandry procedures, potential hazards, manipulations of infectious agents, necessary precautions to prevent exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel must receive annual updates and additional training when procedures or policies change. Records are maintained for all hazard evaluations, employee training sessions and staff attendance.
4. An appropriate medical surveillance program is in place, as determined by risk assessment. The need for an animal allergy prevention program should be considered. Facility supervisors should ensure that medical staff is informed of potential occupational hazards within the animal facility, to include those associated with research, animal husbandry duties, animal care and manipulations. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all personnel and particularly women of childbearing age should be provided information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance. Personnel using respirators must be enrolled in an appropriately constituted respiratory protection program.
5. A sign incorporating safety information must be posted at the entrance to the areas where infectious materials and/or animals are housed or are manipulated. The sign must include the animal biosafety level, all infectious agents in the space, general occupational health requirements, personal protective equipment requirements for entry, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the animal areas. Advance consideration should be given to emergency and disaster recovery plans, as a contingency for man-made or natural disasters.

6. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the facility. All persons including facility personnel, service workers, and visitors are advised of the potential hazards (natural or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.
7. 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. Gloves and personal protective equipment should be removed in a manner that minimizes transfer of infectious materials outside of the areas where infectious materials and/or animals are housed or are manipulated. Persons must wash their hands after removing gloves, and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Eye and face and respiratory protection should be used in rooms containing infected animals, as dictated by the risk assessment.
8. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside of the laboratory in cabinets or refrigerators designed and used for this purpose.
9. All procedures are carefully performed to minimize the creation of aerosols or splatters of infectious materials and waste.
10. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
11. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. When applicable, laboratory supervisors should adopt improved engineering and work practice controls that reduce the risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
    a. Use of needles and syringes or other sharp instruments in the animal facility is limited to situations where there is no alternative for such procedures as parenteral injection, blood collection, or aspiration of fluids from laboratory animals and diaphragm bottles.
    b. Disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal. Used disposable needles must be carefully placed in puncture-resistant containers used for sharps disposal. Sharps containers should be located as close to the work site as possible.
    c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.

e. Equipment containing sharp edges and corners should be avoided.

12. Equipment and work surfaces are routinely decontaminated with an appropriate disinfectant after work with an infectious agent, and after any spills, splashes, or other overt contamination.

13. Animals and plants not associated with the work being performed must not be permitted in the areas where infectious materials and/ or animals are housed or are manipulated.

14. An effective integrated pest management program is required.

15. All wastes from the animal room (including animal tissues, carcasses, and bedding) are transported from the animal room in leak-proof, covered containers for appropriate disposal in compliance with applicable institutional, local and state requirements. Decontaminate all potentially infectious materials before disposal using an effective method.

SAFETY EQUIPMENT (PRIMARY BARRIERS AND PERSONAL PROTECTIVE EQUIPMENT)

1. Gloves are worn to protect hands from exposure to hazardous materials.

   A risk assessment should be performed to identify the appropriate glove for the task and alternatives to latex gloves should be available.

   Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.

   Gloves must not be worn outside the animal rooms.

   Gloves and personal protective equipment should be removed in a manner that prevents transfer of infectious materials.

   Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated waste.

2. Persons must wash their hands after handling animals and before leaving the areas where infectious materials and/or animals are housed or are manipulated. Hand washing should occur after the removal of gloves.

LABORATORY FACILITIES (SECONDARY BARRIERS)

1. 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.

   Access to the animal facility is restricted.

   Doors to areas where infectious materials and/or animals are housed, open inward, are self-closing, are kept closed when experimental animals are present, and should never be propped open. Doors to cubicles inside an animal room may open outward or slide horizontally or vertically.

2. The animal facility must have a sink for hand washing.

   Sink traps are filled with water, and/or appropriate liquid to prevent the migration of vermin and gases.

3. The animal facility is designed, constructed, and maintained to facilitate cleaning and housekeeping. The interior surfaces (walls, floors and ceilings) are water resistant. Floors must be slip resistant, impervious to liquids, and resistant to chemicals.

   It is recommended (required for ABSL-2) that penetrations in floors, walls and ceiling surfaces be sealed, including openings around ducts, doors and doorframes, to facilitate pest control and proper cleaning.

4. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

   Chairs used in animal area must be covered with a non-porous material that can be easily cleaned and decontaminated. Furniture must be capable of supporting anticipated loads and uses. Sharp edges and corners should be avoided.

5. External windows are not recommended; they may pose a security hazard. If present windows must be resistant to breakage. Where possible, windows should be sealed. If the animal facility has windows that open, they are fitted with fly screens. The presence of windows may impact facility security and therefore should be assessed by security personnel.

6. Ventilation should be provided in accordance with the Guide for Care and Use of Laboratory Animals. No recirculation of exhaust air may occur. It is recommended that animal rooms have inward directional airflow.

   Ventilation system design should consider the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

7. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize horizontal surface areas to facilitate cleaning and minimize the accumulation of debris or fomites.

8. If floor drains are provided, the traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.
9. Cages are washed manually or preferably in a mechanical cage washer. The mechanical cage washer should have a final rinse temperature of at least 180°F. If manual cage washing is utilized, ensure that appropriate disinfectants are selected.
10. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
11. Emergency eyewash and shower are readily available; location is determined by risk assessment.

Animal Biosafety Level 1 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. ABSL-1 facilities should be separated from the general traffic patterns of the building and restricted as appropriate. 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. The use of PPE and any specific entry/exit procedures for the animal space is determined by animal species used, and the protocol specific risk assessment.

Animal Biosafety Level 2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1. ABSL-2 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.

ABSL-2 requires that: 1) access to the animal facility is restricted; 2) personnel must have specific training in 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) BSCs or other physical containment equipment is used when procedures involve the manipulation of infectious materials, or where aerosols or splashes may be created.

Appropriate personal protective equipment must be utilized to reduce exposure to infectious agents, animals, and contaminated equipment. Implementation of employee occupational health programs should be considered.

In addition to the individual requirements listed above, the following are requirements that are specific to ABSL-2:

Additional Standard Microbiological Practices for ABSL-2:

1. The safety manual that is prepared or adopted should give special consideration to the specific biohazards that are unique to the animal species and protocols in use.
2. The signage utilized in the animal containment spaces must incorporate the universal biohazard symbol when infectious material and/or animals are housed or are manipulated when infectious agents are present.
3. Access to the animal room is limited. Only those persons required for program or support purposes are authorized to enter the animal facility and the areas where infectious materials and/or animals are housed or manipulated.

   All persons including facility personnel, service workers, and visitors are advised of the potential hazards (physical, naturally occurring, or research pathogens, allergens, etc.) and are instructed on the appropriate safeguards.

Special Microbiological Practices for ABSL-2:

1. Animal care staff, laboratory and routine support personnel must be provided a medical surveillance program as dictated by the risk assessment and administered appropriate immunizations for agents handled or potentially present, before entry into animal rooms.

   When appropriate, a base line serum sample should be stored.
2. Procedures involving a high potential for generating aerosols should be conducted within a biosafety 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 (e.g., physical restraint devices, or chemical restraint medications) should be used whenever possible.
3. Decontamination by an appropriate method (e.g. autoclave, chemical disinfection, or other approved decontamination methods) is necessary for all potentially infectious materials and animal waste before movement outside the areas where infectious materials and/or animals are housed or are manipulated. This includes potentially infectious animal tissues, carcasses, contaminated bedding, unused feed, sharps, and other refuse.

A method for decontaminating routine husbandry equipment, sensitive electronic and medical equipment should be identified and implemented.

Materials to be decontaminated outside of the immediate areas where infectious materials and/or animals are housed or are manipulated must be placed in a durable, leak proof, covered container and secured for transport. The outer surface of the container is disinfected prior to moving materials. The transport container must have a universal biohazard label.

Develop and implement an appropriate waste disposal program in compliance with applicable institutional, local and state requirements. Autoclaving of contents prior to incineration is recommended.

4. 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.

5. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.

6. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the safety manual. All such incidents must be reported to the animal facility supervisor or personnel designated by the institution. Medical evaluation, surveillance, and treatment should be provided as appropriate and records maintained.

Special Safety Equipment (Primary Barriers and PPE) specific to ABSL-2

1. Properly maintained BSCs, personal protective equipment (e.g., gloves, lab coats, face shields, respirators, etc.) and/or other physical containment devices or equipment, are used whenever conducting procedures with a potential for creating aerosols, splashes, or other potential exposures to hazardous materials. These include necropsy of infected animals, harvesting of tissues or fluids from infected animals or eggs, and intranasal inoculation of animals.

   When indicated by risk assessment, animals are housed in primary biosafety containment equipment appropriate for the animal species, such as solid wall and bottom cages covered with filter bonnets for rodents or other equivalent primary containment systems for larger animal cages.

2. A risk assessment should determine the appropriate type of personal protective equipment to be utilized.

Scrub suits and uniforms are removed before leaving the animal facility. Reusable clothing is appropriately contained and decontaminated before being laundered. Laboratory and protective clothing should never be taken home.

Gowns, uniforms, laboratory coats and personal protective equipment are worn while in the areas where infectious materials and/or animals are housed or manipulated and removed prior to exiting. Disposable personal protective equipment and other contaminated waste are appropriately contained and decontaminated prior to disposal.

3. Eye and face protection (mask, goggles, face shield or other splatter guard) are used for manipulations or activities that may result in splashes or sprays from infectious or other hazardous materials and when the animal or 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 when entering areas with potentially high concentrations or airborne particulates.

   Respiratory protection is worn based upon risk assessment.

Laboratory Facility Requirements Specific for ABSL-2

1. A hand-washing sink is located at the exit of the areas where infectious materials and/or animals are housed or are manipulated. Additional sinks for hand washing should be located in other appropriate locations within the facility.

   If the animal facility has segregated areas where infectious materials and/or animals are housed or manipulated, a sink must also be available for hand washing at the exit from each segregated area.

   Sink traps are filled with water, and/or appropriate disinfectant to prevent the migration of vermin and gases.

2. Furniture in ABSL-2 spaces should be minimized. All other furniture requirements are the same as ABSL-1.

3. If BSCs are present, they must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly to the outside through an independent, hard connection. Provisions to assure proper safety cabinet

performance and air system operation must be verified. BSCs should be recertified at least once a year to ensure correct performance.

All BSCs should be used according to manufacturer's specifications to protect the worker and avoid creating a hazardous environment from volatile chemicals and gases.

4. If vacuum service (i.e., central or local) is provided, each service connection should be fitted with liquid disinfectant traps and an in-line HEPA filter placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement.
5. An autoclave should be present in the animal facility to facilitate decontamination of infectious materials and waste.

C. PLANT BIOSAFETY LEVELS (BL-P)

Appendix P of the NIH Guidelines specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant or synthetic nucleic acid molecule-containing plants, plant-associated microorganisms, and small animals.

From the NIH Guidelines: "The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant or synthetic nucleic acid molecule-containing plant genome, including nuclear or organelle hereditary material or release of recombinant or synthetic nucleic acid molecule-derived organisms associated with plants. The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem."

When infectious agents that do pose an infectivity threat to humans and/or animals are used in conjunction with plants, appropriate and corresponding BSL practices and conditions are applied.

Four biosafety levels, referred to as Biosafety Level (BL)1 - Plants (P), BL2-P, BL3-P, and BL4-P, are established in Appendix P of the NIH Guidelines and BL1-P, BL2-P, and BL3-P are summarized in this Manual. **BL4-P work is not permitted at NDSU**.

BIOSAFETY LEVEL 1- PLANT (BL1-P)

Standard Practices (BL1-P)

Greenhouse Access

1. Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress.
2. Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL1-P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.

Records

1. A record shall be kept of experiments currently in progress in the greenhouse facility.

Decontamination and Inactivation

1. Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Control of Undesired Species and Motile Macroorganisms

1. A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and Federal laws.
2. Arthropods 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.

Concurrent Experiments Conducted in the Greenhouse

1. Experiments involving other organisms that require a containment level lower than BL1-P may be conducted in the greenhouse concurrently with experiments that require BL1-P containment, provided that all work is conducted in accordance with BL1-P greenhouse practices.

Facilities Definitions

1. 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 transparent or translucent material to allow passage of sunlight for plant growth.
2. 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.

Greenhouse Design

1. The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.
2. Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

BIOSAFETY LEVEL 2- PLANT (BL2-P)

Standard Practices (BL2-P)

Greenhouse Access

1. Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, to individuals directly involved with the experiments when they are in progress.
2. Personnel shall be required to read and follow instructions on BL2-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Records

1. A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.
2. A record shall be kept of experiments currently in progress in the greenhouse facility.
3. The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, NIH/OBA and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Documentation of any such accident shall be prepared and maintained.

Decontamination and Inactivation

1. Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

2. Decontamination of run-off water is not necessarily required. 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.

Control of Undesired Species and Motile Macroorganisms

1. A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.
2. Arthropods 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.

Concurrent Experiments Conducted in the Greenhouse

1. Experiments involving other organisms that require a containment level lower than BL2-P may be conducted in the greenhouse concurrently with experiments that require BL2-P containment provided that all work is conducted in accordance with BL2-P greenhouse practices.

Signage

1. A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.
2. 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.
3. If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Transfer of Materials

1. 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.

Greenhouse Practices Manual

1. 1. A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms.

Facilities Definitions

1. 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 transparent or translucent material to allow passage of sunlight for plant growth.
2. 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.

Greenhouse Design

1. 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.
2. Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds).

Autoclaves

1. An autoclave shall be available for the treatment of contaminated greenhouse materials.

Supply and Exhaust Air Ventilation Systems

1. 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.

Other

1. BL2-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 foregoing clauses.

BIOSAFETY LEVEL 3 - PLANTS (BL3-P):

The only space on campus that qualifies for BL3-P work is in the AES Greenhouse Facility.

Standard Practices (BL3-P)

Greenhouse Access

1. Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility.
2. Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL3-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Records

1. A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.
2. A record shall be kept of experiments currently in progress in the greenhouse facility.
3. The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/OBA, and other appropriate authorities immediately (if applicable). Reports to the NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). Documentation of any such accident shall be prepared and maintained.

Decontamination and Inactivation

1. 1. All experimental materials shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.

Control of Undesired Species and Motile Macroorganisms

1. A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

2. Arthropods and other motile macroorganisms shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Concurrent Experiments Conducted in the Greenhouse

1. 1. Experiments involving organisms that require a containment level lower than BL3-P may be conducted in the greenhouse concurrently with experiments that require BL3-P containment provided that all work is conducted in accordance with BL3-P greenhouse practices.

Signage

1. A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.
2. If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence should be indicated on a sign posted on the greenhouse access doors.
3. If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Transfer of Materials

1. Experimental materials that are brought into or removed from the greenhouse facility in a viable or intact state shall be transferred to a non-breakable sealed secondary container. At the time of transfer, if the same plant species, host, or vector are present within the effective dissemination distance of propagules of the experimental organism, the surface of the secondary container shall be decontaminated. Decontamination may be accomplished by passage through a chemical disinfectant or fumigation chamber or by an alternative procedure that has demonstrated effective inactivation of the experimental organism.

Greenhouse Practices Manual

1. A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms with recognized potential for serious detrimental impact.

Protective Clothing

1. Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) shall be worn in the greenhouse if deemed

necessary by the Greenhouse Director because of potential dissemination of the experimental microorganisms.

2. Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.

Other

1. Personnel are required to thoroughly wash their hands upon exiting the greenhouse.
2. All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and all experimental manipulations.

Facilities Definitions

1. The term "greenhouse" refers to a structure with walls, roof, and floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.
2. 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 need to maintain negative pressure should be considered when constructing or renovating the greenhouse.

Greenhouse

1. The greenhouse floor shall be composed of concrete or other impervious material with provision for collection and decontamination of liquid run-off.
2. Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).
3. The greenhouse shall be a closed self-contained structure with a continuous covering that is separated from areas that are open to unrestricted traffic flow. The minimum requirement for greenhouse entry shall be passage through two sets of self-closing locking doors.
4. The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.
5. Internal walls, ceilings, and floors shall be resistant to penetration by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

6. Bench tops and other work surfaces should have seamless surfaces that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
7. The greenhouse contains a foot, elbow, or automatically operated sink, which is located near the exit door for hand washing.

Autoclaves

1. An autoclave shall be available for decontaminating materials within the greenhouse facility. A double-door autoclave is recommended (not required) for the decontamination of materials passing out of the greenhouse facility.

Supply and Exhaust Air Ventilation Systems

1. An individual supply and exhaust air ventilation system shall be provided. The system maintains pressure differentials and directional airflow, as required, to assure inward (or zero) airflow from areas outside of the greenhouse.
2. The exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air-HEPA filters and discharged to the outside. The filter chambers shall be designed to allow in situ decontamination before filters are removed and to facilitate certification testing after they are replaced. Air filters shall be 80-85% average efficiency by the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) Standard 52-68 test method using atmosphere dust. Air supply fans shall be equipped with a back- flow damper that closes when the air supply fan is off. Alternatively, a HEPA filter may be used on the air supply system instead of the filters and damper. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times.

Other

1. BL3-P greenhouse containment requirements may be satisfied using a growth chamber or growth room within a building provided that the location, access, airflow patterns, and provisions for decontamination of experimental materials and supplies meet the intent of the foregoing clauses.
2. Vacuum lines shall be protected with high efficiency particulate air/HEPA or equivalent filters and liquid disinfectant traps.

VI. SAFETY CONTROLS

The layers of safety controls incorporated into the biosafety levels will now be discussed. Several primary barriers (PPE, safety equipment) and secondary barriers (facility design features) are utilized to protect personnel and the environment from infectious agents utilized in research. In addition to these barriers, personnel training and workplace practices are incorporated to prepare students, laboratory workers, and others involved in the research to prevent an exposure that may lead to a laboratory associated illness, or a release of an infectious or genetically modified agent or plant into the environment.

A. PERSONAL PROTECTIVE EQUIPMENT (PPE)

The Occupational Safety and Health Administration (OSHA) refers to PPE as "garments and devices designed to protect employees from serious workplace injuries or illnesses resulting from contact with various workplace hazards".

Examples of PPE include, but are not limited to: coveralls, gowns, aprons, lab coats, gloves, face shields, safety glasses, goggles, hair bonnets, safety shoes, booties, and respirators.

OSHA mandates that employers:

- Determine the workplace hazards that require PPE.
- Provide workers with appropriate PPE.
- Ensure proper use and maintenance of PPE.
- Train employees to use PPE correctly, to know when and where PPE is necessary, understand its limitations, and to don (put on) and doff (take off) PPE correctly.

Gloves, and all disposable PPE must be properly discarded as biohazard waste.

Take special care to remove all contaminated PPE, including lab coats, before leaving the lab/going into any common areas to prevent contaminating that area with the agents you are working with.

Clothing

Re-usable outer clothing used as PPE such as coveralls and lab coats **must not** be taken home to be laundered. It must be laundered on site, or laundered professionally. Clothing should be decontaminated as much as possible before being laundered (site chemical decontamination, or autoclaving).

Disposable gowns, coats, and aprons are also available in lieu of reusable clothing when required.

Face and Eye protection: provides eye, mucous membrane, and respiratory protection from splashes, sprays, and droplets.

Types of Eye protection:

- Safety Glasses, preferably with side shields.
- Goggles provide more thorough protection due to the fact that they form a seal around the eyes.
- Face shields are required where facial skin protection is needed, but do not provide adequate eye protection. Safety glasses or goggles must be worn underneath a face shield to provide adequate eye protection.

Personal eye glasses are not designed for laboratory safety. They do not contain side shields and are not made of shatter proof materials and therefore should never be worn for protection when working with biological agents, or other hazardous materials.

Re-usable eyewear must be chemically disinfected before reuse**.**

Respirators

N95 or higher filtering respirators provide partial face, but not eye, protection and will protect from respiratory exposure to aerosols. The NIOSH N95 (N99 & N100 have higher filtration capacity) respirators are designed to filter very small particles. These respirators should fit snugly on the face, require medical clearance, and fit testing through NDSU's Respiratory Protection Program. Please contact the Safety Office (701-231-7759) to discuss respirator selection and enrollment in this program.

Surgical face masks are not the same as a filtering face-piece respirator. Surgical face masks prevent the wearer from shedding or spreading aerosols, but do not protect the wearer from exposure to aerosols.

Powered Air Purifying Respirators (PAPRs) used in some high aerosol generating procedures are full face respirators, which provide eye as well as face protection. Before using a PAPR, medical clearance and training is also required. The EHS Office will provide advice regarding the use and type of respirators required based on the type of hazard anticipated.

There is also helpful information on The National Personal Protective Technology Laboratory (NPPTL) webpage on the Centers for Disease Control (CDC) website that explains the types, and uses of respirators.

Gloves

Disposable gloves must be used whenever direct contact with biohazardous agents or materials is required. A latex free option should be available in each lab for those that may have an allergy to latex.

Gloves must be carefully removed to prevent contaminating hands in the process. You can view a helpful graphic from the CDC that demonstrates proper glove removal.

Wash hands immediately after removing gloves.

Never reuse disposable gloves to prevent contamination of hands and work surfaces from whatever is/was on the gloves.

B. ENGINEERING CONTROLS – BIOLOGICAL SAFETY CABINETS

Engineering controls serve to reduce worker exposure either by removing the hazard or by isolating the worker from exposure. They include, but are not limited to: biological safety cabinets (BSCs), enclosed transport containers, directional airflow indicators, aerosol tight centrifuge cups or rotor lids, micro-isolator tops on animal cages, self- sheathing needles and sharps containers.

THE BIOLOGICAL SAFETY CABINET (PRIMARY CONTAINMENT)

The Biological Safety Cabinet (BSC) is the main device used to provide containment of infectious splashes, droplets, or aerosols generated by many laboratory procedures utilizing biohazardous agents and materials. All BSCs must be certified annually.

Three kinds of BSCs designated as Class I, II, and III, have been developed to meet varying research needs. Most BSCs use high efficiency particulate air (HEPA) filters in their exhaust and/or supply systems.

The types of BSCs commonly used in laboratories are described in detail in the BMBL Appendix A: Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets (beginning on page 367, 6th Edition).

The various types of biosafety cabinets are described below:

The Class I BSC: This type of cabinet is not for aseptic or sterile technique. The Class I BSC provides personnel and environmental protection, but no product protection. It is usually hard ducted (i.e. directly connected to the building exhaust system and is similar in air movement to

a chemical fume hood), but has a HEPA filter in the exhaust system to protect the environment. Those used for animal cage changing allow re-circulation of HEPA filtered air into the room. These require annual certification and more frequent filter changes.

The Class II BSC (most common BSC): The Class II (Types A and B) biological safety cabinets provide personnel, environmental, and product protection. Air flow is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. Because cabinet air has passed through a certified HEPA filter, it is contaminant-free (environmental protection), and may be recirculated back into the laboratory or ducted out of the building via thimble/canopy connection, which maintains a small opening around the cabinet exhaust filter housing (Type A1 and A2), or hard duct connection (Type B1 and B2). With a thimble connection the volume of the exhaust must be sufficient to maintain the flow of room air into the space between the thimble unit and the filter housing. The thimble must be removable or be designed to allow for operational testing of the cabinet. The performance of a cabinet with this exhaust configuration is unaffected by fluctuations in the building exhaust system.

The Class II, Type A1 BSC: Room air is drawn through the front grille via an internal blower to maintain an average inflow velocity of 75 lfpm (A2, B1, and B2 have 100 lfpm) at the face opening of the BSC. HEPA filtered air splits over the work surface to the front and the rear grille. 30% of the air is exhausted while 70% recirculates through the HEPA filter back into the work area. This can cause build-up of toxic fumes-Type II, A1 BSC is not to be used to handle toxic, volatile chemicals.

The Class II, Type A2 (formerly A/B3) BSC: This BSC has a minimum calculated measured inflow velocity of 100 lfpm. Only when this BSC is ducted to the outdoors does it meet the requirements of the former class II type B3. All positive pressure biologically contaminated plenums within the cabinet are surrounded by a negative air pressure plenum. Thus, leakage in a contaminated plenum will be into the cabinet and not into the environment. Beginning in April 2016, Type A BSCs that are either direct-ducted or canopy-connected cannot be re-certified without an exhaust airflow alarm.

The Class II, Type B1 BSC: Some biomedical research requires the use of small quantities of certain hazardous chemicals, such as carcinogens. The powdered form of these carcinogens should be weighed or manipulated in a chemical fume hood or a static-air glove box. Carcinogens used in cell culture or microbial systems require both biological and chemical containment. Type B1 cabinets must be hard-ducted to their own dedicated exhaust system. Typically, 70% of the air is exhausted outside the building through HEPA filter; 30% is recirculated. Blowers on laboratory exhaust systems should be located at the terminal end of the ductwork. A failure in the building exhaust system may not be apparent to the user, as the supply blowers in the cabinet will continue to operate. A pressure-independent monitor should be installed to sound an alarm and shut off the BSC supply fan, should failure in exhaust airflow occur. Since all cabinet manufacturers do not supply this feature, it is prudent to install a sensor in the exhaust system as necessary. To maintain critical operations, laboratories using Type B BSCs should connect the exhaust blower to the emergency power supply.

The Class II, Type B2 BSC: This BSC is a total-exhaust cabinet; no air is recirculated within it. This cabinet provides simultaneous primary biological and chemical containment. Should the building or cabinet exhaust fail, the cabinet will be pressurized, resulting in a flow of air from the work area back into the laboratory. Cabinets built since the early 1980's usually have an interlock system installed by the manufacturer to prevent the supply blower from operating whenever the exhaust flow is insufficient.

Presence of such an interlock system should be verified; systems can be retrofitted if necessary. A pressure-independent device should monitor exhaust air movement.

The Class III BSC: The Class III BSC was designed for work with highly infectious microbiological agents, and provides maximum protection to the environment and the worker. It is a gas-tight enclosure with a non-opening view window. Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow for manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials. The trade-off is clearly on the side of maximizing personal safety. Depending on the design of the cabinet, the supply HEPA filter provides particulate-free, albeit somewhat turbulent, airflow within the work environment.

Operations Within a Class II BSC:

- Only materials and equipment required for the immediate work should be placed in a BSC so as not to disrupt the airflow.
- Frequent inward/outward movement needed to place objects in biohazard collection containers outside the BSC is disruptive to the integrity of the cabinet air barrier and can compromise both personal and product protection. Horizontal pipette discard trays containing a disinfectant (e.g. bleach) are recommended for use inside the BSC.
- Best practices recommend keeping clean materials at least 12 inches away from aerosol-generating activities, which will minimize the potential for cross-contamination.
- The general workflow should be from clean to contaminated (dirty). Materials and supplies should be placed in such a way as to limit the movement of dirty items over clean ones.
- Work at least 4 inches back from the front edge and never cover the front grill with materials.
- When possible, open containers (tubes, bottles) should be held at an angle to prevent contamination. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impact of downward air. Items should be recapped or covered as soon as possible.
- Open flames:
    (1) Create turbulence that disrupts the pattern of HEPA-filtered air supplied to the work surface and are not necessary nor are they permitted in the near microbe-free environment of a biological safety cabinet. Contact the Safety Office regarding alternatives to the use of these devices.
    (2) Small electric furnaces are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops should be used to eliminate the need for heat or flame.
- Aspirator:
    (1) Should be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter. This will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment.
    (2) Sufficient chemical decontamination solution (e.g. 100% bleach) must be placed in the flask to inactivate aspirated material as they are collected. Inactivated liquid material can be disposed of appropriately as noninfectious waste.
    (3) If collection vessel is stored beneath the BSC, it must be in a secondary containment vessel such as a tray or bucket to prevent a spill if tipped or broken.
- Ultraviolet (UV) lamps are not required or necessary in BSC.

(1) If installed, the lamps should be cleaned and checked periodically with a UV meter to confirm appropriate emission.

(2) UV lamps must be turned off when the room is occupied to protect eyes and reduce skin exposure.

(3) Close the sash in BSC when operating UV lamp

- Spills inside the BSC must be handled immediately; see Spills Section of the manual for instructions on handling a spill inside a BSC.
- The BSC must be professionally certified per NSF/ANSI49-2002 Standard when used to handle infectious and potentially infectious material:

(1) After initial installation

(2) At least annually thereafter

(3) After the BSC is relocated or repaired

PPE and Engineering controls are referred to **primary biosafety barriers**. **Secondary biosafety barriers** would include facility design features in these laboratories such as separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities. Depending on risk assessment, the nature of the infectious agent(s), or the containment levels required, additional secondary barriers such as directional airflow, air or liquid treatment systems to decontaminate or remove agents from exhaust air or waste water, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules to isolate the laboratory may be required.

C. WORK PRACTICES

Just as important as using equipment or barriers as protection against exposure or to prevent release of biohazardous agents, behavior, awareness, and conduct in the workplace serve critical roles in maintaining biosafety.

Strict adherence to standard microbiological practices and techniques must be emphasized when working with biohazards.

Persons working with or near biohazardous agents or animals that may carry zoonotic diseases must not only be aware of the potential hazards, but also must be trained and proficient in the practices and techniques required for handling materials and animals safely.

1. Hand Hygiene

   Hand washing is one of the simplest, yet most often overlooked biosafety practices that can be employed to prevent exposure or contamination of surfaces with whatever biohazards one is working with. People working with biohazards should wash their hands immediately upon glove removal, upon completion of any task that involves contact with biohazardous agents and materials, before leaving the lab, and before eating, drinking, applying cosmetics, or touching their eyes or face. Hand washing should be performed with running, warm water, and soap, for no less than 20 seconds. Be sure to wash the backs of hands, in between the fingers, and include wrists.

2. Training

   The principal investigator or supervisor of the laboratory/facility is responsible for the safe conduct of work with any biohazardous agents or materials and providing, or arranging for, the appropriate training of personnel. There should be an evaluation of the technical competency and proficiency of each worker before they are allowed to work unsupervised with biohazardous agents or materials. The PI or supervisor must make this determination.

   The NDSU Safety Office provides Laboratory Safety Training modules, including a training module on working with biohazards. The IBC requires all participants on a protocol registered with the committee to complete CITI Biosafety training. The NDSU Safety Office is also available for to consult for specialized training that may be agent specific, related to utilization and proper use of safety equipment, or selection and usage of PPE.

3. Lab Manual

   Each laboratory should develop a laboratory-specific biosafety manual which identifies the hazards that exist or may be encountered, agent specific hazards, specifies the practices and procedures that will be used to minimize or eliminate exposures or releases of these hazards, and contains the lab specific emergency procedures. This laboratory-specific safety manual is required for all labs at NDSU that operate at BSL-2 or above. The manual must be updated (if necessary) and reviewed with lab personnel annually. An electronic copy of each lab's manual will be held in the Safety Office.

4. Lab Hygiene

   You are referred to the CDC's Guideline for Disinfection and Sterilization in Healthcare Facilities for additional details and a thorough description of disinfection processes and chemicals.

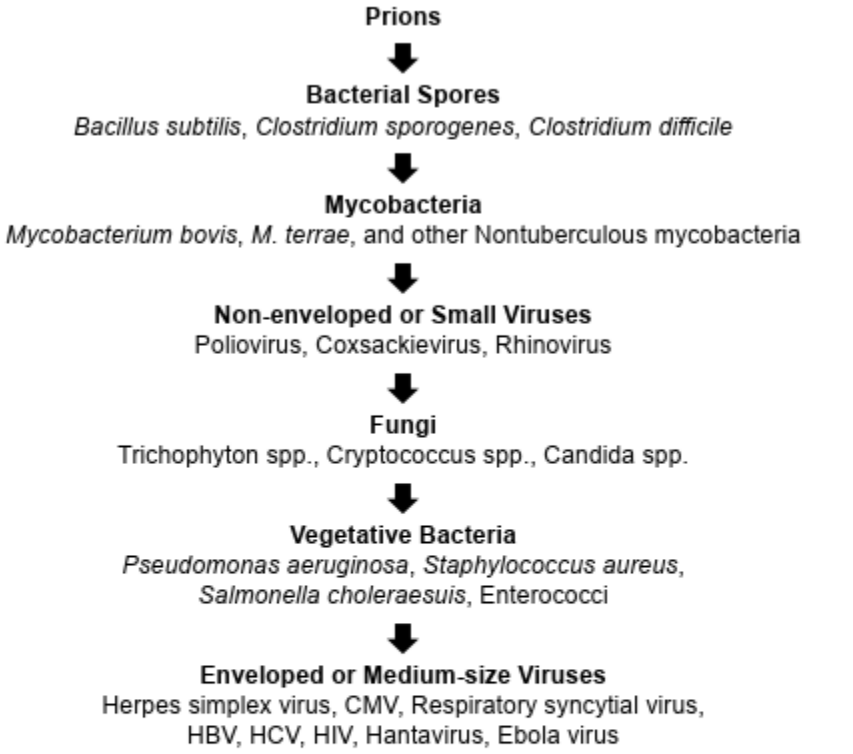
A few definitions, according to the CDC's Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008:

- **Cleaning** is the removal of visible soil (e.g., organic and inorganic material) from objects and surfaces and normally is accomplished manually or mechanically using water with detergents or enzymatic products. Thorough cleaning is essential before high-level disinfection and sterilization because inorganic and organic materials that remain on the surfaces interfere with the effectiveness of these processes.
- **Decontamination** removes pathogenic microorganisms from objects so they are safe to handle, use, or discard.
- **Sterilization** describes a process that destroys or eliminates all forms of microbial life.
- *Terms with the suffix ***cide*** or ***cidal*** for killing action are commonly used. Virucide, fungicide, bactericide, sporicide, and tuberculocide can kill the type of microorganism identified by the prefix. For example, a bactericide is an agent that kills bacteria.

Labs have to achieve these three processes, cleaning, decontamination, and sterilization, routinely, if not daily. Cleaning is relatively straight forward and requires only soap or some detergent. Sterilization of products including wastes is also straight forward and is almost always achieved by autoclaving; however, when sterilization of surfaces is required, it can also be achieved in some cases with chemical submersion with long contact times, or in some specialized spaces on campus with vaporized hydrogen peroxide treatment.

Therefore, the choice of an effective, routine surface decontamination method is what most labs are faced with. The method is, of course, dependent on the agent(s) being utilized in the space. Different organisms have varying levels of innate resistance to chemical inactivation:

Figure 1. Descending Order of Relative Resistance to Disinfectant Chemicals



Note: There are exceptions to this list. *Pseudomonas* spp. are sensitive to high-level disinfectants. However, in biofilms, the protected cells and those within free-living amoeba, or existing as persister cells (viable but not culturable) within the biofilm, can approach the resistance of bacterial spores to the same disinfectant. The same is true for the resistance to glutaraldehyde by some nontuberculous mycobacteria, some fungal ascospores of *Microascus cinereus* and *Chaetomium globosum*, and the pink-pigmented Methylobacteria. Prions are also resistant to most liquid chemical germicides and are discussed in the last part of this appendix.

The above figure is from the BMBL 6th ed., page 404.

In addition to the resistance of the organism to chemical inactivation, these factors also contribute to the ability of a chemical to effectively decontaminate a surface:

- the number of organisms (level of contamination)
- the presence of a biofilm
- concentration of the chemical
- water hardness/pH

Optimal **contact time** is critical to achieving desired decontamination

- Appropriate contact times for decontamination differ depending on the organisms, the chemical, and the method of decontamination being used.

- Be sure to follow instructions that came with the chemical if supplied.
- As a general rule, a contact time of 10-30 minutes should be used

CHEMICALS COMMONLY USED FOR DECONTAMINATION:

ALCOHOL

***Overview*.** "Alcohol" refers to two water-soluble chemical compounds—ethyl alcohol and isopropyl alcohol—that have generally underrated germicidal characteristics. FDA has not cleared any liquid chemical sterilant or high-level disinfectant with alcohol as the main active ingredient. These alcohols are rapidly bactericidal rather than bacteriostatic against vegetative forms of bacteria; they also are tuberculocidal, fungicidal, and virucidal but do not destroy bacterial spores. Their cidal activity drops sharply when diluted below 50% concentration, and the optimum bactericidal concentration is 60%–90% solutions in water (volume/volume).

***Mode of Action*.** The most feasible explanation for the antimicrobial action of alcohol is denaturation of proteins. This mechanism is supported by the observation that absolute ethyl alcohol, a dehydrating agent, is less bactericidal than mixtures of alcohol and water because proteins are denatured more quickly in the presence of water. Protein denaturation also is consistent with observations that alcohol destroys the dehydrogenases of *Escherichia coli*, and that ethyl alcohol increases the lag phase of *Enterobacter aerogenes* and that the lag phase effect could be reversed by adding certain amino acids. The bacteriostatic action was believed caused by inhibition of the production of metabolites essential for rapid cell division.

***Uses*.** Alcohols are not recommended for sterilizing medical and surgical materials principally because they lack sporicidal action and they cannot penetrate protein-rich materials. Alcohols are flammable and consequently must be stored in a cool, well-ventilated area. They also evaporate rapidly, making extended exposure time difficult to achieve unless the items are immersed.

CHLORINE AND CHLORINE COMPOUNDS

***Overview*.** Hypochlorites, the most widely used of the chlorine disinfectants, are available as liquid (e.g., sodium hypochlorite) or solid (e.g., calcium hypochlorite). The most prevalent chlorine products in the United States are aqueous solutions of 5.25%–6.15% sodium hypochlorite, usually called household bleach. They have a broad spectrum of antimicrobial activity, do not leave toxic residues, are unaffected by water hardness, are inexpensive and fast acting, remove dried or fixed organisms and biofilms from surfaces, and have a low incidence of serious toxicity. Sodium hypochlorite at the concentration used in household bleach (5.25-

6.15%) can produce ocular irritation or oropharyngeal, esophageal, and gastric burns. Other disadvantages of hypochlorites include corrosiveness to metals in high concentrations (>500 ppm), inactivation by organic matter, discoloring or "bleaching" of fabrics, release of toxic chlorine gas when mixed with ammonia or acid (e.g., household cleaning agents), and relative instability. The microbicidal activity of chlorine is attributed largely to undissociated hypochlorous acid (HOCl). The dissociation of HOCl to the less microbicidal form (hypochlorite ion OCl-) depends on pH. The disinfecting efficacy of chlorine decreases with an increase in pH that parallels the conversion of undissociated HOCl to OCl-.

***Mode of Action*.** The exact mechanism by which free chlorine destroys microorganisms has not been elucidated. Inactivation by chlorine can result from a number of factors: oxidation of sulfhydryl enzymes and amino acids; ring chlorination of amino acids; loss of intracellular contents; decreased uptake of nutrients; inhibition of protein synthesis; decreased oxygen uptake; oxidation of respiratory components; decreased adenosine triphosphate production; breaks in DNA; and depressed DNA synthesis. The actual microbicidal mechanism of chlorine might involve a combination of these factors or the effect of chlorine on critical sites.

***Uses**:* A 1:10–1:100 dilution of 5.25%–6.15% sodium hypochlorite (i.e., household bleach) is recommended for most laboratory needs, or for decontaminating blood spills. Make fresh bleach dilutions ideally every day. Less dilute bleach dilutions (1:10) can be kept for 1 week. More dilute dilutions must be made daily.

Bleach Solution	Dilution	Chlorine (ppm)
5.25-6.15%	None	52,500-61,500
	1:10	5,250-6,150
	1:100	525-615
	1:1000	53-62

GLUTARALDEHYDE

***Overview*.** Glutaraldehyde is a saturated dialdehyde that has gained wide acceptance as a high-level disinfectant and chemical sterilant. Aqueous solutions of glutaraldehyde are acidic and generally in this state are not sporicidal. Only when the solution is "activated" (made alkaline) by use of alkalinating agents to pH 7.5–8.5 does the solution become sporicidal. Once activated, these solutions have a shelf-life of minimally 14 days because of the polymerization of the glutaraldehyde molecules at alkaline pH levels. This polymerization blocks the active sites (aldehyde groups) of the glutaraldehyde molecules that are responsible for its biocidal activity. Novel glutaraldehyde formulations (e.g., glutaraldehyde-phenol-sodium phenate, potentiated acid glutaraldehyde, stabilized alkaline glutaraldehyde) produced in the past 30 years have overcome the problem of rapid loss of activity (e.g., use-life 28–30 days) while generally maintaining excellent microbicidal activity. However, antimicrobial activity depends not only on

age but also on use conditions, such as dilution and organic stress. Manufacturers' literature for these preparations suggests the neutral or alkaline glutaraldehydes possess microbicidal and anticorrosion properties superior to those of acid glutaraldehydes. However, two studies found no difference in the microbicidal activity of alkaline and acid glutaraldehydes. The use of glutaraldehyde-based solutions in health-care facilities is widespread because of their advantages, including excellent biocidal properties; activity in the presence of organic matter (20% bovine serum); and noncorrosive action to endoscopic equipment, thermometers, rubber, or plastic equipment.

***Mode of Action*.** The biocidal activity of glutaraldehyde results from its alkylation of sulfhydryl, hydroxyl, carboxyl, and amino groups of microorganisms, which alters RNA, DNA, and protein synthesis. The mechanism of action of glutaraldehydes are reviewed extensively elsewhere.

Uses. Glutaraldehyde is noncorrosive to metal. Personnel can be exposed to elevated levels of glutaraldehyde vapor when using this chemical in poorly ventilated rooms, when spills occur, when glutaraldehyde solutions are activated or changed, or when open immersion baths are used. Acute or chronic exposure can result in skin irritation or dermatitis, mucous membrane irritation (eye, nose, mouth), or pulmonary symptoms. Epistaxis, allergic contact dermatitis, asthma, and rhinitis also have been reported in healthcare workers exposed to glutaraldehyde.

HYDROGEN PEROXIDE

Overview. The literature contains several accounts of the properties, germicidal effectiveness, and potential uses for stabilized hydrogen peroxide for decontamination. Hydrogen peroxide is active against a wide range of microorganisms including bacteria, yeasts, fungi, viruses, and spores.

Mode of Action. Hydrogen peroxide works by producing destructive hydroxyl free radicals that can attack membrane lipids, DNA, and other essential cell components. Catalase, produced by aerobic organisms and facultative anaerobes that possess cytochrome systems, can protect cells from metabolically produced hydrogen peroxide by degrading hydrogen peroxide to water and oxygen. This defense is overwhelmed by the concentrations used for disinfection.

Uses. A 0.5% hydrogen peroxide demonstrated bactericidal and virucidal activity in 1 minute and mycobactericidal and fungicidal activity in 5 minutes. Commercially available 3% hydrogen peroxide is a stable and effective disinfectant when used on inanimate surfaces.

QUATERNARY AMMONIUM COMPOUNDS

Overview. Quaternaries sold as hospital disinfectants are generally fungicidal, bactericidal, and virucidal against lipophilic (enveloped) viruses; they are not sporicidal and generally not tuberculocidal or virucidal against hydrophilic (nonenveloped) viruses. The quaternary ammonium compounds are widely used as disinfectants. The quaternaries are good cleaning agents, but high water hardness can make them less microbicidal because of insoluble precipitates. Chemically, the quaternaries are organically substituted ammonium compounds in which the nitrogen atom has a valence of 5, four of the substituent radicals (R1-R4) are alkyl or heterocyclic radicals of a given size or chain length, and the fifth (X-) is a halide, sulfate, or similar radical. Each compound exhibits its own antimicrobial characteristics, hence the search for one compound with outstanding antimicrobial properties. Some of the chemical names of quaternary ammonium compounds used in healthcare are alkyl dimethyl benzyl ammonium chloride, alkyl didecyl dimethyl ammonium chloride, and dialkyl dimethyl ammonium chloride. The newer quaternary ammonium compounds (i.e., fourth generation), referred to as twin-chain or dialkyl quaternaries (e.g. didecyl dimethyl ammonium bromide and dioctyl dimethyl ammonium bromide), purportedly remain active in hard water and are tolerant of anionic residues.

Mode of Action. The bactericidal action of the quaternaries has been attributed to the inactivation of energy-producing enzymes, denaturation of essential cell proteins, and disruption of the cell membrane.

Uses will depend on the exact formulation of the chemical.

VII. ERGONOMICS

Ergonomics is the scientific study of people at work and the goal of ergonomics is to reduce stress and eliminate injuries and disorders associated with the overuse of muscles, bad posture, and repeated tasks. This is accomplished by designing tasks, work spaces, controls, displays, tools, lighting, and equipment to fit the employee´s physical capabilities and limitations.

Research includes many laboratory tasks that are repetitive and can include some work spaces/conditions that place physical stresses on workers. It is important that measures be taken to minimize these circumstances to prevent musculoskeletal or other injuries to workers, as well as fatigue and distraction from being uncomfortable or in pain. Poor ergonomic design of a lab, or laboratory tasks pose biosafety concerns because they may contribute to potential accidents which may result in spills, exposures, or environmental releases.

Common laboratory tasks that pose ergonomic challenges are:

- Pipetting

- Working in a BSC
- Working with animals
- Work at extreme temperatures such as in a cold room
- Work requiring repetition of the same task for a large number of samples

A video that addresses pipetting, which is a very common, repetitive, ergonomically challenging laboratory task is available on YouTube. It gives advice on how to adjust your posture, set up your workspace, modify your practices, and select the correct equipment to avoid developing injury from conducting this common, yet necessary laboratory task.

Please read NDSU's Ergonomics Program for tips and ideas on how to properly adjust workspaces with ergonomics in mind. Also, complete NDSU's training module on Ergonomics which can be found on the Safety Office's training webpage. You may always contact the EHS Office (701-231-7759) for an Ergonomics evaluation of your laboratory space, and advice on making positive changes to improve the functionality of your work environment.

VIII. EMERGENCY PROCEDURES

IMPORTANT CONTACT INFORMATION:

EMERGENCY

You may always dial 911 for Emergency Assistance

NDSU Police Call Center	701-231-8998

ENVIRONMENTAL HEALTH & SAFETY

Brandon Gustafson - Associate Director EHS	701-231-6299
brandon.gustafson@ndsu.edu	
Zachary Smith - Environmental Health Technician	701-231-9536
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Robert Cuchna - Hazardous Materials Technician	701-231-8040
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Olivia Stiller, Ph.D. – Laboratory Safety Inspector	701-231-4294

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Christina Rodenbiker - Administrative Operations Specialist 701-231-5166

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PUBLIC HEALTH AND SAFETY

Jennifer Quenette- Associate Director 701-231-6740

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Monty Botschner- Compliance Specialist 701-231-5637

montgomary.botschner@ndsu.edu

Lois Christianson- Loss Control and Claims Specialist 701-231-9587

lois.christianson@ndsu.edu

FACILITIES MANAGEMENT

Contact Number 701-231-7911

To file a work order:

MEDICAL SERVICES

Occupational Health - Sanford 701-234-4700

(hours are 8:00-4:30; M-F; 3838 12th Ave N, Fargo)

Occupational Health - Essentia 701-364-5757

(NO walk-ins; hours are 8:00-4:30; M-F; 1100 19th Ave N Fargo)

EMERGENCY RESPONSE PROCEDURES

Below are general spill and emergency situation response descriptions. Please note that exact emergency response details will vary depending on the agent(s) involved, as well as the type and severity of the spill.

However:

- **Always** notify your PI and/or your supervisor immediately in the event an accident or spill involving a biohazardous agent or material.

- **Always** notify the Environmental Health and Safety Office if you want or need assistance handling the accident or spill.
- **Always** seek proper medical attention immediately if an exposure happened, or was suspected to happen. The Exposure Risk section of your Laboratory Biosafety Manual is a helpful tool to be sent with someone who has had an exposure to an agent in the lab when they are going to be seen by an Occupational Health Provider.
- **Always** fill out an incident report, or a near miss report, within 24 hours if required. Contact the Safety Office for assistance (701-231-7759).
- **Always** report accidents, spills, or releases involving recombinant or synthetic nucleic acids to your PI, the Environmental Health and Safety Office, the IBC, and the Greenhouse Manger (if working in the greenhouse) as soon as possible to facilitate reporting to the NIH.
- A document on incident reporting can be found on the NIH OSP website here:
- **Always** review what actions/inactions led to the accident or spill, provide additional training, revise protocols, and change procedures as necessary.

SPLASH TO FACE, EYES OR MUCOUS MEMBRANES WITH A BIOHAZARDOUS AGENT OR MATERIAL, OR WITH A SAMPLE POTENTIALLY CONTAINING A BIOHAZARD

- Proceed to the nearest eyewash station and activate it
- Rinse face/mouth/nose/eyes
- Eyes should be flushed for at least 15 minutes.
- Forcibly hold eye open to ensure effective rinsing behind eyelids.
- Move eye side-to-side and up-down during rinsing.
- Remove contact lenses if possible.
- Place contaminated clothing in a red bag or biohazard bag for decontamination.
- Obtain medical treatment if required. If during normal working hours go to Sanford or Essentia Occupational Medicine. If after hours, go to the Emergency Room.
- File an Incident Report with the Safety Office.
- Watch for symptoms of exposure or delayed onset effects.
- Report incident to PI/supervisor and the Safety Office (701-231-7759)

NEEDLESTICKS, CUTS OR NON-INTACT SKIN BIOHAZARD EXPOSURE

- Remove protective clothing or PPE as needed to gain access to the affected area.
- Wash hands.

- Wash the affected part while allowing the wound to bleed freely (if applicable). Use soap if available, but avoid strong chemical disinfectants that can damage skin like bleach.
- Apply an appropriate disinfectant from the first aid kit (e.g. antibiotic ointment).
- Notify the PI or lab supervisor and inform them of the circumstances of the injury, including the biohazardous agent or material that was being handled at the time.
- For known biohazard exposures:
    - During normal business hours, go to Sanford or Essentia Occupational Health. Take agent information from your lab-specific biosafety manual with you.
    - After normal business hours, go to the Emergency Room.
- File an Incident Report with the Safety Office within 24 hours.
- Watch for symptoms of exposure or delayed onset effects.
- Report incident to PI/supervisor and Safety Office (701-231-7759).

Each lab that works with biohazardous agents and materials should put together a spill kit, must generate written, agent specific emergency procedures for their own space, and practice them on a routine basis. Every member of the lab must know where the emergency procedures SOPs are stored and they should be readily accessible. If the lab keeps and maintains a laboratory biosafety manual (required for all labs that work at BSL-2 and above), the emergency procedures must be part of the manual.

IN GENERAL, A SPILL KIT FOR BIOHAZARDOUS MATERIALS SHOULD INCLUDE:

- An easy-to-read outline of the spill response SOP
- Gloves (of several different sizes)
- Face protection
- Respirator (if you work with agents that pose an exposure hazard via aerosolization)
- Safety glasses or goggles
- Clean lab coat or disposable gown
- Paper towels to absorb contaminated liquids
- Disinfectant appropriate for agents used in the lab (for example, undiluted bleach; do not store diluted bleach)
- Tongs or forceps to pick up broken glass
- A biohazard waste container large enough to handle wet, contaminated paper towels

PROCEDURE FOR HANDLING SMALL SPILLS INSIDE THE BSC:

1. Leave the BSC running. First, wait 5 minutes to allow the blower to move aerosols through the HEPA filter. Do not disrupt air flow within the BSC during this time.

2. Check to see if the spill is fully contained within the BSC, if any PPE has become contaminated, or if any breach of containment has occurred (e.g., a splash where droplets have escaped the BSC and fallen on the floor). If there has been a breach of containment, response should be as for a spill outside the BSC.

3. Small spills (≤25 ml) can be decontaminated by layering paper towels soaked in appropriate disinfectant on top of the spill, allowing 20 minutes for the disinfectant to inactivate the agent, then depositing the paper towels in the biohazard waste bag in the BSC. If using bleach, residual bleach can be wiped off with paper towels sprayed with 70% EtOH, and the towels deposited in the biohazard waste bag.

   *Note: a spill of media or buffer not containing the agent does not represent a biohazard, but paper towels used to wipe it up should still be deposited in the biohazard bag in the BSC.

PROCEDURE FOR HANDLING LARGE SPILLS INSIDE THE BSC (SPILLS OVER 25 ML, WITH LIKELY SPLATTER DROPLETS OUTSIDE THE BSC):

1. Large spills should be treated more cautiously. Leave the BSC running. Remove PPE and any contaminated clothing (check the sleeves of your lab coat) and place it in sealable plastic container or a biohazard bag.

2. Notify the PI. If you must leave the room to do so, close the door to the room as you leave - make sure you have removed your gloves and washed your hands before you touch the door knob.

3. If you are absolutely sure that there has been no exposure and no breach of containment, proceed as for a small spill inside the BSC.

4. If there has been overt exposure (e.g., actual contact of bare skin with the agent(s) you were working with), wash the exposed skin with soap and water for 15 minutes, and contact the Safety Office (701-231-7759). After hours, contact the NDSU Police Department for assistance (701-231-8998).

5. Allow 20 minutes for any potential aerosols to settle from the spill. Don clean PPE, cover the spill with paper towels, soak with appropriate diluted disinfectant, starting at the perimeter

and working inward toward the center. Allow 20 minutes contact time with the disinfectant to inactivate the agent. Deposit soaked towels in biohazard waste.

6. The interior of the BSC should be decontaminated by wiping down the walls, sash, and equipment with disinfectant. Autoclavable equipment (e.g., racks, some pipettors, and tube containers) should be autoclaved, if feasible.

7. If the spill has entered the BSC drain pan, more extensive decontamination must be performed. The drain pan should be emptied into a collection vessel containing disinfectant. A hose barb and flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. The drain pan should be decontaminated, flushed with water and the drain tube removed.

8. After decontamination with corrosive disinfectants (e.g., bleach), remember to wipe down the BSC with 70% EtOH to remove residual chemicals.

9. If no overt exposure has occurred, and the spill was completely contained within the BSC, the Biological Safety Officer does not need to be informed. The PI should review the incident to revise procedures to minimize the risk of recurrence.

PROCEDURE FOR HANDLING SMALL SPILLS OUTSIDE THE BSC:

A small spill, in this circumstance, is defined as a spill with low potential to aerosolize, presents no inhalational hazard, and no endangerment to people or the environment. As a practical consideration, volumes less than 10 ml fall into this category.

1. First, ascertain the extent of the spill. Simply dropping a 150 mm dish contained inside a closed secondary container does not constitute a spill outside the BSC, since there is no breach of containment—as long as the secondary container stays closed.

2. If other personnel are present, alert them immediately. Keep in mind: spills generate aerosols.

3. Quickly check to ascertain the extent of the spill: Is PPE contaminated? (Gloves, lab coat, pants cuffs, shoes?). Is bare skin exposed? Has liquid splashed over a large area? If shoes are visibly contaminated, decontaminate them with appropriate disinfectant, then evacuate the room, closing the door. Remove gloves before touching the door knob. Remove any potentially contaminated PPE, place it in a biohazard bag, wash hands and face thoroughly.

4. Post a sign on the door warning personnel not to enter. Allow 20 minutes for aerosols to settle. During this time, notify the PI (and the Safety Office 701-231-7759). After hours, contact the NDSU Police for assistance (701-231-8998).

5. After 20 minutes, don fresh PPE, re-enter the room, use tongs to remove any sharps in the spill and transfer them to a biohazard sharps container, cover the spill with paper towels, then soak them with disinfectant starting at the periphery and moving inward toward the center. Be sure to check for and decontaminate small splashes beyond the main affected area. Leave the soaked towels in place for 20 minutes to inactivate the agent. After the 20 minutes inactivation time, transfer soaked paper towels to biohazard waste. Wipe up the residual spill with more paper towels.

6. Give the area a final wipe-down with paper towels using the appropriate disinfectant.

PROCEDURE FOR HANDLING LARGE SPILLS OUTSIDE THE BSC:

A large spill, in this circumstance, is defined as a spill that spreads rapidly, presents an inhalational hazard, endangers people or the environment, and/or involves personal injury or rescue and should be handled as an emergency. In practical terms, this might be a spill of more than 10 ml splattering over a large area, thus presenting the possibility of aerosolization and widespread contamination.

1. If other personnel are present, alert them immediately. Keep in mind: spills generate aerosols.

2. Ascertain the extent of the spill: possible overt exposure, splash on shoes or soles of shoes, contamination of PPE. If shoes are contaminated, disinfect them before evacuating the room (if shoes are extensively contaminated, you should remove them as you leave the room).

3. After removing gloves evacuate the room, closing the door as you leave. Remove PPE. Wash hands and face thoroughly.

4. Post a sign on the door warning personnel not to enter. Allow 20 minutes for aerosols to settle. During this time, notify the PI. If a spill of this magnitude occurs, you should notify the Safety Office (701-231-7759) for assistance, or the NDSU Police if it is after hours (701-231-8998).

5. After 20 minutes don fresh PPE, re-enter the room. If there is any broken glass associated with the spill, pick it up with tongs or forceps, and transfer it to a biohazardous broken glass container. Cover the spill with paper towels, and soak the towels with appropriate disinfectant, working from the outside toward the center. Allow 20 minutes for the agent(s) involved in the spill to be completely inactivated. Pick up soaked paper towels, and transfer to a biohazard bag.

6. Give the area a final wipe-down with paper towels using the appropriate disinfectant.

7. All large spills outside of the BSC that involve breach of containment, regardless of exposure, should be reported to the Safety Office (701-231-7759).

HANDLING SPILLS IN A CENTRIFUGE:

1. If tube failure is suspected (sudden clunking or automatic shut-down due to imbalance), leave the centrifuge lid closed for 30 minutes to allow aerosols to settle.

2. During this time, notify the PI. Open the lid cautiously to check the integrity of the rotor/tubes. If the rotor looks intact, spray the rotor with 70% EtOH, and transport it into the BSC before unloading centrifuge tubes.

3. If a tube has cracked or collapsed within a swinging bucket (e.g., SW28), decontaminate the tube and bucket inside the BSC. (Use your own judgment regarding recovery of samples that contain biohazardous agents or materials).

4. If there appears to be a leak or spill inside the centrifuge, decontaminate the centrifuge chamber by cautiously opening the centrifuge, adding paper towels to soak up any contaminated liquids, then liberally spraying disinfectant onto the walls and inside the lid of the centrifuge, so that disinfectant pools at the bottom of the chamber. (e.g., about 0.5-1 liter). Close the centrifuge for 20 minutes. Clean up the soaked paper towels as for a major spill outside the BSC.

5. In the event of a catastrophic failure in the centrifuge (e.g., swinging bucket coming off the rotor at 22,000 rpm, damaging the centrifuge, and releasing agent(s) into the centrifuge chamber), keep the centrifuge lid closed for 30 minutes. During this time, notify the PI and if the contamination is too extensive to manage alone, ask the Biosafety Officer for assistance (231-9543). Decontamination is similar to a major spill outside the BSC. Lay paper towels inside the centrifuge chamber, and soak with 10% bleach (or other appropriate disinfectant). Spray the inside of the centrifuge jacket with 70% EtOH. Close the lid for 20 minutes. Clean up following the same procedure as for a major spill outside the BSC.

IX. BIOHAZARDOUS WASTE PROCEDURES

The following materials are defined as biohazardous waste:

a) **Sharps waste**: Contaminated sharps must not be disposed of as regular waste. The term "sharps" is a regulatory waste classification associated with those instruments used to puncture, cut, or scrape body parts and that, in a waste container, can cause punctures or

cuts to solid waste handlers or to the public. This means that all sharps waste should be placed in appropriate, biohazard sharps containers and decontaminated prior to disposal.

Sharps include the following:

i) Needles, including syringes with needles attached to them
ii) Syringes without needles, when removed from their original sterile container
iii) Lancets
iv) Scalpel blades
v) Glass tubes/vials that can be broken during handling, such as Pasteur pipettes, ampoules, and capillary tubes
vi) Razor blades and other sharp items not defined above, when contaminated with biohazardous materials, including recombinant or synthetic nucleic acids.
vii) *If not contaminated with biohazardous materials, sharps can be disposed of like other laboratory sharps or broken glassware.

b) **Human blood, blood products, body fluids, tissues, and cells**. Human and non- human primate cell lines, regardless of origin, are also defined as waste.

c) **Cultures and stocks of etiologic agents and associated biologicals**: included but are not limited to specimen cultures, discarded live and attenuated vaccines, cultures and stocks of etiologic agents, and wastes from the production of biologicals and serums.

d) **Recombinant and synthetic nucleic acids**: included but is not limited to waste products from laboratory research procedures involving recombinant and synthetic nucleic acids in plasmids, viral vectors, and organisms used to propagate recombinant and synthetic nucleic acids, cell cultures, as well as naked DNA from polymerase chain reaction (PCR) and sequencing reactions. This also includes tissue and cells harvested from animals containing recombinant and synthetic nucleic acids (e.g., transgenic animals).

e) **Laboratory waste that has come in contact with a biohazard as listed in a, b, c, or d.** above: includes but is not limited to culture dishes, tissue culture plastics, blood specimen tubes, devices used to transfer, inoculate, and mix cultures, and other materials that have come in contact with biohazards (**includes disposable PPE and clothing**).

f) **Animal waste, animal carcasses, and body parts exposed to pathogens or recombinant and synthetic nucleic acids**: includes animal bedding and other wastes from such animals.

g) **Human pathological waste**: includes human source biopsy materials, tissues, and anatomical parts. This does not include teeth, human corpses, remains, and anatomical parts that are intended for interment or cremation.

The PI of each lab is responsible for developing a procedure for segregating and preparing biohazardous waste for appropriate disposal.

HANDLING SHARPS BIOHAZARDOUS WASTE:

Sharps that are or may be contaminated with a biohazard must be contained in leak proof, rigid, puncture-resistant, durable plastic containers. These containers are red in color, marked with the universal biohazard symbol, and equipped with a tight-fitting lid for use during handling and transport. Do not overfill sharps containers.

All sharps waste is picked up by the Safety Office and transported off campus for incineration. You are no longer allowed to autoclave and dispose of your own biohazard sharps waste. We must comply with the North Dakota State regulation which requires that sharps that originated as biohazard waste and are not incinerated must be rendered non-sharp before disposal. We do not have a mechanism for rendering sharps non-sharp before disposal on campus. Therefore, all biohazard sharps waste will be transported off campus for disposal.

To have your biohazard sharps waste picked up by the Safety Office follow this procedure:

1. When ¾ full, follow instructions on sharps container to lock the lid shut.
2. Completely close the sharps container using the locking lid mechanism on the container.
3. Decontaminate the outside of the sharps container using an appropriate decontamination chemical.
4. Complete a Biohazard Waste form, and notify the Safety Office that you have biohazard sharps for pick up (1-7759).

The Safety Office will not pick up sharps containers if the outside of the container has not been decontaminated, and/or if the lids are not completely locked closed.

HANDLING LIQUID BIOHAZARDOUS WASTE

Liquid biohazardous waste (including recombinant and synthetic nucleic acids) generated during work must be contained in leak proof, rigid, durable container labeled with what agent(s) are in the waste, the word "biohazard", and the universal biohazard symbol. These containers are closed when not actively in use, and must be placed in leak proof containers if/when transport is required.

Liquid wastes must not be disposed of in solid waste containers/together with solid waste.

All liquid biohazardous wastes must be decontaminated prior to disposal EITHER by:

- Bleaching - Add freshly prepared chlorine bleach to a final concentration of 10% bleach (or another appropriate liquid chemical decontaminant if your agent requires it). The solution must sit for at least 30 minutes; the resulting bleach solution should be collected as liquid chemical waste and disposed of through the Safety Office.

  Or,

- Autoclaving - Remove or loosen cap of collection vessel before loading into the autoclave. Run autoclave cycle with liquid exhaust. After the autoclaved liquid has cooled, dispose of the fluid in the sewer. Autoclaved waste can only be disposed of in the sewer if the liquid does not contain any hazardous materials. Contact the Safety Office for approval to sewer autoclaved liquid waste.

  - If you are using this method of sterilizing liquid waste you cannot use the liquid waste containers obtained from the Safety Office as they are not autoclave safe. Use an autoclave safe collection vessel such as glass to collect liquid biohazard waste for this purpose.

  - If you use this method of decontaminating liquid biohazard waste, you are strongly encouraged to verify the performance of your autoclave by regularly testing it using biological indicators. You can find out how to obtain biological indicators from the Safety Office by accessing the Autoclave Standard Operating Procedure.

If you have a need to dispose of human blood products, or body fluids in 10 liter or greater volumes, contact the Safety Office 701-231-7759 prior to conducting this work.

Animal blood: Small quantities may be flushed into the sewer system without treatment. Due to coagulation when handling large quantities, flushing is impractical. Contact the Safety Office for additional information on the disposal of large volumes of animal blood. If the blood and/or body fluid is potentially infected with a pathogen, handle according to the guidelines for human blood.

MIXED WASTE

Try and avoid generating mixed liquid waste as much as possible, such as biohazard liquid waste mixed with hazardous chemical, radiation, or nanomaterial waste. If your experimental protocol makes this unavoidable, please contact the Safety Office for assistance ahead of time to develop a waste management strategy (1-7759).

HANDLING SOLID BIOHAZARDOUS WASTE

Solid biohazardous waste (for example: tissue culture plastics, contaminated PPE, agar plates, etc.) must be collected in the laboratory in plastic, autoclavable, biohazard waste bags. The waste must be marked as a biohazard in some way, either on the bag, with a sticker, or on a secondary collection vessel such a burn up incinerator box or solid waste bucket. If you use the solid waste buckets obtained from the Safety Office to collect solid biohazard waste, you must remove the hazardous waste tag and place a biohazard sticker on the container.

As little liquid as possible must enter the solid biohazardous waste stream to prevent leakage and so that it does not interfere with downstream sterilization processes.

Also, no glass or sharps can be added to solid biohazardous waste containers.

Do not overfill your waste containers. Take care to not have sharp items such as stripettes poking through collection bags as these pose a biosafety hazard to people walking by the work area.

If your intention is to dispose of your solid waste in the Fargo Landfill, then the waste must be autoclaved and, in its final state, it cannot be in a red bag. Put the bag containing the waste in a secondary tray or container inside the autoclave during the sterilization process. Use some method to verify the autoclaving process was successful such as autoclave tape or SteriGage, and keep that evidence with your bag/waste. Deface the biohazard symbols on your autoclave bag with a heavy black marker or tape and double bag it in two black garbage bags. Your waste can now be sent to the landfill.

If you process your own solid biohazard waste, you are strongly encouraged to participate in the autoclave verification program, which you can learn about on the Biological Safety page of the University Police and Safety Office website. This program involves regular testing of autoclave performance using biological indicators, which can be obtained from the Safety Office free of charge.

If your intention is to have the Safety Office pick up your solid biohazardous waste for incineration, your waste must meet these criteria:

- It must be in a solid walled, outer container, such as burn-up bin which are available by contacting the Safety Office 701-231-7759.
- The box cannot be over-filled or weigh more than 30 lbs.
- There can be no signs, or evidence of leaking and nothing can be protruding from the box.
- The inner bag must be twisted, and taped shut; no bag items should be able to come out during transport.
- The lid must be secured with sturdy tape.

- The bottom must be secured/supported with tape if the weight of the box requires extra support.
- There can be nothing protruding out the sides of the box, such as stripettes.
- The biohazardous waste form must be properly filled out.

X. TRANSPORTING AND SHIPPING

Shipping biological materials is a highly regulated activity. A large number of people will handle or be in proximity to your package as it travels to its destination and all that protects these people from any hazard within the package is the information you provide on or with your package and the packaging itself. To meet IATA regulations, anyone who ships biological materials must be certified to do so and renew the certification every two years or when regulations change. DOT hazardous materials shipping training must be obtained every three years, or within 90 days of when regulation revisions are issued.

Regulations that apply to the packaging and shipment of biological materials:

- U.S. Department of Transportation, 49 CFR Parts 171-180 and amendments
- U.S. Public Health Service, 42 CFR Part 72, Interstate Shipment of Etiologic Agents
- U.S. Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Bloodborne Pathogens
- International Air Transport Association (IATA), Dangerous Goods Regulations
- U.S. Postal Service, 39 CFR Part 111, Mailability of Etiologic Agents, Mailability of Sharps and Other Medical Devices, and Publication 52, Acceptance of Hazardous, Restricted or Perishable Matter
- International Civil Aviation Organization, Technical Instructions for the Safe Transport of Dangerous Goods by Air
- United Nations, Recommendations of the Committee of Experts on the Transportation of Dangerous Goods

All North American airlines and FedEx, the largest shipper of infectious materials, use the IATA regulation (also referred to as the Dangerous Goods Regulation or DGR) as their standard.

Meeting the conditions of this standard will ensure meeting the provisions of the other US regulations. Many biological materials fall into the category of dangerous goods for shipping

purposes. All individuals involved in the transport of dangerous goods or the preparation of dangerous goods for transport must be trained to do so properly and safely. If you need to ship biological materials, please contact the Safety Office at 1-7759 and we will do whatever we can to assist you in this process.

For safe transport of biohazardous agents and materials on campus, these guidelines must be followed:

- Double contain the items in plastic leak-proof containers within sturdy outer packaging.
- Include absorbent material within the containers as well as padding to minimize movement of the container(s) within the outer packaging.
- Wipe the outer container with an appropriate disinfectant before removing it from the laboratory and apply a biohazard sticker if applicable (if the agent poses an infectivity threat to humans).
- Place your name and contact information on the package.
- The person doing the transporting must be knowledgeable on how to handle spills.
- A state fleet vehicle should be used.

XI. PERMITS AND EXPORT CONTROL

Research with certain infectious agents may require a permit. The Principal Investigator is responsible for obtaining and maintaining valid permits, and supplying a copy to the Environmental Health and Safety Office.

CDC

The Centers for Disease Control and Prevention's Import Permit Program (IPP) regulates the importation of infectious biological agents, infectious substances, and vectors of human disease into the United States. Prior to issuing an import permit, IPP reviews all applications to ensure that entities have appropriate safety measures in place for working safely with these imported materials. You can find more information regarding the CDC's permitting program for importation of infectious biological agents, infectious substances, and vectors of human disease on the Center for Disease Control website.

USDA-APHIS

APHIS issues permits for the import, transit and release of regulated animals, animal products, veterinary biologics, plants, plant products, pests, organisms, soil, and genetically engineered organisms. This process can be complex, however there is information available on the U.S. Department of Agriculture website that pertains to APHIS permits and certifications, as well as some instructional videos.

EXPORT CONTROL

NDSU is committed to pursuing its mission in teaching, research, and service in a manner consistent with all U.S. export laws and regulations. The export of etiologic agents of humans, animals, plants, and related materials primarily is regulated by the U.S. Departments of Commerce, State, and Treasury. A wide variety of etiologic agents of human, plant, and animal diseases, including genetic material and products that might be used for culture or production of biological agents, will require an export license(s). Furthermore, physical export of these agents to certain countries is prohibited. In addition, disclosing (including oral or visual disclosure) controlled information or technologies to a non-U.S. person in the U.S. (also known as a deemed export) or abroad – and/or providing certain technical assistance, training, or other defense services for/on behalf of a non-U.S. person, whether in the U.S (deemed export) or abroad – also may implicate export control laws and regulations. For more information on export compliance and biological agents, visit NDSU's Export Controls Compliance Manual, or send a request to ndsu.exportcontrols@ndsu.edu.