Northeastern University
Biological Safety Manual

Northeastern University

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Introduction

Northeastern University (NU) is committed to providing a safe and healthful learning, teaching and research environment.

The goals of the University's biological safety program are to:

- Protect staff and students from exposure to infectious agents.
- Prevent environmental contamination.
- Protect experimental materials.
- Comply with federal and local regulations.

The Office of Academic and Research Safety (OARS) under the direction of the University's Institutional Biosafety Committee and the Office of the Vice Provost for Research developed the NU Biological Safety Manual. The manual provides university-wide safety guidelines for those working with biohazards. It outlines general policies and procedures for using and disposing of infectious or potentially infectious materials. Federal and state regulations and guidelines mandate these practices. Updates to this manual are available at OARS website. If procedures currently in practice in your laboratory do not comply with those in this manual, please make the necessary changes to do so. Principal investigators (PIs) or laboratory supervisors must call the Office of Academic and Research Safety at 617-373-2769, if they are uncertain how to categorize, handle, store, treat or discard any biologically derived material.

I. Program Administration: Responsibilities and Accountability

A. The Office of the President and Office of the Provost.
The Office of the President and Office of the Provost have ultimate responsibility for biological safety within the University and must along with other officials, provide support for biological safety.

B. The Institutional Biosafety Committee (IBC).
The IBC is charged by the Vice Provost for Research to formulate policy and procedures related to the use of biohazardous agents, including: human pathogens, oncogenic viruses, other infectious agents and recombinant or synthetic nucleic acids (r/sNA). As mandated by the National Institutes of Health, experiments involving human gene therapy, formation of transgenic animals and work with r/sNA must be reviewed and approved by the IBC. (See section IIC below). In addition, work with human blood, blood products and tissues are also reviewed and approved by the IBC.

C. The Office of Academic and Research Safety (OARS).
The Office of Academic and Research Safety performs the following oversight:

- OARS monitors compliance with NU's safety policies and procedures regarding potentially infectious and biohazardous materials.
- Assists Principal Investigators (PIs) in the selection of laboratory practices, equipment and controls.
- Provides technical guidance to all personnel on matters related to laboratory safety.
- Develops and conducts appropriate training programs to promote techniques for the safe handling and disposal of biohazardous materials.
- Approves the use of biohazardous materials by PIs and sets safety criteria for the handling of those agents.
- Investigates all reported accidents which may result in personnel or environmental exposure to biohazardous materials.
- Coordinates the off-site treatment of infectious wastes.

D. Deans/Department Chairs.
Deans/Department Chairs are responsible for the implementation of safe practices and procedures in their colleges or departments.

E. Principal Investigators (PIs).
Principal Investigators (PIs) are responsible for identifying potentially infectious and biohazardous materials and carrying out specific control procedures within their own laboratories. This responsibility may not be shifted to inexperienced or untrained personnel. PIs are also responsible for the instruction of students and staff in the potential hazards of biologically derived materials. All protocols involving work with potentially infectious agents must be submitted to NU OARS for review and approval. For more information call OARS at 373-2769.

If an incident occurs in a laboratory which includes violations to established safety control procedures, the IBC may recommend to the Vice Provost for Research that the PI and the entire laboratory group be required to receive or participate in additional specialized training.

F. Employees.
Employees have the responsibility to:

- Comply with safety guidelines and procedures required for the task(s) performed.
- Report unsafe conditions to the PI, supervisor or NU OARS.
- Seek guidance from their PI, supervisor or NU OARS when they are uncertain how to handle, store or dispose of any hazardous or biohazardous material.

II. Biohazards and Potentially Infectious Material

Definition:

Biohazards are infectious agents or biologically derived infectious materials that present a risk or potential risk to the health of humans or animals, either directly through infection or indirectly through damage to the environment. Infectious agents have the ability to replicate and give rise to the potential of large populations in nature when small numbers are released from a controlled situation.

Categories of Biohazards or Potentially Infectious Materials

1. Human, animal and plant pathogens:
    - Bacteria, including those with drug resistance plasmids
    - Fungi
    - Viruses, including oncogenic viruses
    - Parasites

The NIH has compiled a general list of human pathogens or etiologic agents and has classified them according to risk. This list can be accessed in Appendix B of the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, April 2019 or the latest edition.

Select Agents are a subset of human and animal pathogens and toxins that have been identified by the U.S. Government as agents with potential for use in biological terrorism or warfare. The Department of Health and Human Services (DHHS), through the U.S. Centers for Disease Control and Prevention (CDC), and the Animal Plant Health Inspection Service (APHIS), through the United States Department of Agriculture (USDA), regulate select agents in the United States and its territories. Work with these agents require approval by the United States Government prior to initiation. It may take months to acquire authorization. Please contact the NU OARS Biosafety Office if you are interested in pursuing research with Select Agents

or amounts of select agent toxins that exceed the permissible levels. A list of Select Agents and toxins and the permissible amounts of Select Agent toxins can be found at the following websites.

2. All human blood, blood products, tissues and body fluids

3. Cultured cells (all human or certain animal) and potentially infectious agents these cells may contain

4. Allergens

5. Toxins (bacterial, fungal, plant, etc.)

6. Certain recombinant products

7. Clinical specimens

8. Infected animals and animal tissues

Recombinant or Synthetic Nucleic Acid Molecules (r/sNA)

1. Experiments Involving r/sNA

Experiments involving r/sNA may require registration and approval by the appropriate campus IBC. The National Institutes of Health (NIH) Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) is the definitive reference for r/sNA research in the United States. There may be experiments which are not covered by the NIH Guidelines that do require review and approval by outside agencies before initiation or funding. These experiments are not generally associated with biomedical research but are more common in the agricultural and environmental sciences. If the experimental protocol is not covered by the NIH Guidelines contact the Biosafety Officer at 373-2769 for determination of further review.

If you have any specific questions about a particular host-vector system not covered by the NIH Guidelines, please call the NIH Office of Science Policies at (301) 496-9838 or FAX (301) 496-9839. Updates to the NIH Guidelines are published in the Federal Register and are available at NU OARS website.

2. Human Gene Therapy

All protocols involving the r/sNA for human gene therapy must be approved locally by the IBC and the Institutional Review Board (IRB) prior to submission to outside agencies and the initiation of experimentation. For more details about IBC approval of human gene therapy protocols, call 373- 2769. For information about IRB submissions, call 617-373-4025.

3. Transgenic Animals

PIs who create transgenic animals must complete a r/sNA registration in BioRAFT and submit it to NU's OARS for IBC approval prior to initiation of experimentation. In addition, the Institutional Animal Care and Use Committee (IACUC) protocol must be approved by OARS prior to its being given full approval by the IACUC.

4. Transgenic Plants

Experiments to genetically engineer plants by recombinant DNA methods may require registration with the IBC. The NIH Guidelines provide specific plant biosafety containment recommendations for experiments involving the creation and/or use of genetically engineered plants.

To submit a r/sNA registration visit your PI profile on the BioRAFT website. To obtain a copy of current NIH Guidelines, call NU OARS at 373-2769. A copy of the NIH Guidelines can also be accessed from the website of the National Institutes of Health's Office of Science Policy.

Other Potentially Hazardous Biological Materials

1. Human Blood, Blood Products, Body Fluids and Tissues

Biosafety Level 2 practices and procedures must be followed when handling human blood, blood products, body fluids and tissues because of the infectious agents they may contain. Biosafety Level 2 practices and procedures are consistent with the concept known as Universal Precautions; which requires all specimens of human blood or other potentially infectious materials to be treated as if they are infectious. In 1991, the Occupational Safety and Health Administration (OSHA) promulgated a standard to eliminate or minimize occupational exposure to Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV) and other bloodborne pathogens. This federal regulation, Occupational Exposure to Bloodborne Pathogens, mandates a combination of engineering and work practice controls, training, Hepatitis B vaccination, and other provisions to help control the health risk to employees resulting from occupational exposure to human blood and other potentially infectious materials which may contain these or other specified agents.

Free Hepatitis B vaccination is available to all occupationally at-risk University employees through Mount Auburn Hospital. Mandatory safety training which provides information on protection from occupational exposure to infectious materials is offered by NU OARS on a monthly basis university wide. For more information on training or the availability of free Hepatitis B vaccine, call OARS at 373-2769.

PIs using human blood, blood products, body fluids or tissues must complete an Exposure Control Plan, which may be obtained from NU OARS. The plan is also available on the NU OARS website. The completed plan must be readily available in the laboratory for all workers. In addition, PIs must consult with NU's IRB (617-373-4025) to ensure that all regulatory requirements relating to the use of human materials or subjects in research are met.

Laboratory personnel (faculty and staff) in HIV or HBV research laboratories must fulfill additional OSHA requirements as follows:

a. The employee must attend an annual general biosafety training offered by NU's OARS.

b. The employee must have prior experience in the handling of human pathogens or tissue cultures before working with HIV or HBV.

c. In the laboratory, the employee must demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the laboratory to the satisfaction of the PI or laboratory supervisor before being allowed to work with HIV or HBV.

d. An employee with no prior experience in handling human pathogens must be trained in the laboratory prior to handling infectious materials. Initial work activities shall not include handling of infectious agents. A progression of work activities will be assigned as techniques are learned and proficiency is developed. Participation in work activities involving infectious agents will be allowed only after proficiency has been demonstrated to the satisfaction of the PI or laboratory supervisor.

e. The employee must view the video Working Safely with HIV in the Laboratory. A copy of the video is available in the Snell Library for viewing.

2. Use of Animals

The use of animals in research requires compliance with the "Animal Welfare Act" and any state or local regulations covering the care or use of animals. Facilities for laboratory animals used for studies of infectious or non-infectious disease should be physically separate from clinical laboratories and facilities that provide patient care.

Vertebrate animal biosafety level criteria must be adhered to where appropriate. All animal protocols involving the use of r/sNA; infectious or transmissible agents; human blood, body fluids or tissues; toxins; carcinogenic, mutagenic, teratogenic chemicals; or physically hazardous chemicals (reactive, explosive, etc.) must be submitted to NU OARS for review and approval prior to final approval by the Institutional Animal Care and Use Committee (IACUC). The PI must notify Animal Care Office (ACO) and OARS in writing prior to initiation of experimentation at Animal Biosafety Level 2 or Animal Biosafety Level 3. IACUC "guidelines" are available from ACO (373-3958). PIs who are uncertain how to categorize agents should call OARS (373-2769).

3. Tissue Culture/Cell Lines

When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line can be classified as the same level as that recommended for the agent.

The Centers for Disease Control and Prevention (CDC) and OSHA recommend that all cell lines of human origin be handled at Biosafety Level 2.

Cell lines which are non-primate or are of normal primate origin, which do not harbor a primate virus, which are not contaminated with bacteria, mycoplasma or fungi and which are well established may be considered Risk Group 1 cell lines and handled at Biosafety Level 1.

Primate cell lines derived from lymphoid or tumor tissue, all cell lines exposed to or transformed by a primate oncogenic virus, all clinical material (e.g., samples of human tissues and fluids obtained after surgical resection or autopsy), all primate tissue, all cell lines new to the laboratory (until proven to be free of all adventitious agents) and all virus and mycoplasma-containing primate cell lines are classified as Risk Group 2 and should be handled at Biosafety Level 2.

Studies involving suspensions of HIV prepared from T cell lines must be handled at Biosafety Level 3.

4. Guidelines for Preventing the Transmission of Tuberculosis

Since 1985, the incidence of tuberculosis in the United States has been increasing steadily, reversing a 30-year downward trend. Recently, drug resistant strains of *Mycobacterium tuberculosis* have become a serious concern. Outbreaks of tuberculosis, including drug resistant strains, have occurred in healthcare environments. Several hundred employees have become infected after workplace exposure to tuberculosis, requiring medical treatment. A number of health-care workers have died.

In October 1994, CDC published its "Guidelines for Preventing the Transmission of Tuberculosis in Health-Care Facilities, 1994". The guidelines contain specific information on ventilation requirements, respiratory protection, medical surveillance and training for those personnel who are considered at-risk for exposure to tuberculosis. For more information, contact NU OARS at 373-2769.

PIs intending to work with *Mycobacterium tuberculosis* in the laboratory must obtain written approval from NU OARS before beginning work. Propagation and manipulation of *Mycobacterium tuberculosis* cultures must be performed at Biosafety Level 3. An agent summary statement for this organism may be found in Appendix A (a link to the NU OARS website is provided at the end of this document).

5. Use of Vaccinia Virus

PIs wishing to use vaccinia virus must obtain written approval to do so from NU OARS. Biosafety Level 2 practices and procedures must be followed. Experiments involving the r/sNA in vaccinia virus must be registered with the IBC. A registration for r/sNA experiments must be submitted online via BioRAFT. The registration must be approved by the IBC.

All employees who directly handle cultures or animals contaminated or infected with vaccinia, recombinant vaccinia viruses or other orthopox viruses that infect humans, must be offered small pox vaccine. The Lane Health Center requires that PIs complete a "Request For Small Pox Vaccine" form. The completed form must be returned to the Lane Health Center. Lane will notify individuals when the vaccine is available.

E. Clinical Laboratories

Clinical laboratories receive clinical specimens with requests for a variety of diagnostic services. The infectious nature of this material is largely unknown. In most circumstances, the initial processing of clinical specimens and identification of microbial isolates can be done safely at Biosafety Level 2. A primary barrier, such as a biosafety cabinet, should be used:

- when it is anticipated that splashing, spraying or splattering of clinical materials may occur,
- for are aerosol generating procedures (e.g. pipetting, (un)loading of centrifugation buckets)
- for initial processing of clinical specimens where it is suggested that an agent transmissible by infectious aerosols may be present (e.g., *M. tuberculosis*),
- to protect the integrity of the specimen.

All laboratory personnel who handle human source materials are required to comply with the OSHA bloodborne pathogens standard as stated in section II-D1. Universal precautions must be followed when handling human blood, blood products, body fluids or tissues.

The segregation of clinical laboratory functions and restricting access to specific areas is the responsibility of the laboratory director. It is also the director's responsibility to establish standard, written procedures that address the potential hazards and the required precautions to be implemented. An Exposure Control Plan must be completed and be available in the laboratory. Additional recommendations specific for clinical laboratories may be obtained from the National Committee for Clinical Laboratory Standards.

Biological Agents/Biohazard Classification

Biological agents are classified according to risk as follows:

Risk Group 1

Agents of no or minimal hazard under ordinary conditions of handling.

Risk Group 2

Agents of ordinary potential hazard. This Risk Group (RG) includes agents which may produce disease of varying degrees of severity from accidental inoculation or injection or other means of cutaneous penetration but which are contained by ordinary laboratory techniques.

Risk Group 3

Agents involving special hazard or agents derived from outside the United States which require a federal permit for importation unless they are specified for higher classification. This Risk Group includes pathogens which require special conditions for containment.

Risk Group 4

Agents that require the most stringent conditions for their containment because they are extremely hazardous to laboratory personnel or may cause serious epidemic disease. This group includes RG 3 agents from outside the United States when they are employed in entomological experiments or when other entomological experiments are conducted in the same laboratory area.

High-risk Agricultural Pathogens

Foreign animal pathogens that are excluded from the United States by law or whose entry is restricted by USDA administrative policy.

NOTE: Federally licensed vaccines containing live bacteria or viruses are not subject to these classifications. These classifications are applicable, however, to cultures of the strains used for vaccine production, or further passages of the vaccine strains.

A list of Biological Agents classified according to risk may be found in Appendix A (a link to the NU OARS website is provided at the end of this document where the Appendix can be downloaded). CDC Agent summary statements on retroviruses (including HIV), hepatitis viruses (including HBV) and *Mycobacterium tuberculosis* may also be found in Appendix A. If the biological agent of interest is not listed, contact NU OARS.

III. Principles of Biosafety

A. Risk Assessment

Risk assessment is a structured process that takes the following factors into consideration:

- Identification of biohazards
- Properties of biohazards (including the availability of treatment and pre- and post-exposure prophylaxis for human and animal pathogens)
- Personnel engaged in the research or working in the laboratory (considering their health status, contraindications for treatment or prophylaxis, training and proficiency, and safety record)
- Proposed laboratory procedures (factoring in the inherent risks associated with the experimental protocol such as the use of sharps and the unpredictability of animals)

Gather as much information as possible on the biohazard utilized. If proposed research involves the use of a human pathogen, excellent risk assessment information can be obtained from the agent summary statements in the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 6th Edition and Health Canada's Pathogen Safety Data Sheet website. This will assist in gathering as much relevant information on the potential risks of the biohazards that will be utilized. It is critical that all handling biohazards are provided with the following information, including, but not limited to:

- The signs and symptoms of infection.
- What preventive therapies (immunizations and post-exposure treatment) exist.
- All clinical syndromes that can be caused by the agent.
- What medical conditions may make a person more susceptible or at higher risk of infection or serious disease.
- How to seek counsel from Employee Health prior to starting work with biohazards for a private consult regarding their health status if applicable.
- The incubation period, the infectious dose, the starting Risk Group.
- Any laboratory-acquired infections with the proposed biohazard and identify how it was transmitted if known.
- How the biohazard is transmitted in nature and in a lab setting.
- How long the biohazard can survive on surfaces or the environment.
- The disinfectants that are effective at inactivating the biohazard and identify the concentration of the disinfectant and contact time required for kill.

In addition, researchers must be aware of procedural hazards that can contribute to risk. All procedures, equipment and supplies that will be used in your research or lab protocol should be identified. Consider every step involved, including the removal of the biohazard from the freezer, transport to work areas, all protocol steps, through decontamination and disinfection and/or return to frozen storage. Also consider procedures that must be performed outside your lab, such as in core facilities (i.e. specialized microscopy, flow cytometry) and any shared equipment locations.

Identify all of the risks and potential exposures that could possibly occur during the course of research, such as splashes, splatter, spills, aerosols, cuts, lacerations, punctures, bites, scratches, etc. Pay particular attention to punctures from contaminated sharps (if not working with animals – eliminate sharps and use plastic alternatives. Any step with a liquid can generate splash or splatter that could contact facial mucous

membranes, skin, or personal clothing and contact surrounding work areas. Procedures that impart energy to a culture (basically all of them) from pipetting to vortexing to centrifugation may produce aerosols. For animal experiments consider the use of sharps for inoculation, bites and scratches from the animal, exposure to contaminated bedding from excretion of the biohazard. Identify the potential for spills (dropped flasks, broken flasks in shakers, leaks in centrifuges, etc.).

Finally, evaluate the personnel who will conduct work with biohazards. Consider the following:

- Do all staff have prior experience working with this biohazard or very similar biohazards? If not, an internship to gain hands-on experience with the agent can be arranged with another lab or within your lab.
- Have all staff completed all required Biosafety and other applicable laboratory safety trainings prior to initiating work?
- Do staff have a positive safety attitude and a healthy amount of respect for the risks involved with the proposed biohazard?
- Have the proposed staff exhibited a strong safety record in the lab?
- Are all staff informed of the risks presented by this work and the proposed procedures?
- Are any staff at greater risk due to their health status?
- Have they met with and been cleared by Employee Health?
- This discussion of likely elevated risks and review of proposed participation is critical.
- Do any staff have contraindications with any of the pre- or post-exposure treatment options?
- Has a suitable treatment been identified for them if they are?
- Have you documented the proficiency of the staff with the lab protocols and the associated biocontainment practices required to mitigate risks?

Once a risk assessment has been conducted for proposed research with biohazards, the risk management portions of this biosafety manual are assembled into standard operating procedures to work safely with the biohazards. Risk management elements include safe work practices when handling biohazards, personal protective equipment and engineering controls and the laboratory itself. These are all elements that contribute to minimizing exposure and laboratory biosafety containment.

B. Containment

The term *"containment"* is used in describing safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other people and the outside environment to potentially hazardous agents. The three elements of containment include laboratory practice and technique, safety equipment, and facility design.

Biosecurity

The CDC and NIH define biosecurity as measures designed to prevent loss, theft, or deliberate misuse of biological material, technology, or research-related information from laboratories or laboratory-associated facilities. Biosecurity practices ensure that access to the laboratory facility and biological materials are limited and controlled as necessary. An inventory or material management process for control and tracking of

biological stocks or other sensitive materials is also a component of both programs. Elements of biosecurity include assessment of the value or sensitivity of research materials and information; physical protection of these items; an inventory that is established and periodically monitored; awareness to report suspicious persons or activities; and discussions with staff to raise awareness of the importance of protecting research materials and sensitive information.

Biosecurity threats can be from inside or outside of the institution, but awareness of basic biosecurity measures, such as locking doors when the lab is unoccupied, locking freezers when the lab is unoccupied, and keeping and checking inventories periodically can help reduce potential threats. More detailed information on Biosecurity can be accessed in the CDC/NIH Biosafety in Microbiological Laboratories, 6th Edition in Section VI – Laboratory Biosecurity.

Primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by good microbiological technique and the use of appropriate safety equipment. The use of vaccines may provide an increased level of personal protection.

Secondary containment, the protection of the environment external to the laboratory from exposure to infectious materials is provided by a combination of facility design and operational practices. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

Laboratory Practice and Technique. The most important element of containment is strict adherence to standard microbiological practices and techniques. Researcher working with infectious agents or infected materials must be aware of potential hazards and must be trained and proficient in the practices and techniques required for handling such material safely. The PI or laboratory supervisor is responsible for providing or arranging for appropriate training of personnel.

Each laboratory should develop an operational manual which identifies specific hazards that will or may be encountered, and which specifies practices and procedures designed to minimize or eliminate risks. Personnel should be advised of special hazards and should be required to read and to follow the required practices and procedures. A scientist trained and knowledgeable in appropriate laboratory techniques, safety procedures and hazards associated with the handling of infectious agents must direct laboratory activities.

When standard laboratory practices are not sufficient to control the hazard associated with a particular agent or laboratory procedure, additional measures may be needed. The PI is responsible for selecting additional safety practices, which must be in keeping with the hazard associated with the agent or procedure.

Laboratory personnel safety practices and techniques must be supplemented by appropriate facility design and engineering features, safety equipment and management practices.

Safety Equipment (Primary Barriers). Safety equipment includes biosafety cabinets, enclosed containers and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The biosafety cabinet (BSC) is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. More information on BSCs may be found in section IV-B1 and IV-C3.

Safety equipment also may include items for personal protection such as personal protective clothing, respirators, face shields, safety glasses or goggles. Personal protective equipment is often used in combination with other safety equipment when working with biohazardous materials. In some situations, personal protective clothing may form the primary barrier between personnel and the infectious materials.

Facility Design (Secondary Barriers). The design of a facility is important in providing a barrier to protect people working inside and outside the laboratory, and to protect people or animals in the community from infectious agents which may be accidentally released from the laboratory. Facilities must be commensurate with the laboratory's function and the recommended biosafety level for the agent being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in Biosafety Level 1 and 2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave) and handwashing facilities.

As the risk for aerosol transmission increases, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features could include specialized ventilation systems to assure directional airflow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks at laboratory entrances, or separate buildings or modules for isolation of the laboratory.

C. Biosafety Levels

There are four biosafety levels (BSLs) which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity. The recommended biosafety level for an organism represents the conditions under which the agent can be ordinarily handled safely.

Biosafety Level 1 is appropriate for work done with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. It represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for handwashing.

Biosafety Level 2 is applicable to work done with a broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. Agents can be used safely on the open bench, provided the potential for producing splashes or aerosols is low. Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures or ingestion of infectious materials. Procedures with high aerosol or splash potential must be conducted in primary containment equipment such as BSC. Primary barriers such as splash shields, face protection, gowns and gloves should be used as appropriate. Secondary barriers such as handwashing and waste decontamination facilities must be available.

Biosafety Level 3 is applicable to work done with indigenous or exotic agents with a potential for respiratory transmission and which may cause serious and potentially lethal infection. Primary hazards to personnel

working with these agents (i.e., *Mycobacterium tuberculosis*, St. Louis encephalitis virus and *Coxiella burnetii*) include autoinoculation, ingestion and exposure to infectious aerosols. Greater emphasis is placed on primary and secondary barriers to protect personnel in adjoining areas, the community and the environment from exposure to infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment. Secondary barriers include controlled access to the laboratory and a specialized ventilation system that minimizes the release of infectious aerosols from the laboratory.

Biosafety Level 4 is applicable for work with dangerous and exotic agents which pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with close or identical antigenic relationship to Biosafety Level 4 agents should also be handled at this level. Primary hazards to workers include respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets and autoinoculation. All manipulations of potentially infected materials and isolates pose a high risk of exposure and infection to personnel, the community and the environment. Isolation of aerosolized infectious materials is accomplished primarily by working in a Class III BSC or a full-body, air-supplied positive pressure personnel suit. The facility is generally a separate building or a completely isolated zone within a complex with specialized ventilation and waste management systems to prevent release of viable agents to the environment.

D. Vertebrate Animal Biosafety Levels.

There are four animal biosafety levels, designated Animal Biosafety Level 1 through 4, for work with infectious agents in mammals. The levels are combinations of practices, safety equipment and facilities for experiments on animals infected with agents which produce or may produce human infection. In general, the biosafety level recommended for working with an infectious agent in vivo and in vitro is comparable.

Animal biosafety levels have been adapted for work in agricultural animals by NU OARS and University Laboratory Animal Resources (ULAR). The use of any barn for research with infectious agents in farm animals must be approved by OARS and ULAR prior to experimentation.

Summaries of Biosafety Levels may be found in Table 1 at the end of this document. Complete descriptions of Biosafety Levels 1 through 3 and Animal Biosafety Levels 1 through 3 may be found in Appendices B and C (links for the appendices are provided at the end of this document).

IV. Practices and Procedures

A . Administrative Controls

1. Biohazard Warning Signs and Posting

Each laboratory must have a room sign that provides safety information to visitors and service personnel. Room signs must contain designations for all laboratory hazards in use within the laboratory (carcinogens, acutely toxic agents, reproductive hazards, biohazards, radioactive materials, lasers and magnetic fields). Contact NU OARS for more information.

a. All areas and laboratories which contain biohazardous agents must be posted with a biohazard sign. The sign must be red/orange in color with a biohazard symbol and lettering in black.

b. All areas and laboratories which contain biohazardous, radioactive or toxic agents must be posted with signs stating "EATING, DRINKING, SMOKING AND APPLYING COSMETICS ARE PROHIBITED IN THIS AREA."

2. Biosafety Levels

The essential elements of the four biosafety levels for activities involving infectious microorganisms are summarized in Table 1 at the end of this document. In general, RG 1 agents are handled at Biosafety Level 1, RG 2 agents at Biosafety Level 2 and so on. The levels are designated in ascending order, by degree of protection provided to personnel, the environment and the community. Complete descriptions of Biosafety Levels 1 through 3 may be found in Appendix B (a link to the NU OARS website is provided at the end of this document where the document can be downloaded).

3. Vertebrate Animal Biosafety Levels

There are four animal biosafety levels for experiments on animals infected with agents which produce or may produce human infection. As with Biosafety Levels, increasing levels of protection to personnel and the environment are provided as the order ascends. Complete descriptions of Animal Biosafety Levels 1 through 3 may be found in Appendix C (a link to the NU OARS website is provided at the end of this document where the document can be downloaded).

4. Medical Surveillance

Medical surveillance requirements are developed to address the risks associated with the proposed research. At a minimum, all researchers must be cleared to work with the biohazards they handle by the Principal Investigator, using the restrictions developed for their protocols. The IBC may require additional medical surveillance elements based on their review of the protocol. Medical surveillance takes into account the health of the people who will have exposure to biohazards, and the potential risks to colleagues who work in the same laboratory, those on the same floor, and any family members or other close contacts.

Health conditions that may elevate risk include temporary or permanent immunosuppressive conditions, such as T and B cell disorders, cancer, cancer chemotherapy, HIV infection, and treatment with antimicrobials or corticosteroids. Other conditions, such as asthma, diabetes, inflammatory bowel disease, lupus, splenectomy, gastrectomy, and connective tissue diseases also may place an individual at increased susceptibility of infection if exposed. Eczema, dermatitis, psoriasis and other skin disorders also increase the risk for workers as the normal intact skin barrier may be compromised in these individuals. Pregnancy must also be evaluated for potential risk to the mother and to the developing fetus. Individuals with lacerations, burns, or open wounds are also at increased susceptibility of exposure to biohazards via open skin. Researchers with such conditions should not conduct research with biohazards until these conditions have resolved.

All those working with biohazards must understand the signs and symptoms of infection to ensure control of the infection and prompt treatment and management of the illness. Researchers should also be aware of the post-exposure prophylaxis options available.

a. A medical surveillance program will be provided for those personnel having substantial direct animal contact through the Lane Health Center.

b. Vaccines for which the benefits (levels of antibody considered to be protective) clearly exceed the risk (local or systemic reactions) will be offered to all clearly identified at-risk personnel, because immuno- prophylaxis may provide an additional level of protection. The following immunizations may be considered.

- Diphtheria-tetanus: Every 5 years for adults, and should be re-assessed in the case of a research animal bite or other wound. Employee Tetanus immunization status should be evaluated at each incident (Employees should be checked for their immunization status at time of hire and immunized if the last record of immunization was more than 10 years ago).
- MMR-Measles/Mumps/Rubella: Adults should have received two doses of live attenuated vaccine.
- Rabies: For researchers handling rabies virus in the laboratory or experiments involving infected animals; also for field workers, handlers of feral animals, possibly dogs from non-quarantined sources, cats.
- Hepatitis B: For employees with exposure to human blood or body fluids.
- Hepatitis A: For those handling Hepatitis A virus in the research or clinical laboratory.
- Vaccinia immunization (poxviruses): For researchers with exposure to Vaccinia virus, recombinant Vaccinia viruses, and other Orthopox viruses that can infect humans (e.g., smallpox).
- Typhoid Vaccine: Recommended for researchers and clinical laboratory workers who are handling Salmonella typhi in the laboratory.
- Meningococcal vaccine: For consideration for researchers handling Neisseria meningitidis.
- Varicella: For researchers who may be susceptible to exposure to chickenpox (Varicella virus).
- Influenza virus (annual flu shot): Recommended for general wellness during flu season, and consideration for researchers handling high-risk strains of influenza.

c. A medical surveillance program will be provided through the Lane Health Center for those personnel who are occupationally at-risk of exposure to bloodborne pathogens. The program will include free Hepatitis B vaccination, post-exposure evaluation and follow-up. For a more detailed explanation of this program, consult the University's Exposure Control Plan.

B. Engineering Controls

1. Biosafety cabinets (BSCs).

BSCs are designed to contain aerosols generated during work with infectious material through the use of laminar air flow and high efficiency particulate air (HEPA) filtration. Three types of BSCs (Class I, II and III) are used in microbiological laboratories. Open-fronted Class I and Class II BSCs are partial containment devices which provide a primary barrier offering significant levels of protection to laboratory personnel and to the environment when used in combination with good microbiological techniques.

The Class I BSC is suitable for work involving low to moderate risk agents, where there is a need for containment, but not for product protection. It provides protection to personnel and the environment from contaminants within the cabinet. The Class I BSC does not protect the product from "dirty" room air.

The Class II BSC protects the material being manipulated inside the cabinet (e.g., cell cultures, microbiological stocks) from external contamination. It meets requirements to protect personnel, the environment and the product. There are three basic types of Class II BSCs: Type A, Type B and 100% Exhaust. The major differences between the three types may be found in the percent of air that is exhausted or recirculated, and the manner in which exhaust air is removed from the work area.

The gas-tight Class III BSC or glove box, provides the highest attainable level of protection to personnel, the environment and the product. It is the only cabinetry which provides a total physical barrier between the product and personnel. It is for use with *high risk* biological agents and is used when absolute containment of highly infectious or hazardous material is required.

It is important to note that laminar flow clean benches must not be utilized for work with biohazardous or chemically hazardous agents. Clean benches provide product protection by ensuring that the product is exposed only to HEPA-filtered air. They do not provide protection to personnel or the ambient environment.

2. Safety equipment

Safety equipment includes items for personal protection such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses or goggles. Personal protective equipment (PPE) is often used in combination with BSCs and other devices which contain the biohazardous agents, animals or materials. When it is impractical to work in BSCs, PPE may form the primary barrier between personnel and infectious materials. Examples include certain animal studies, animal necropsy, agent production activities and activities relating to maintenance, service or support of the laboratory facility.

Other safety equipment such as safety centrifuge cups and safety blenders are enclosed containers designed to prevent aerosols from being released during centrifugation or homogenization of infectious material.

Containment controls such as BSCs, safety centrifuge cups and blenders must be used for handling infectious agents that can be transmitted through the aerosol route of exposure. A description of effective use of BSCs and information on other safety equipment may be found in the Recommended Work Practices section below. For more information on proper use and selection of a BSC or other safety equipment, call OARS at 373-2769.

C. Recommended Work Practices

1. Pipettes and Pipetting Aids

Pipettes are used for volumetric measurements and transfer of fluids that may contain infectious, toxic, corrosive or radioactive agents. Laboratory associated infections have occurred from oral aspiration of infectious materials, mouth transfer via a contaminated finger and inhalation of aerosols. Exposure to aerosols may occur when liquid from a pipette is dropped onto the work surface, when cultures are mixed by pipetting, or when the last drop of an inoculum is blown out. A pipette may become a hazardous piece of equipment if improperly used. The safe pipetting techniques which follow are required to minimize the potential for exposure to hazardous materials.

a. Never mouth pipette. Always use a pipetting aid.
b. If working with biohazardous or toxic fluid, confine pipetting operations to a BSC.
c. Always use cotton plugged pipettes or filtered pipette tips when pipetting biohazardous or toxic materials, even when safety pipetting aids are used.
d. Do not prepare biohazardous materials by bubbling expiratory air through a liquid with a pipette.
e. Do not forcibly expel biohazardous material out of a pipette.
f. Never mix biohazardous or toxic material by suction and expulsion through a pipette.
g. When pipetting, avoid accidental release of infectious droplets. Place a disinfectant soaked towel on the work surface and autoclave the towel after use.
h. Use “to deliver” pipettes rather than those requiring “blowout”.
i. Do not discharge material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.
j. Place contaminated pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them. Do not place pipettes vertically into a cylinder. Autoclave the pan and pipettes as a unit before processing them for reuse.
k. Discard contaminated disposable pipette tips in an appropriate sharps container.
l. Pans or sharps containers for contaminated pipettes should be placed inside the BSC, if possible.

2. Syringes and Needles

Syringes and hypodermic needles are dangerous instruments. *The use of needles and syringes should be restricted to procedures for which there is no alternative*. Blunt cannulas should be used as alternatives to needles wherever possible (i.e., procedures such as oral or intranasal animal inoculations). Needles and syringes should never be used as a substitute for pipettes. When needles and syringes must be used, the following procedures are recommended:

a. Use disposable needle locking syringe units whenever possible.
b. When using syringes and needles with biohazardous or potentially infectious agents:
    1. Work in a BSC whenever possible.
    2. Wear gloves.
    3. Fill the syringe carefully to minimize air bubbles.
    4. Expel air, liquid and bubbles from the syringe vertically into a cotton pledget moistened with disinfectant.
    5. Do not use a syringe to mix infectious fluid forcefully.
    6. Do not contaminate the needle hub when filling the syringe in order to avoid transfer of infectious material to fingers.
    7. Wrap the needle and stopper in a cotton pledget moistened with disinfectant when removing a needle from a rubber-stoppered bottle.

c. Bending, recapping, clipping or removal of needles from syringes is prohibited. If it is essential that a contaminated needle be recapped or removed from a syringe, the use of a mechanical device or the one-handed scoop method must be used. The use of needle nipping devices is prohibited and the devices must be discarded as infectious waste.
d. Use a separate pan of disinfectant for reusable syringes and needles. Do not place them in pans containing pipettes or other glassware in order to eliminate sorting later.
e. Used disposable needles and syringes must be placed in appropriate sharps disposal container immediately after use and discarded as infectious waste. (See Section IV-F for more information).

3. Safe and Effective Use of Biosafety Cabinets

In general:

- Make sure your BSC is certified when it is installed or after it is moved, and annually thereafter. (For information on cabinet certification call OARS at 373-2769). Check the magnehelic gauge regularly for an indication of a problem.
- Understand how your cabinet works.
- Do not disrupt the protective airflow pattern of the BSC. Such things as rapidly moving your arms in and out of the cabinet, people walking rapidly behind you, and open lab doors may disrupt the airflow pattern and reduce the effectiveness of the BSC.
- Plan your work.
- Minimize the storage of materials in and around the BSC.
- Always leave the BSC running during use.

Operational directions:

- Before using, wipe work surface with 70% alcohol. Wipe off each item you need for your procedures and place in cabinet.
- DO NOT place objects over the front air intake grille. DO NOT block the rear exhaust grille.
- Segregate contaminated and clean items. Work from “clean to dirty”.
- Place a pan with disinfectant and/or a sharps container inside the BSC for pipette discard. DO NOT use vertical pipette discard canisters on the floor outside cabinet.
- It is not necessary to flame items. This creates turbulence in airflow and will compromise sterility; heat buildup may damage the HEPA filters of the BSC.
- Move arms slowly when removing or introducing new items into the BSC in a straight backwards movement.
- If you use a piece of equipment that creates air turbulence in the BSC (such as a centrifuge, blender) place equipment in the back 1/3 of the cabinet; stop other work while equipment is operating.
- Protect the building vacuum system from biohazards by placing a cartridge filter between the vacuum trap and the source valve in the cabinet.
- Clean up all spills in the cabinet immediately. Wait 10 minutes before resuming work.

- When work is finished, remove all materials and wipe all interior surfaces with the appropriate disinfectant.
- Remove lab coat and wash hands thoroughly before leaving laboratory.

4. Cryostats

Frozen sections on unfixed human tissue or animal tissue infected with an etiologic agent pose a risk because accidents can occur. Freezing tissue does not necessarily inactivate infectious agents. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material. Gloves should be worn during preparation of frozen sections. When working with biohazardous material in a cryostat, the following is recommended:

- Consider the contents of the cryostat to be contaminated and decontaminate it frequently with 70% ethanol or appropriate disinfectant.
- Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.
- Defrost and decontaminate the cryostat with a tuberculocidal hospital disinfectant once a week and immediately after tissue known to contain bloodborne pathogens, *M. tuberculosis* or other infectious agents is cut.
- Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
- Consider solutions for staining potentially infected frozen sections to be contaminated.

5. Centrifuge Equipment

Hazards associated with centrifuging include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users should be properly trained and operating instructions that include safety precautions should be prominently posted on the unit.

Aerosols are created by practices such as filling centrifuge tubes, removing plugs or caps from tubes after centrifugation, removing supernatant, and resuspending sedimented pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation. To minimize the generation of aerosols when centrifuging biohazardous material, the following procedures should be followed:

- Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Fill and open centrifuge tubes, rotors and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
- Add disinfectant to the space between the tube and the bucket to disinfect material in the event of breakage during centrifugation.

- Always balance buckets, tubes and rotors properly before centrifugation.
- Do not decant or pour off supernatant. Use a 2-flask vacuum system with appropriate in-line reservoirs and filters (See Figure 5 at the end of this document).
- Work in a BSC when resuspending sedimented material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
- Small low-speed centrifuges may be placed in a BSC during use to reduce the aerosol escape. High-speed centrifuges pose additional hazards. Precautions should be taken to filter the exhaust air from vacuum lines, to avoid metal fatiguing resulting in disintegration of rotors and to use proper cleaning techniques and centrifuge components. Manufacturers' recommendations must be meticulously followed to avoid metal fatigue, distortion and corrosion.
- Avoid the use of celluloid (cellulose nitrate) tubes with biohazardous materials. Celluloid centrifuge tubes are highly flammable and prone to shrinkage with age. They distort on boiling and can be highly explosive in an autoclave. If celluloid tubes must be used, an appropriate chemical disinfectant must be used to decontaminate them.

6. Personal Protective Equipment (PPE)

PPE is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may also protect the experiment from contamination. PPE must be provided without cost to personnel. The following PPE is recommended for regular use:

a. Face Protection

Goggles or safety glasses with solid side shields in combination with masks, or chin length face shields or other splatter guards are required for anticipated splashes, sprays or splatters of infectious or other hazardous materials to the face. Information on the availability of low cost prescription safety eyewear may be obtained by calling OARS at 373-2769. Wearing of contact lenses is inappropriate in the laboratory setting.

b. Laboratory Clothing

This category includes: laboratory coats, smocks, scrub suits, and gowns. Long sleeved garments should be used to minimize the contamination of skin or street clothes and to reduce shedding of microorganisms from the arms. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect clothing from contamination. If the garment is not disposable, it must be capable of withstanding sterilization, in the event it becomes contaminated. Additional criteria for selecting clothing are: comfort, appearance, closure types and location, antistatic properties and durability. Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas. Disposables should be available for visitors, maintenance and service workers in the event it is required. All protective clothing should be either discarded in the laboratory or laundered by the facility. Personnel must not launder laboratory clothing at home.

c. Gloves

These must be selected based on the hazards involved and the activity to be conducted. Gloves must be worn when working with biohazards, toxics and other physically hazardous agents. Temperature resistant gloves must be worn when handling hot material or dry ice. Delicate work requiring a high degree of precision

dictates the use of thin walled gloves. Protection from contact with toxic or corrosive chemicals may also be required. For assistance in glove selection, call OARS at 373-2769.

When working with hazardous materials, the lower sleeve and the cuff of the laboratory garment should be overlapped by the glove. A long-sleeved glove or disposable arm-shield may be worn for further protection of the garment.

In some instances, double gloving may be appropriate. If a spill occurs, hands will be protected after the contaminated outer gloves are removed. Gloves must be disposed of when contaminated, removed when work with infectious materials is completed and not worn outside the laboratory. Disposable gloves must not be washed or reused.

d. Respirators.
In certain instances, additional PPE may be required. Respirator selection is based on the hazard and the protection factor required. Personnel who require respiratory protection must contact OARS for assistance in selection of equipment and training in its proper usage. Contact OARS for assistance in selection of other personal protective equipment as well.

7. Blenders, Ultrasonic Disrupters, Grinders and Lyophilizers
The use of any of these devices results in considerable aerosol production. Blending, cell-disrupting and grinding equipment should be used in a BSC when working with biohazardous materials.

Safety blenders, although expensive, are designed to prevent leakage from the bottom of the blender jar, provide a cooling jacket to avoid biological inactivation and to withstand sterilization by autoclaving. If blender rotors are not leak-proof, they should be tested with sterile saline or dye solution prior to use with biohazardous material. The use of glass blender jars is not recommended because of the breakage potential. If they must be used, glass jars should be covered with a polypropylene jar to prevent spraying of glass and contents in the event the blender jar breaks. A towel moistened with disinfectant should be placed over the top of the blender during use. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle. The device should be decontaminated promptly after use.

Lyophilizers and ampoules. Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material should be loaded in a BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized and vapor traps should be used wherever possible.

Opening ampoules containing liquid or lyophilized culture material should be performed in a BSC to control the aerosol produced. Gloves must be worn. To open, nick the neck of the ampoule with a file, wrap it in disinfectant soaked towel, hold the ampoule upright and snap it open at the nick. Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the contents without bubbling and withdraw it into a fresh container. Discard the towel and ampoule top and bottom as infectious waste (See Section IV-F for more information).

Ampoules used to store biohazardous material in liquid nitrogen have exploded causing eye injuries. The use of polypropylene tubes eliminates this hazard. These tubes are available dust-free or pre-sterilized and are fitted with polyethylene caps with silicone washers. Heat sealable polypropylene tubes are also available.

8. Loop sterilizers and Bunsen burners

Sterilization of inoculating loops or needles in an open flame generates small-particle aerosols which may contain viable microorganisms. The use of a shielded electric incinerator minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work where electric incinerators or gas flames are not available. The loops are semiquantitative and can be used for counting bacteria.

Continuous flame gas burners should not be used in BSCs. These burners can produce turbulence which disturbs the protective airflow patterns of the cabinet. Additionally, the heat produced by the continuous flame may damage the HEPA filter. If a gas burner must be used, one with a pilot light should be selected.

9. Laundry

All personal protective clothing must be cleaned, laundered and disposed of by the employer at no cost to employees. Apparel contaminated with blood or other potentially infectious materials should be handled as little as possible and decontaminated, preferably by autoclaving, before being sent to the laundry for cleaning. Appropriate PPE must be worn by employees who handle contaminated laundry.

10. Housekeeping

Good housekeeping in laboratories is essential to reduce risks and protect the integrity of biological experiments. Routine housekeeping must be relied upon to provide work areas free of significant sources of contamination. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected.

Laboratory personnel are responsible to clean laboratory benches, equipment and areas that require specialized technical knowledge. Additional laboratory housekeeping concerns include:

- Keeping the laboratory neat and free of clutter - surfaces should be clean and free of infrequently used chemicals, glassware and equipment. Access to sinks, eyewashes, emergency showers and fire extinguishers must not be blocked.
- Proper disposal of chemicals and wastes - old and unused chemicals should be disposed of promptly and properly. Call OARS at 373-2769 for details.
- Providing a workplace that is free of physical hazards - aisles and corridors should be free of tripping hazards. Attention should be paid to electrical safety, especially as it relates to the use of extension cords, proper grounding of equipment, avoidance of overloaded electrical circuits and avoidance of the creation of electrical hazards in wet areas.
- Removing unnecessary items on floors, under benches or in corners.
- Properly securing all compressed gas cylinders.
- Never using fume hoods for storage of chemicals or other materials.

Practical custodial concerns include:

- Dry sweeping and dusting which may lead to the formation of aerosols is not permitted.
- The usual wet or dry industrial type vacuum cleaner is a potent aerosol generator and, unless equipped with high efficiency particulate air (HEPA) filter, must not be used in the biological research laboratory. Their use is prohibited to protect personnel as well as the integrity of the experiment. Wet and dry units with HEPA filters on the exhaust are available from a number of manufacturers.

11. Biohazard Spill Clean-up Procedures

The following procedures are provided as a guideline to biohazardous spill cleanup.

a. Inside the BSC:

- Wear lab coat, safety glasses and gloves during cleanup.
- Allow cabinet to run during cleanup.
- Apply disinfectant and allow a minimum of 20 minutes contact time.
- Wipe up spillage with disposable disinfectant-soaked cloth.
- Wipe the walls, work surface and any equipment in the cabinet with a disinfectant-soaked cloth.
- Discard contaminated disposable materials in appropriate biohazardous waste container(s) and autoclave before discarding as infectious waste.
- Place contaminated reusable items in biohazard bags, autoclavable pans with lids or wrap in newspaper before autoclaving and cleanup.
- Expose non-autoclavable materials to disinfectant, 20 minute contact time, before removal from the BSC.
- Remove protective clothing used during cleanup and place in a biohazard bag for autoclaving.
- Run cabinet 10 minutes after cleanup before resuming work or turning cabinet off.

b. In the lab, outside the BSC:

1. Clear area of all personnel.
2. Label all doors with 'DO NOT ENTER' signs to prevent personnel from entering.
3. Notify NU OARS for any spill outside of biosafety cabinet or other primary containment. NU OARS will consider the extent of contamination within the laboratory prior to decontaminating your lab. If NU OARS determines researchers can clean up the biohazard spill themselves continue as follows:
4. Wait for aerosol to settle before entering spill area, at least 30 minutes.
5. Remove any contaminated clothing and place in biohazard bag to be autoclaved.
6. Wear a disposable gown or lab coat, goggles and two pair of gloves during cleanup.
7. Place paper towels over spill and spray with disinfectant.
8. Encircle the spill with additional disinfectant being careful to minimize aerosolization while assuring adequate contact.

9. Decontaminate all items within the spill area.
10. Allow 20 minutes contact time to ensure germicidal action of disinfectant.
11. Use tongs or forceps for collecting debris and place in biohazard waste container.
12. Wipe equipment with freshly prepared 1:10 bleach followed by water than 70% alcohol.
13. Place disposable contaminated spill materials in appropriate biohazardous waste container(s) for autoclaving.
14. Place contaminated reusable items in biohazard bags, autoclavable pans with lids before autoclaving and cleanup.
15. Remove PPE used during cleanup and place in a biohazard bag for autoclaving.
16. Wash your hands.

c. Inside Centrifuge

- Clear area of all personnel. Wait 30 minutes for aerosol to settle before attempting to clean up spill.
- Wear a lab coat, safety glasses and gloves during cleanup.
- Remove rotors and buckets to nearest BSC for cleanup.
- Thoroughly disinfect inside of centrifuge.
- Remove contaminated debris with tongs or forceps after disinfection, place in appropriate biohazardous waste container(s) and autoclave before disposal as infectious waste.

d. Outside lab, during transport

- The transport of biohazardous material should occur in two leak-proof containers containing of an unbreakable well-sealed primary container placed inside of a second unbreakable leak-proof sealed container labeled with a biohazard symbol.
- Should a spill occur in a public area, evacuate immediately the area for at least 30 minutes and notify NU OARS. NU OARS will determine the appropriate spill response. Do not attempt to clean it up without appropriate personal protective equipment.
- As an interim measure, wear gloves and place paper towels, preferable soaked in disinfectant, directly on spilled materials to prevent spread of contamination. To assure adequate contact, surround the spill with disinfectant, if available, taking care to minimize aerosols.
- Call OARS at 373-2769 to assist in cleanup.

Standard Operating Procedures (SOPs) on certain laboratory tasks can be found on NU OARS website, a link is provided in Appendix E at the end of this document to assist PIs when filling out their Biological registration in BioRAFT.

D. Emergency Procedures

If an exposure to biohazardous materials occurs, the following procedure will be followed: Remove PPE (such as gloves or face protection) before using the eye wash or washing hands. For wounds, skin, and needle

stick exposure: Thoroughly scrub the area for 15 minutes with warm water and soap. For eye and mucous membrane exposure: Flush immediately at nearest eyewash station for 15 minutes. Seek care immediately. Use the eye wash immediately outside the lab in the event that this exposure was related to a spill or aerosol creating event inside the lab. While you are treating your exposure area, have a co-worker contact NU's campus police for help. Campus police will triage you and get you to a medical care facility for further treatment if necessary. Be prepared to provide information for the healthcare providers. Bring information about the agent and/or animal or material involved in your injury or exposure. This information should include agent description, route of exposure, dose and concentration, and any unusual characteristics of the agent. Notify your PI/supervisor and NU's OARS immediately. Submit an injury report form after you have sought medical evaluation.

E. Decontamination

Decontamination is a term used to describe a process or treatment that renders a medical device, instrument, or environmental surface safe to handle. A decontamination procedure can range from sterilization to simple cleaning with soap and water. Sterilization, disinfection and antisepsis are all forms of decontamination.

Sterilization is the use of a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores.

Disinfection eliminates virtually all pathogenic non-spore forming microorganisms but not necessarily all microbial forms on inanimate objects (work surfaces, equipment, etc.). Effectiveness is influenced by the kinds and numbers of organisms, the amount of organic matter, the object to be disinfected and chemical exposure time, temperature and concentration.

Antisepsis is the application of a liquid antimicrobial chemical to skin or living tissue to inhibit or destroy microorganisms. It includes swabbing an injection site on a person or animal and hand washing with germicidal solutions. Although some chemicals may be utilized as either a disinfectant or an antiseptic, adequacy for one application does not guarantee adequacy for the other. Manufacturers' recommendations for appropriate use of germicides should always be followed.

1. General Procedures

a. All infectious materials and all contaminated equipment or apparatus should be decontaminated before being washed, stored or discarded. Autoclaving is the preferred method. Each individual working with biohazardous material should be responsible for its proper handling.

b. Biohazardous materials should not be placed in autoclaves overnight in anticipation of autoclaving the next day.

c. Autoclaves should not be operated unattended or by untrained personnel.

d. Special precautions should be taken to prevent accidental removal of material from an autoclave before it has been sterilized or simultaneous opening of both doors on a double door autoclave.

e. Dry hypochlorites, or any other strong oxidizing material, must not be autoclaved with organic materials such as paper, cloth or oil:
OXIDIZER + ORGANIC MATERIAL + HEAT = MAY PRODUCE AN EXPLOSION.

2. Methods

There are four main categories of physical and chemical means of decontamination. They are heat, liquid disinfection, vapors and gases and radiation. Each category is discussed briefly below.

a. Heat

1. **Wet heat** is the most dependable method of sterilization. Autoclaving (saturated steam under pressure of approximately 15 psi to achieve a chamber temperature of at least 250° F for a prescribed time) is the most convenient method of rapidly achieving destruction of all forms of microbial life. In addition to proper temperature and time, prevention of entrapment of air is critical to achieving sterility. Material to be sterilized must come in contact with steam and heat. Chemical indicators, e.g. autoclave tape, must be used with each load placed in the autoclave. The use of autoclave tape alone is not an adequate monitor of efficacy. Autoclave sterility monitoring should be conducted on a regular basis using appropriate biological indicators (B. stearothermophilus spore strips) placed at locations throughout the autoclave. The spores, which can survive 250° F for 5 minutes but are killed at 250° F in 13 minutes, are more resistant to heat than most, thereby providing an adequate safety margin when validating decontamination procedures. Each type of container employed should be spore tested because efficacy varies with the load, fluid volume, etc.

2. **Dry heat** is less efficient than wet heat and requires longer times and/or higher temperatures to achieve sterilization. It is suitable for the destruction of viable organisms on impermeable nonorganic surfaces such as glass, but it is not reliable in the presence of shallow layers of organic or inorganic materials which may act as insulation. Sterilization of glassware by dry heat can usually be accomplished at 160-170° C for periods of 2-4 hours. Dry heat sterilizers should be monitored on a regular basis using appropriate biological indicators [*B. subtilis (globigii)* spore strips].

3. **Incineration** is another effective means of decontamination by heat. As a disposal method incineration has the advantage of reducing the volume of the material prior to its final disposal.

b. Liquid disinfection

The most practical use of liquid disinfectants is for surface decontamination and, when used in sufficient concentration, as a decontaminate for liquid wastes prior to final disposal in the sanitary sewer. If liquid disinfectants are used, they must have been shown to be effective against the organism(s) present.

Liquid disinfectants are available under a wide variety of trade names. In general, these can be classified as: halogens, acids, alkalis, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols and amines. The more active a compound is, the more likely it is to have undesirable characteristics such as corrosivity. No liquid disinfectant is equally useful or effective under all conditions and for all viable agents. Properties of common disinfectants may be found in Appendix D (a link to the NU OARS website is provided at the end of this document). The activity levels of selected liquid chemical disinfectants are listed in Table 2 at the end of this document.

c. Vapors and Gases

A variety of vapors and gases possess decontamination properties. Vapors and gases are primarily used to decontaminate BSC and associated systems, bulky or stationary equipment not suited to liquid disinfectants, instruments or optics which might be damaged by other decontamination methods, and rooms, buildings and associated air-handling systems. Agents included in this category are glutaraldehyde and formaldehyde vapor, ethylene oxide gas, peracetic acid and hydrogen peroxide vapor. When used in closed systems and under controlled conditions of temperature and humidity, excellent disinfection can be obtained. Great care

must be taken during use because of the hazardous nature of many of these compounds. Contact OARS for monitoring requirements if these compounds are to be used.

d. Radiation

Although ionizing radiation will destroy microorganisms, it is not a practical tool for laboratory use. Nonionizing radiation in the form of ultraviolet radiation (UV) is used for inactivating viruses, bacteria and fungi. It will destroy airborne microorganisms and inactivate microorganisms on exposed surfaces or in the presence of products of unstable composition that cannot be treated by conventional means.

Because of the low penetrating power of UV, microorganisms inside dust or soil particles will be protected from its action, limiting its usefulness. UV is used in air locks, animal holding areas, ventilated cabinets and laboratory rooms to reduce levels of airborne microorganisms and maintain good air hygiene. Because UV can cause burns to the eyes and skin of people exposed for even a short period of time, proper shielding should be maintained when it is in use. UV lamps that are used for space decontamination should be interlocked with the general room or cabinet illumination, so that turning on the lights extinguishes the UV.

UV lamps are not recommended for decontamination unless they are properly maintained. Because UV lamp intensity or destructive power decreases with time, it should be checked with a UV meter yearly. Frequent cleaning every few weeks is necessary to prevent accumulation of dust and dirt on the lamp which also reduces its effectiveness drastically. If UV must be used, it should be used when areas are not occupied.

F. Infectious Waste Management

1. Categories of infectious waste as defined by the Commonwealth of Massachusetts, Department of Public Health include:

a. Blood and blood products: discarded bulk human blood products in free draining, liquid state; body fluids contaminated with visible blood; and materials saturated/dripping with blood.

b. Pathological waste: human anatomical parts, organs, tissues, and body fluids removed and discarded during surgery or autopsy, or other medical procedures and specimens of body fluids and their containers.

c. Culture and stocks of infectious agents and associated biologicals: all discarded cultures and stocks of infectious agents and associated biologicals, biotechnological by-product effluents (any discarded preparations made from genetically altered living organisms and their products), cultures of specimens from medical and pathological laboratories, cultures and stocks of infectious agents from research laboratories, wastes from the production of biologicals, and discarded live attenuated vaccines intended for human use.

d. Contaminated animal carcasses, body parts and bedding: the contaminated carcasses and body parts and bedding of all research animals known to be exposed to pathogens.

e. Sharps: discarded medical articles that may cause puncture or cuts, including but not limited to all used and discarded hypodermic needles and syringes, Pasteur pipettes, broken medical glassware, scalpel blades, disposable razors, and suture needles.

2. Handling

All infectious waste from University laboratories must be autoclaved by the generator prior to disposal in appropriate infectious waste bags with labels. Treatment of infectious waste, other than by autoclaving, must be reviewed by the Office of Academic and Research Safety.

The primary responsibility for identifying and disposing of infectious material rests with PIs or laboratory supervisors. This responsibility cannot be shifted to inexperienced or untrained personnel.

Potentially infectious and biohazardous waste must be separated from general waste at the point of generation (i.e., the point at which the material becomes a waste) by the generator into the following three classes as follows:

a. Used Sharps
b. Autoclavable Material
c. Non-Autoclavable Material for Incineration

Used sharps must be segregated into sharps containers that are non-breakable, leakproof, impervious to moisture, rigid, tightly lidded, puncture resistant, red in color and marked with the universal biohazard symbol.

Fluids in volumes greater than 20 cc that are discarded as infectious waste must be segregated in containers that are leakproof, impervious to moisture, break-resistant, tightly lidded or stoppered, red in color and marked with the universal biohazard symbol. To minimize the burden of this waste category, fluids in volumes greater than 20 cc, may be decontaminated (by autoclaving or exposure to an appropriate disinfectant), then flushed into the sanitary sewer system. The pouring of these wastes must be accompanied by large amounts of water. The empty fluid container may be discarded with other infectious waste if it is disposable or autoclaved and washed if reusable.

Other infectious waste must be discarded directly into containers or plastic (polypropylene) autoclave bags which are clearly identifiable and distinguishable from general waste. Containers must be marked with the universal biohazard symbol (Figure 1; at the end of this document). Autoclave bags must be distinctly colored red or orange, and marked with the universal biohazard symbol. These bags must not be used for any other materials or purpose.

Decontaminated infectious waste must be put into black plastic bags and labeled appropriately. Infectious waste must be properly packaged prior to offsite transport for destruction and disposal.

For specific information on infectious waste disposal procedures and pickup locations in your facility, call OARS. Any off-site treatment of infectious waste must be coordinated through OARS (373-2769).

3. Mixed Waste

Provisions must be made for potentially infectious waste with multiple hazards, e.g., radioactive material contaminated wastes or wastes substantially contaminated with toxic/carcinogenic compounds. Contact OARS regarding the disposal of these wastes.

4. Storage

Infectious waste must not be allowed to accumulate. Contaminated material should be inactivated and disposed of daily or on a regular basis as required. If the storage of contaminated material is necessary, it must be done in a rigid container away from general traffic.

Infectious waste, excluding used sharps, may be stored at room temperature until the storage container is full, but no longer than 30 days from the date of generation. It may be refrigerated for up to 30 days and frozen for up to 90 days from the date of generation. Infectious waste must be dated when refrigerated or frozen for storage. Storage of infectious waste in a freezer must be approved by NU OARS.

If infectious waste becomes putrescent during storage it must be moved off site within 24 hours for processing and disposal.

Sharps containers may be used until 3/4 full, at which time they must be disposed of as infectious waste.

5. Monitoring Treatment of Infectious Waste

Autoclaving of infectious waste should be monitored to assure the efficacy of the treatment method (See section IV-F and Section IV-D2). A log noting the date, test conditions and the results of each test of the autoclave must be kept.

6. Animals

University Laboratory Animal Resources must approve the disposal of research animals and animal parts that are considered to be infectious waste.

G. Packaging and Shipping of Biomedical Materials

Etiologic agents, infectious materials and vectors that may contain them are recognized by the federal government and state government as hazardous materials. Infectious materials are regularly transported from one location to another by common land and air carriers. Containers of infectious materials must be carefully packaged to prevent leakage or breakage and consequent exposure to package contents. Packaging instructions are provided below.

Packaging and shipping of biomedical material must meet federal requirements. Regulations governing the interstate shipment of etiologic agents are currently under revision. The shipper (i.e., person with direct knowledge of what is being shipped) must be acquainted with the most current requirements. It is the intent of the regulation that biomedical material which may contain etiologic agents will be packaged and shipped in such a way that the contents will not leak and will arrive in good condition. The following definitions apply:

Biomedical materials that are known to contain or could contain, etiologic agents are divided into two groups: “diagnostic specimens and biological products” and “materials containing certain etiologic agents”.

Etiologic agents are those viable microorganisms that cause disease in humans and include bacteria, bacterial toxins, viruses, fungi, rickettsia, protozoans and parasites. These disease-causing microorganisms may also be referred to as infectious agents or infectious substances.

Infectious substances are those substances containing viable microorganisms or their toxins which are known, or are suspected to cause disease in animals or humans.

Diagnostic specimens are any human or animal material including but not limited to, excreta, secreta, blood and its components, tissue, tissue fluids, etc., which the shipper reasonably believes may contain an etiologic agent and that is being shipped for purposes of diagnosis.

Biological product means a product prepared in accordance with regulations that govern the manufacture of vaccines, reagents, etc.

Materials containing certain etiologic agents means materials known to contain or reasonably believed by the shipper to contain an etiologic agent from a list included in the regulation. The list contains most of the RG 2, 3 and 4 agents but any etiologic agent should be handled according to the regulation even if it is not on the list. Patient specimens that are expected to contain an etiologic agent should be shipped according to these requirements.

Interstate shipping is interpreted to include intrastate shipping.

Packaging of diagnostic specimens and biological products should be such that the package will withstand leakage of contents, shocks, pressure changes and other conditions incident to ordinary handling in transportation. Contents should not leak to the outside of the shipping container, even if leakage of the primary container occurs. Packages should be able to withstand rough handling and passage through cancellation machines, sorters, conveyers, etc.

Packaging of materials containing etiologic agents varies depending on the volume shipped.

For volumes not exceeding 50 ml:
The material to be shipped must be placed in a securely closed, watertight primary container. The primary container must be placed in a durable, watertight secondary container. Several primary containers may be placed in a single secondary container, so long as the total contents does not exceed 50 ml. Absorbent material must be placed in the spaces between the primary and secondary containers, so that there is enough absorbent to absorb the entire contents of the primary container(s) should breakage or leakage occur. Each set of primary and secondary containers must be placed in an outer shipping container constructed of corrugated fiberboard, cardboard, wood or other material of equivalent strength. (Most bags and envelopes are not acceptable). See Figure 2 at the end of this document.

For volumes greater than 50 ml:
Packaging of these larger volumes must comply with the above-mentioned requirements. In addition, shock absorbent material in volume at least equal to that of the absorbent material must be placed between the secondary container and the outer shipping container. Single primary containers must not contain more than one liter of material. However, two or more primary containers, whose volumes do not exceed one liter may be placed in a single secondary container. The maximum amount of etiologic agent that may be enclosed within a single outer shipping container may not exceed four liters.

If dry ice is used, it must be placed between the secondary container(s) and the outer shipping container and the shock absorbent material placed so that the secondary container(s) do not become loose within the outer shipping container as the dry ice sublimates.

A special label, illustrated in Figure 2 and 4 at the end of this document, must be placed on the outer shipping container. This label identifies the package as containing etiologic agents and directs anyone observing damage to the package or leakage of its contents to call CDC.

Certain etiologic agents require special handling in addition to that stated above. Most of these agents are in RG 3 and RG 4. They must be shipped by registered mail or an equivalent system which requires or provides for sending notification of receipt to the sender immediately upon delivery. When this notice of receipt is not received within 5 days following anticipated delivery the sender must notify CDC.

Questions pertaining to proper shipping and packaging of etiologic agents should be directed to NU OARS at 617-73-2769 or the Centers for Disease Control and Prevention, Office of Health and Safety at (404) 639-3883.

For shipments made internationally or domestically by carriers such as FEDEX, the International Air Transport Association (IATA) Dangerous Goods Regulations must be followed. Appropriate labels must be applied to the outer shipping container for packages that contain dry ice and/or infectious substances as shown in Figure 3 and Figure 4 at the end of this document respectively. More information on additional packaging and labeling requirements may be obtained by contacting the specific carrier's dangerous goods agent prior to shipment.

H. Importation/Exportation of Etiologic Agents

Importation of infectious materials, etiologic agents and vectors that may contain them is governed by federal regulation. In general, an import permit is required for any infectious agent known to cause disease in man. This includes but is not limited to bacteria, viruses, rickettsia, parasites, yeasts and molds. In some instances, an agent which is suspected of causing human disease also requires a permit.

The following vectors require import permits:

- Animals known or suspected of being infected with any disease transmissible to man. Importation of turtles less than 4 inches in shell length and all nonhuman primates requires an importation permit issued by the United States Public Health Service (USPHS) Division of Quarantine, (617)-561-2837.

- Biological materials: Unsterilized specimens of human and animal tissue (including blood), body discharges, fluids, excretions or similar material, when known or suspected to be infected with disease transmissible to man.

- Insects: Any living insect or other living arthropod, known or suspected of being infected with any disease transmissible to man. Also, if alive, any fleas, flies, lice, mites, mosquitoes or ticks, even if uninfected. This includes eggs, larvae, pupae, and nymphs as well as adult forms.

- Snails: Any snails capable of transmitting schistosomiasis. No mollusks are to be admitted without a permit from either CDC or the Department of Agriculture (see below for phone numbers). Any shipment of mollusks with a permit from either agency will be cleared immediately.

- Bats: All live bats. Bats may also require a permit from the U. S. Department of the Interior, Fish and Wildlife Services (USDI; see below for phone number).

When an etiologic agent, infectious material or vector containing an infectious agent is being imported to the United States it must be accompanied by an importation permit issued by the USPHS. Importation permits are issued only to the importer, who must be located in the United States. The importation permit, with the proper packaging and labeling, will expedite clearance of the package of infectious materials through the USPHS Division of Quarantine and release by U.S. Customs.

Shipping labels containing the universal biohazard symbol, the address of the importer, the permit number and the expiration date are issued to the importer with the permit. The importer must send the labels and one or more copies of the permit to the shipper. The permit and labels inform the U. S. Customs Service and the U.S. Division of Quarantine personnel of the package contents.

The importer bears responsibility for assuring that the foreign shipping personnel pack and label the infectious materials according to USPHS regulations. Transfers of previously imported material within the United States also require a permit.

Instead of an importation permit, a "Letter of Authorization" may be issued by the issuing officer after review of an "Application to Import an Etiological Agent". The letter is issued for materials that are judged to be noninfectious, but which might be construed to be infectious by U. S. Customs inspection personnel. Letters of Authorization may be issued for items such as formalin fixed tissues, sterile cell cultures, clinical materials such as human blood, serum, plasma, urine cerebrospinal fluid, and other tissues or materials of human origin when there is no evidence or indication that such materials contain an infectious agent. Letters of Authorization are in effect for two years, and do not require a shipping label to be issued by CDC.

Etiologic agents, infectious materials and vectors that may contain them must be carefully packaged to prevent leakage or breakage and consequent exposure to the package contents. The package must be labeled with the universal biohazard sign to warn package handlers of the hazardous contents. (See Section IV-F regarding packaging instructions.)

Importation permits and Letters of Authorization are issued by the Biosafety Branch, Office of Health and Safety, CDC, after review of a completed application form. Application forms may be obtained directly from OARS (898-4453) or by calling CDC at (404)-718-2077.Completed forms may be returned to CDC by mail or FAX. Application to CDC for the importation permit should be made 10 working days in advance of the shipment date to allow time for processing, issuance and delivery of the permit and shipping labels to the permittee.

Other permits:
U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) permits are required for infectious agents of livestock and biological materials containing animal, particularly livestock, material. Tissue (cell) culture techniques customarily use bovine material as a stimulant for cell growth. Tissue culture materials, and suspensions of cell culture grown viruses or other etiologic agents containing growth stimulants of bovine or other livestock origin are, therefore, controlled by the USDA due to the potential risk of introduction of exotic animal disease into the U. S. Applications for USDA/APHIS permits may be

obtained from OARS (373-2769). Further information may be obtained by calling the USDA/APHIS at (508)-829-4477.

USDI permits are required for certain live animals and all live bats. Call (800)358-2104 for further information.

Export of infectious materials may require a license from the Department of Commerce. Call (978)-526-1531 further information.

Appendix

Table 1: Summary of Laboratory Biosafety levels (BSLs)

From CDC/NIH BMBL, 6th edition, June 2020

BSL	Agents	Special Practices[a]	Primary Barrier and Personal Protective Equipment[a]	Facilities (Secondary Barriers)[a]
1	Well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment	Standard microbiological practices	No primary barriers required; protective laboratory clothing; protective face, eyewear, as needed	Laboratory doors; sink for handwashing; laboratory bench; windows fitted with screens; lighting adequate for all activities
2	Agents associated with human disease and pose moderate hazards to personnel and the environment	Limited access; occupational medical services including medical evaluation, surveillance, and treatment, as appropriate; all procedures that may generate an aerosol or splash conducted in a BSC; decontamination process needed for laboratory equipment	BSCs or other primary containment device used for manipulations of agents that may cause splashes or aerosols; protective laboratory clothing; other PPE, including respiratory protection, as needed	Self-closing doors; sink located near exit; windows sealed or fitted with screens; autoclave available
3	Indigenous or exotic agents; may cause serious or potentially lethal disease through the inhalation route of exposure	Access limited to those with need to enter; viable material removed from laboratory in primary and secondary containers; opened only in BSL-3 or ABSL-3 laboratories; all procedures with infectious materials performed in a BSC	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls; two pairs of gloves, when appropriate; protective eyewear, respiratory protection, as needed	Physical separation from access corridors; access through two consecutive self-closing doors; hands-free sink near exit; windows are sealed; ducted air ventilation system with negative airflow into laboratory; autoclave available, preferably in laboratory.
4	exotic agents that pose high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that are frequently fatal, for which there are no vaccines or treatments; and related agents with unknown risk of transmission	Clothing change before entry; daily inspections of essential containment and life support systems; all wastes decontaminated prior to removal from laboratory; shower on exit	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls;[b] gloves;[b] full-body, air-supplied, positive-pressure suitc	Entry sequence; entry through airlock with airtight doors;[c] walls, floors, ceilings form sealed internal shell; dedicated, non-recirculating ventilation system required; double-door,

				pass-through autoclave required

Legend:

a. BSL contains the recommendations of the preceding level(s) and the criteria in the cell.

b. Applies to Cabinet Laboratory

c. Applies to Suit Laboratory

Table 2. Activity Levels of Selected Liquid Chemical Disinfectants

From CDC/NIH BMBL, 6th edition, June 2020

Chemical[a]	Concentration	Activity level
Glutaraldehyde	Variable	Sterilization
Glutaraldehyde	Variable	Intermediate to high-level disinfection
Ortho-phthalaldehyde (OPA)	0.55%	High-level disinfection
Hydrogen peroxide	6–30%	Sterilization
Hydrogen peroxide	3–6%	Intermediate to high-level disinfection
Formaldehyde[b]	6–8%	Sterilization
Formaldehyde	1–8%	Low- to high-level disinfection
Chlorine dioxide	Variable	Sterilization
Chlorine dioxide	Variable	High-level disinfection
Peracetic Acid	0.08%–0.23% with peroxide concentrations of 1–7.35%	Sterilization
Peracetic acid	Variable	High-level disinfection
Hypochlorites[c]	500–6000 mg/L Free available	Intermediate to high-level disinfection
Alcohols (ethyl, Isopropyl)[d]	70%	Intermediate-level disinfection
Phenolics	0.5–3%	Low- to intermediate-level disinfection
Iodophors[e]	30–50 mg/L Free	Low- to intermediate-level disinfection
Quaternary Ammonium Compounds	Variable	Low-level disinfection

Legend:

a. This list of chemical disinfectants centers on generic formulations. A large number of commercial products based on these generic components can be considered for use. Users should ensure that commercial formulations are registered with EPA or by the FDA. Users can search for EPA-registered products at https://www.epa.gov/pesticide-labels.
b. Because formaldehyde is classified as a known human carcinogen and has a low permissible exposure limit (PEL), the use of formaldehyde is limited to certain specific circumstances under carefully controlled conditions (e.g., for the disinfection of certain hemodialysis equipment). There are no FDA-cleared liquid chemical sterilant/disinfectants that contain formaldehyde.
c. Generic disinfectants containing chlorine are available in liquid or solid form (e.g., sodium or calcium hypochlorite). The indicated concentrations are rapid-acting and broad-spectrum (i.e., tuberculocidal, bactericidal, fungicidal, and virucidal). Note: Common household bleach is an excellent and inexpensive source of sodium hypochlorite. Concentrations between 500 and 1000 ppm chlorine are appropriate for the vast majority of uses requiring an intermediate-level of germicidal activity; higher concentrations are extremely corrosive as well as irritating to personnel, and their use should be limited to situations where there may be spores or there is an excessive amount of organic material or unusually high concentrations of microorganisms (e.g., spills of cultured material in the laboratory). In situations where there is an excessive amount of organic material present, the surfaces should be thoroughly cleaned to remove as much organic material as possible before applying sodium hypochlorite solution to disinfect the surface (see product label instructions). The concentration of the sodium hypochlorite should be determined in advance of use and the solution should be made fresh each day.

d. The effectiveness of alcohols as intermediate-level germicides is limited because they evaporate rapidly, resulting in short contact times, and because they lack the ability to penetrate residual organic material. They are rapidly tuberculocidal, bactericidal, and fungicidal, but may vary in spectrum of virucidal activity. Items to be disinfected with alcohols should be carefully pre-cleaned then totally submerged for an appropriate exposure time.
e. Only those iodophors registered with EPA as hard-surface disinfectants should be used, closely following the manufacturer's instructions regarding proper dilution and product stability. Antiseptic iodophors are not suitable to disinfect devices, environmental surfaces, or medical instruments.

Figure 1: Universal Biohazard symbol

Figure 2: Packaging of materials containing etiologic agents

From CDC/NIH BMBL, 6th edition, June 2020

The figure shows an example of the UN standard triple packaging system for materials known or suspected of being a Category A infectious substance as outlined in the Packaging Instruction of the IATA Dangerous Goods Regulations.[3] The package consists of a watertight primary receptacle or receptacles; a water-tight secondary packaging; and a rigid outer packaging of adequate strength for its capacity, mass, and intended use. Note that for liquid materials, the secondary packaging must contain absorbent material in sufficient quantities to absorb the entire contents of all primary receptacles. A list of contents must be located on or near the secondary packaging. Each surface of the external dimension of the packaging must be 100 mm (3.9 inches) or more. The completed package must pass specific performance tests, including a drop test and a water-spray test, and must be capable of withstanding, without leakage, an internal pressure producing a pressure differential of not less than 95 kPa (0.95 bar, 14 psi). The completed package must also be capable of withstanding, without leakage, temperatures in the range of -40ºC to +55ºC (-40ºF to 131ºF). The completed package must be marked "UN 2814, Infectious substance, affecting humans," or "UN 2900, Infectious substance, affecting animals," and labeled with a Division 6.2 (infectious substance) label. In addition, the package must be accompanied by appropriate shipping documentation, including a shipping paper and emergency response information.

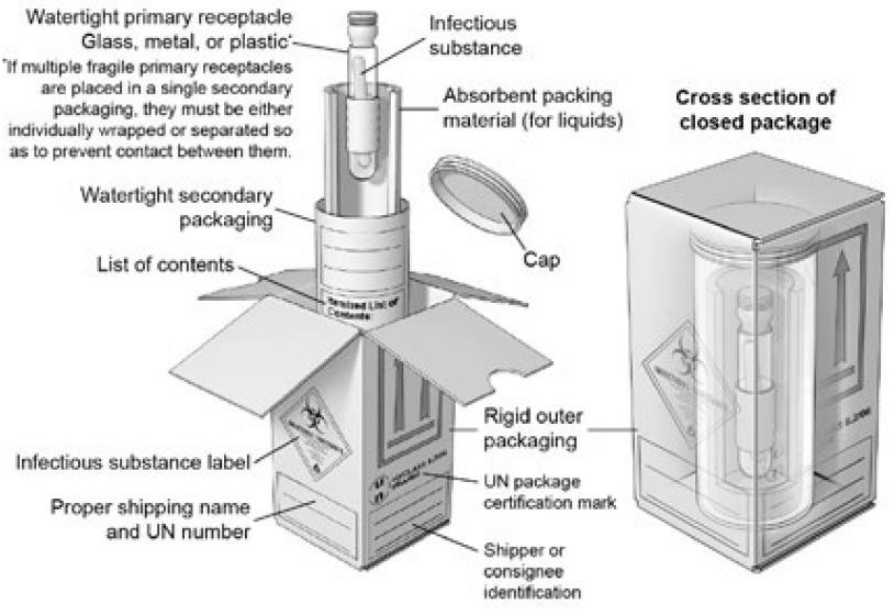

Figure 3: Dry Ice shipping label

A shipping package containing dry ice must be labeled with a dry ice label that identifies the Class 9 ('miscellaneous' hazards).

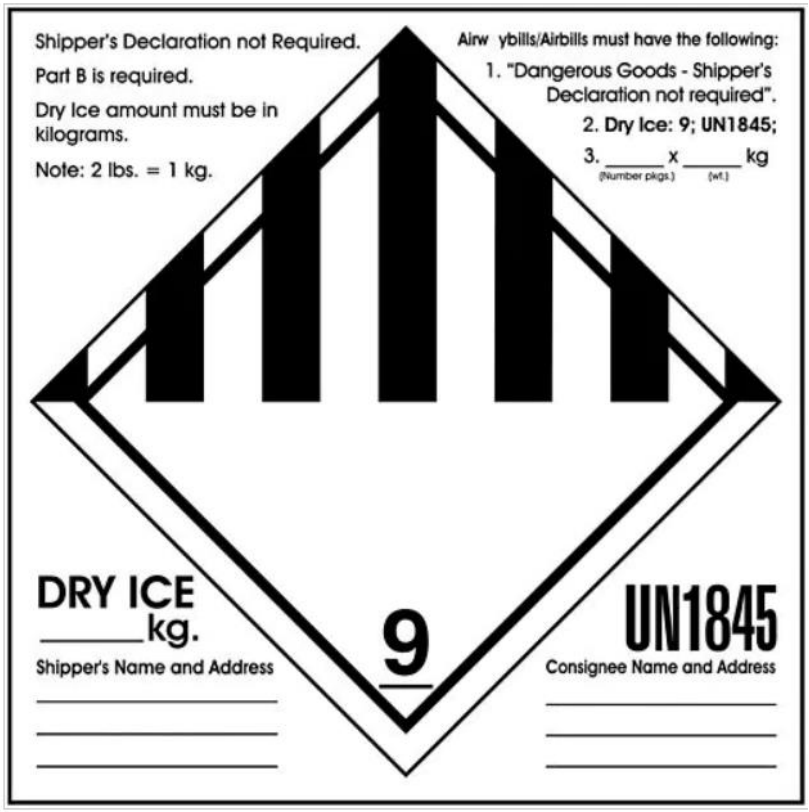

Figure 4: Infectious Substances shipping label

A shipping package containing infectious materials must be labeled with a Division 6.2 (infectious substance) label.

Figure 5: Protection of a house vacuum line

From CDC/NIH BMBL, 6th edition, June 2020

Example method to protect a house vacuum system during aspiration of infectious fluids. The suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask (B) serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms.

Appendix A:

A summary of classifications of biological agents according to risk (Risk Groups 1 to 4), as well as agent summary statements can be found under:
https://oars.northeastern.edu/home/biological-safety/

In addition, a Pathogen Safety Data Sheet for human pathogens such as Mycobacterium tuberculosis can be accessed through the following website:
https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/mycobacterium-tuberculosis-complex.html

Appendix B: Description of Biosafety Levels 1 to 3

A description of biosafety levels 1 to 3 can be found under:
https://oars.northeastern.edu/home/biological-safety/

Appendix C: Description of Animal Biosafety Levels 1-3

A description of animal biosafety levels 1 to 3 can be found on NU OARS website:
https://oars.northeastern.edu/home/biological-safety/

Appendix D: Table for disinfectant applications and characteristics

A table for disinfectant applications and characteristics can be found on NU OARS website:
https://oars.northeastern.edu/home/biological-safety/

Appendix E: Standard Operating Procedures

The NU IBC has created the following Standard Operating Procedures (SOP) to assist PIs in filling out the Biosafety Registration Form. You may customize the forms to your specific protocols. The list of available SOPs can be found on NU OARS website:
https://oars.northeastern.edu/home/biological-safety/