Environmental Health & Safety
MICHIGAN STATE UNIVERSITY

BIOSAFETY AND BIOSECURITY MANUAL

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Environmental Health & Safety
Michigan State University
4000 Collins Road, Room B20
Lansing, MI 48910
517-355-0153
ehs.msu.edu

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CONTACTS

EMERGENCY: Dial 911

Non – Emergency Medical Attention

Faculty, staff, and paid student employees

Lansing Urgent Care
www.lansingurgentcare.com

Non-employee students

Olin Student Health Center
http://olin.msu.edu/
(517) 353-5557
Or nearest emergency room or after – hours urgent care facility

Outside of the Lansing area:

Go to the nearest emergency room.

Assistance involving occupational, environmental, radiation, chemical and/or biological safety

Environmental Health & Safety (EHS)

ehs.msu.edu
Monday – Friday
8:00 am – 5:00 pm
(517) 355-0153

Nights and weekends

MSU Police
(517) 355-2221

FOREWORD

This biosafety manual has been developed by Environmental Health and Safety (EHS) Department at Michigan State University as part of MSU's biosafety and biosecurity program with the following goals:

- Protect personnel from exposure to biological materials and infectious agents
- Prevent environmental contamination
- Provide an environment for high quality research while maintaining a safe work – place
- Comply with applicable federal, state, and local requirements

The manual provides university-wide safety guidelines, policies and procedures for the use and manipulation of biohazards. The implementation of procedures is the responsibility of the Principal Investigator (PI), its success depends on the efforts of laboratory supervisors and employees. Planning for and implementation of biological safety is part of every laboratory activity in which biohazardous materials are used.

The handling and manipulation of biological agents and toxins, as well as recombinant or synthetic nucleic acid molecules, requires the use of precautionary measures that depend on the material(s) involved. This manual assists in the evaluation, containment, and control of biohazards. Risk assessments are performed and communicated with all involved in the work. It is imperative that all parties involved or working with these materials seek additional advice and training when necessary. EHS is available at MSU to assist.

UPDATES THIS YEAR INCLUDE:

- Adding “Biosecurity” to the title, in alignment with increased biosecurity importance by regulators.
- Removal of biohazardous waste handling information as this is covered in the MSU Biohazardous and Medical Waste Program guide available on the EHS website at www.ehs.msu.edu.
- Changes to the wording to be consistent with the BMBL using more definitive language, removing “should”, “would”, “may”, “could”, etc.
- Long hair, ties, or long sleeves are secured.
- Lab coats are required for all work at BSL-1.
- Street clothing worn follow the chemical hygiene plan.
- Appendix M includes a new reference article.

BIOHAZARD DEFINITION

Biohazards include infectious or etiologic (disease causing) agents of humans, animals and plants, toxins of biological origin, human-derived materials, recombinant DNA, and any materials potentially containing infectious agents or biohazards.

Biohazardous agents may include but are not limited to: Certain bacteria, fungi, viruses, parasites, recombinant products, allergens, cultured human or animal cells and the potentially infectious agents these cells may contain viroids, prions and other infectious agents as outlined in laws, regulations, or guidelines.

RULES, REGULATIONS & GUIDELINES

This is a summary of the regulatory authorities that either regulate or provide guidelines for the use of biological materials, infectious agents, and recombinant DNA molecules. Copies of these documents are available from the EHS.

National Institute of Health (NIH): Guidelines on Research Involving Recombinant or Synthetic Nucleic Acid Molecules

These guidelines address the safe conduct of research involving construction and handling of recombinant DNA molecules and organisms containing them. In 1974, a recombinant DNA Advisory Committee (RAC) was established to determine appropriate biological and physical containment practices and procedures for experiments that potentially posed risks to human health and the environment. As a result of the committee's activity, the initial version of the NIH Guidelines was published in 1976. The latest amendment was April 2019. Included in the Guidelines is a requirement for the institution to establish an Institutional Biosafety Committee (IBC) with authority to approve or disapprove proposed research using the NIH Guidelines as a minimum standard. Refer to the following section of this manual: *Biosafety and Recombinant DNA or Synthetic Nucleic Acid Molecule Technology,* the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules and the Biosafety in Research website.

Biosafety in Microbiological and Biomedical Laboratories (BMBL)

In 1984, the Centers for Disease Control and Prevention (CDC) and the National Institute of Health (NIH) published the first edition of the BMBL. This document describes combinations of standard and special microbiological practices, safety equipment, and facilities that constitute Biosafety Levels 1-4, which are recommended for working with infectious agents in different laboratory settings. This document also outlines requirements for animal biosafety levels. The BMBL has been revised several times and is commonly seen as the standard for biosafety in the United States. MSU is using the BMBL as the basis for this manual.

Michigan Occupational Safety and Health Administration: Bloodborne Infectious Disease Standard

In 1992, the Occupational Safety and Health Administration (OSHA) published a rule to deal with the occupational health risk caused by exposure to human blood and other potentially infectious materials. OSHA's rule includes a combination of engineering and work practice controls, personal protective clothing and equipment, training and medical follow-up of exposure incidents, vaccination, and other provisions. The Michigan Occupational Safety and Health Administration (MIOSHA) enforced its standard

for Bloodborne Infectious Diseases in 1993. Consequently, MSU established an Exposure Control Plan to protect employees at MSU from exposure to HIV, Hepatitis B and other bloodborne pathogens. For more information, please refer to the MSU Exposure Control Plan.

OSHA Standard
MIOSHA Standard

Department of Health and Human Services (HHS) Possession, Use, and Transfer of Select Agents and Toxins

In 1996, HHS published a set of rules that require facilities and institutions to be registered and approved to transfer or receive certain biological agents and toxins. These rules have been revised several times since then. HHS requires MSU to comply with the BMBL (see above) and OSHA's Laboratory Safety Standard 29 CFR 1910.1450. The list of restricted agents and toxins in Appendix B. The most current list can be found on HHS website.

United States Department of Agriculture (USDA) Possession, Use, and Transfer of Biological Agents and Toxins

Agricultural Bioterrorism Protection Act of 2002; United States Department of Agriculture (USDA)

The USDA has also established a set of rules that require facilities and institutions to be registered and approved to transfer or receive certain biological agents and toxins. A copy of the restricted agents and toxins covered under this rule is included in Appendix B. Select Agents and Toxins Rules

Michigan Department of Public Health: Michigan Medical Waste Regulatory Act (MMWRA)

In 1990, the MMWRA was created to establish a program regulating the handling and disposal of medical waste. The rule mandates how producing facilities (generators of medical waste) must handle medical waste from the point at which it becomes medical waste, to the point of its ultimate disposal. MSU's compliance is outlined in the MSU Biohazardous Waste Management Plan, used in conjunction with the MSU Hazardous Waste Disposal Guide.

Packaging, shipment, and transportation requirements for biologicals

Packaging, shipment and transportation requirements for infectious substances, diagnostic specimens, biological products, and genetically modified organisms are addressed in the following rules and guidelines:

- United Nations
  Recommendations of the Committee of Experts on the Transportation of Dangerous Goods
- International Civil Aviation Organization (ICAO)
  Technical Instructions for the Safe Transport of Dangerous Goods by Air
- International Air Transport Association (IATA)
  Dangerous Goods Regulations
- U.S. Department of Transportation

49 CFR Parts 171-178

- U.S. Public Health Service
  42 CFR Part 72
- U.S. Postal Service
  39 CFR Part 111
- U.S. Department of Labor, OSHA
  29 CFR 1910.1030

Importation permits

Importation permits are required for certain infectious agents, biological materials and animals as outlined in U.S. Public Health Service, 42 CFR Part 71, Foreign Quarantine. In addition, the Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) requires permits for importation and transportation of controlled materials, certain organisms, or vectors. This includes animal and plant pathogens, certain tissue cultures and live animals. APHIS also regulates the importation, interstate movement, or environmental release of genetically engineered organisms as regulated under 7 CFR Part 340.

RISK ASSESSMENT

Risk assessment is an ongoing process used to examine the factors associated with a procedure involving biological materials, to identify the hazardous characteristics of the material, the activities that can result in a person's exposure to an infectious agent, the likelihood that exposure will cause a laboratory acquired infection, and the probable consequences of an infection. Environmental release consequence as well as biosecurity regarding export control are also examined. To be successful, this process is a collaborative process inclusive of all stakeholders. Beginning by recognizing a hierarchy of controls by first elimination or reduction of hazards, then progress to implement appropriate engineering/administrative controls, and finally personal protective equipment (PPE) identified to protect the worker. The information identified by risk assessment will provide a guide for the selection of biosafety levels, microbiological practices, safety equipment, and facility safeguards that can prevent laboratory acquired infections and reduce environmental contamination risk. Appendix C is a form to assist with risk assessments as well as the BMBL 6th Edition Section II. Section VIII of BMBL 6th Edition provides agent summary statements for many agents associated with LAIs (Laboratory Acquired Infections) or are of increased public concern.

Factors to consider in a risk assessment include both agent hazards and laboratory procedure factors.

Agent Hazards:

- *Capability to infect and cause disease in a susceptible host or population*
- *Virulence as measured by the severity of disease*
- *Availability of preventive measures and effective treatments for the disease*
- *Probable routes of transmission of laboratory infection*
  (mucous membrane exposure, parenteral inoculation, ingestion, and inhalation of infectious aerosols)
- *Infective dose*
- *Stability in the environment*
- *Host range*
- *Its endemic nature*
- *Existing reports of laboratory acquired infections*

- *Origin of the agent*

Risk Groups: Classification of Infectious Agents on Basis of Hazard

Risk groups are classifications describing relative hazards posed by infectious agents or toxins regarding laboratory work. There are four risk groups. These correlate to but are not equivalent to biosafety levels. Determining the risk group of a biological agent is part of the biosafety risk assessment and helps in assigning the correct biosafety level. In general, RG-2 agents are handled at BSL- 2, and RG-3 agents at BSL-3. However, the use of certain RG-2 agents in large quantities might require BSL-3 conditions, while some RG-3 agents may be safely manipulated at a BSL-2 under certain conditions.

A listing of the risk group definitions for many countries, including the US, can be found on the American Biological Safety Association website and the OIE Terrestrial Manual. The WHO Laboratory Biosafety Manual is a document that is also used as a standard for guidelines and is based on the risk assessment process with the specific institution's available resources.

NIH Guidelines Definition of Risk Groups

Risk Group 1 (RG1) - Agents that are not associated with disease in healthy adult humans. This group includes a list of animal viral etiologic agents in common use. These agents represent no or little risk to an individual and minimum risk to the community.

Risk Group 2 (RG2) - Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available. These agents represent a moderate risk to an individual but a low risk to the community.

Risk Group 3 (RG3) - Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available. These agents represent a high risk to an individual and a low to moderate risk to the community depending on the pathogen.

Risk Group 4 (RG4) –Agents ("Exotic Agents") that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available. These agents represent a high risk to the individual and a high risk to the community.

Appendix B, NIH Guidelines

Hazards of Genetically Modified Agents

Conducting a risk assessment addresses the possibility that the genetic modification could alter (i.e., increase or decrease) the pathogenicity of the agent or affect its susceptibility to antibiotics or other treatments. Investigators have reported observed unanticipated enhanced virulence in studies with engineered agents. Important information may not be available for a newly engineered agent and the risk assessment may be difficult or incomplete. Due diligence should be practiced, and the biosafety level assignment should be made conservatively. Another risk assessment should be completed as research progresses.

Hazards of Cell Cultures

Human and animal cells and tissues have the potential to harbor latent infectious agents and personnel who handle these materials are at risk for possible exposure. For additional information and requirements for working with human cell cultures refer to the MSU Exposure Control Plan and to the following section of this manual: *Guidelines for Working with Tissue Culture/Cell Lines*.

Laboratory Procedure Hazards

Parenteral Inoculations
Injection of potentially hazardous materials can occur by contaminated needle or other sharps object, or by bites from infected animals or arthropod vectors.

Spills and Splashes into Skin and Mucous Membranes
Mucous membranes include the eyes, nose, and mouth.

Ingestion through mouth pipetting

Animal bites and scratches

Inhalation exposures to infectious aerosols or droplets
Aerosols, or respirable sized particles, are extremely hazardous. They are generated in many lab procedures and are usually undetected. Creation of infectious aerosols can place persons in the laboratory at risk. Any procedure that breaks the surface tension of a liquid produces aerosols. Pipetting, blenders, non – self – contained centrifuges, sonicators, vortex mixers, cell sorters, and matrix – assisted laser desorption/ionization – time of flight mass spectrometers, all produce respirable – size particles that remain airborne for protracted periods. Procedures and equipment that create aerosols also create larger droplets that rapidly settle out of the air. These droplets can settle on surfaces contaminating gloved hands, workspaces, and mucous membranes.

BIOLOGICAL SAFETY AND BIOSAFETY LEVELS

Biological safety is the application of knowledge, techniques, and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or biohazards. Biosafety defines the containment conditions under which infectious agents can be safely manipulated. The objective of containment is to confine biohazards and to reduce the potential exposure of the laboratory worker, the community, and the environment to potentially infectious agents.

Primary Containment: Protection of personnel and the immediate laboratory environment through good microbiological technique (laboratory practice) and the use of appropriate safety equipment.

Secondary Containment: Protection of the environment external to the laboratory from exposure to infectious materials through a combination of facility design and operational practices.

Combinations of laboratory practices, containment equipment, and special laboratory design are made to achieve different levels of physical containment. Four biosafety levels (1- 4) define the levels of containment to protect personnel and the environment. Biosafety level 1 (BSL-1) is the least restrictive; while biosafety level 4 (BSL-4) requires a special containment laboratory or facility, which is not available at MSU. Most research at MSU is conducted at biosafety levels 1 and 2 with few experiments at BSL-3, this manual will mainly focus on these three biosafety levels. Information on biosafety level 4: refer to the appropriate literature or contact the Biological Safety Officer. A summary of the different biosafety level requirements can be found in Appendix G.

The most important element in maintaining a safe work environment is strict adherence to good microbiological and laboratory practices and techniques. Everyone working with infectious agents or potentially infectious materials are made aware of the potential risks. In addition, they are trained and proficient in the practices and techniques required for handling such material. It is the responsibility of the Principal Investigator or person in charge of the laboratory to provide or arrange for appropriate training of all personnel.

GENERAL LABORATORY PRACTICES

The following information applies to all laboratories housing biological materials. Information for specific biosafety levels will follow.

Routes of Infection

Infection occurs when disease-causing microorganisms enter the human body with sufficient concentration and by a particular route and overcome the body's defense system. Routes of infection reported for laboratory-acquired infections:

Laboratory Acquired Infection Routes

Mouth
Eating, drinking, and smoking in the laboratory
Mouth pipetting
Transfer of microorganisms to mouth by contaminated fingers or articles

Skin
Accidental inoculation with contaminated hypodermic needle or other sharp instrument including broken glass.
Applying cosmetics.
Cuts, scratches, bites

Eye
Splashes of infectious material into the eye
Transfer of microorganisms to eyes by contaminated fingers

Lungs
Inhalation of airborne microorganisms

Many laboratory-acquired infections (LAI's) reported point to accidents during work with infectious agents. The general laboratory procedures outlined in this manual address the issues and provide for guidance in handling infectious or potentially infectious materials.

Access

When procedures are in progress, the lab door must remain closed and when no one is present in the lab the doors are locked. Anyone requesting access to the laboratory is questioned as to their purpose and identification is provided.

Signage: Biohazard Communication

A biohazard label is required for all areas or equipment in which BSL-2 or higher agents are handled or stored and BSL-2 procedures are performed. Labels are posted at the main entrance door(s) to laboratories and animal rooms, on equipment such as refrigerators, freezers, biological safety cabinets, incubators, and transport containers. Labels and door signage are obtained from the EHS (355-0153).

Signage for BSL-2 or higher labs include the following information:
- Biosafety level

- Supervisor's or another responsible person's name
- Telephone number for emergency responders to contact in case of emergency
- Procedures required for entering and exiting the lab with potential biosafety hazard

Personal Protective Equipment (PPE)

Personal protective equipment is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may also protect the materials from contamination. Personal protective devices and safety equipment as well as training in the proper use of those, is provided to all employees under the appropriate circumstances. The employees have the responsibility of properly using the equipment.

Eye and Face Protection

Safety glasses are worn in the lab whenever procedures are underway involving a low probability of splash, work with low hazard chemicals, and impact hazards.

Whenever possible, lab operations are performed in containment devices such as a biological safety cabinet or fume hood, or behind a bench-top shield to minimize the potential for skin or mucous membrane contact with a hazardous splash. If procedures do not permit containment of the hazard with a containment device, the safety procedures are reviewed by the Biosafety Officer and Institutional Biosafety Committee, and then appropriate controls and PPE must be worn as outlined:

- Splash goggles are the only form of eye protection approved for splash hazards. If a chemical (including bleach) or biological splash hazard exists, splash goggles are worn.
- Full face protection (i.e., face shield) are used for procedures that have anticipated splashes or sprays of infectious or other hazardous materials to the face or if there is a high potential for aerosol generation. Face shields are not a replacement for eye protection. Refer to the EHS Chemical Safety website for further information regarding eye & face protection.
- Information on the availability of low-cost prescription safety eyewear may be obtained by calling the EHS at 355-0153.

Laboratory Clothing

This category includes laboratory coats, smocks, scrubs, uniforms, and gowns. Long-sleeved garments are used to minimize the contamination of skin or street clothes. In circumstances where it is anticipated that splashes may occur, the garment is resistant to liquid penetration. If the garment is not disposable, it is capable of withstanding sterilization, in the event it becomes contaminated. The garment is flame resistant when working with or around open flames or high temperature heat sources.

A laboratory coat is required for all work at BSL-.1 Additionally, other suitable protective clothing is required when handling potentially infectious materials at BSL-2 or higher. Additional criteria for selecting clothing are for comfort, appearance, closure types and location, antistatic properties, and durability. Disposables are available for visitors, maintenance, and service workers in the event it is required.

Protective clothing is removed and left in the laboratory before leaving for non-laboratory areas. All protective clothing is either discarded in the laboratory or laundered (Department facilities or MSU's laundry – Spartan Linen Services). Personnel will not take laboratory clothing home.

Long hair and loose clothing (like neck ties, dangling jewelry, scarves, or long sleeves) are secured to avoid contamination and material getting caught in equipment.

Personal street clothing worn in the laboratory must also follow the chemical hygiene plan as disinfectants are chemicals and required for work with biological materials. Long pants or long skirt, and closed toed shoes are some of the requirements. See the Chemical Hygiene Plan for more information.

Gloves

Gloves are selected based on the hazards involved and the activity to be conducted. They are worn when working with biohazards, toxic substances, hazardous chemicals, and other physically hazardous agents. Temperature resistant gloves are worn when handling hot material or dry ice. Delicate work requiring a high degree of precision dictates the use of thin-walled gloves. When latex gloves have been chosen, alternatives are made available.

Gloves are changed as soon as possible after they have become contaminated, when their integrity has been compromised or when necessary.

Hands are properly washed with soap and water after removing gloves. Disposable gloves are not washed or reused and are disposed of as soon as removed.

When transporting potentially infectious materials (i.e., cultures, waste, etc.) to another part of the building use the one glove rule: use one gloved hand for handling the materials and use the other ungloved hand for touching common surfaces such as doorknobs and elevator buttons.

The US Food and Drug Administration has issued a ban on all powdered gloves. Exposure to starch powder from gloves can cause undesirable reactions, which vary from well-known allergy symptoms and upper respiratory-tract disorders to surgical adhesions and infections. The presence of glove powder can also result in many other undesirable effects, such as interference in laboratory testing causing false results (i.e., PCR – Polymerase Chain Reaction, enzyme immunoassay or some serology tests).

For assistance in glove selection, contact the EHS at 355-0153.

Respirators

For certain protocols and projects, additional PPE such as respiratory protection may be required. Respirator selection is based on the hazard and the protection factor required. Personnel who require respiratory protection contact the EHS for assistance in selection of proper equipment and training in its usage. All personnel wearing respirators are included in **MSU's Respiratory Protection Program** which includes a medical evaluation, initial training, and fit-testing and annual retraining. Do not wear N95 respirators without enrolling in the program or if it is not indicated.

Laundry

All personal protective clothing is cleaned, laundered, and disposed of by the employer at no cost to employees. Apparel contaminated with human blood or other potentially infectious materials is handled as little as possible and is collected in special hampers (labeled or color coded) or in biohazard bags. Laundry will be cleaned by MSU's laundry facility – Spartan Linen Services (or department facility). Appropriate PPE is worn by employees who handle contaminated laundry.

Food and Drink Policy

The following statement is the accepted practice for food and drink items when working in MSU laboratories.

There shall be no food, drink, smoking or applying cosmetics in laboratories which have radioactive materials, biohazardous materials or hazardous chemicals present. There shall be

no storage, use or disposal of these 'consumable' items in laboratories (including refrigerators or freezers within laboratories). Rooms which are adjacent, but separated by floor to ceiling walls, and do not have any chemical, radioactive or biological agents present, may be used for food consumption, preparation, or applying cosmetics at the discretion of the principal investigator responsible for the areas.

Housekeeping

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

Laboratory personnel are responsible for cleaning laboratory benches, equipment and areas that require specialized technical knowledge. To facilitate decontamination, the laboratory is kept neat and free of clutter - surfaces are clean and free of infrequently used chemicals, glassware, and equipment. Access to sinks, eyewash stations, emergency showers and exits, and fire extinguishers are not blocked.

Inventory Log

A written or computerized inventory log is kept. The inventory is complete enough so that the PI would know if materials were missing, what those materials are, the quantity of materials, and the potential hazards of the materials. The log is reconciled with the physical inventory on a periodic basis. Refer to Appendix H for an example.

Training

Good microbiological and laboratory practices are essential for a safe work environment. It is ideal if training and education on these practices and procedures starts at the undergraduate level. All personnel working with RG-2 or 3 agents or at BSL-2 or 3 have received adequate laboratory specific training from the Principal Investigator (PI) or laboratory supervisor. A proficiency in following good microbiological practices, correct use of safety equipment, consistent use of standard operating procedures (SOPs) for specific lab activities, ability to respond to emergencies, and willingness to accept responsibility for protecting oneself and others. See Appendix K for a site-specific training checklist that can facilitate and document this training annually or more frequently if processes or hazards change.

Trainings include but not limited to:

- Good laboratory and animal practices as applicable.
- Site specific information on risks, hazards, and procedures; and
- Laboratory or environment specific BSL-2 or 3 procedures as applicable.

All personnel working in a laboratory handling biological materials take the appropriate biological safety-related trainings offered by the EHS. Current listing can be found on EHS website.

Biosafety Principles Training: This online course covers general training requirements for working in environments at Biosafety Level 1 or higher. There are modules for different disciplines or work environments (see the list below).

- **Plant Module**: Handling plant material, especially recombinant DNA and genetically modified plant material including but not limited to seeds, proteins, as well as whole plants, for the purposes of plant genetic manipulation, field research using genetically modified plants or plant disease, and/or plant disease research.
- **Farms**: Handling live animals in a farm setting, such as a MSU agricultural employee whose job duties involve contact with and care of agricultural animals or diagnostic products.

- **Laboratory Animal Module:** Contact with and care of animals, or animal diagnostic products, or other animal materials (e.g., animal-derived cell lines).
- **Other**: Handling microbes, arthropods, or other organisms that are not classified as animal or human. Human derived materials such as blood, body fluids, unfixed tissues and/or cell lines. If your activities include insects, bacteria, viruses, viroids, fungi, parasites, and prions.

Biosafety Principles Refresher Training: Online course required each year after taking the Biosafety Principles Training initially.

Bloodborne Pathogens Initial: A MIOSHA required online class for anyone handling human-derived materials, including blood and cell lines. Different groups (healthcare workers, medical students, custodial) will select the module that fits the description of their work duties best. Questions regarding which module to take, contact EHS.

Bloodborne Pathogens Refresher: Online course that is required by MIOSHA each year after taking the Bloodborne Pathogens Initial course once.

Medical Waste Training: Medical Waste Regulatory Act (MMWRA) training requirements apply to every employee who generates, handles, treats and/or disposes of biohazardous waste (including sharps) at MSU. It is available online.

Autoclave Safety Training: This training is now required for those individuals who operate an autoclave as part of their job duties.

Security Awareness Training: Online course required for anyone who works in or who has access to a laboratory.

Others: Biosafety Office offers specialized courses as requested. For example: Non-Primate Biohazard training, Infectious Substance and Biological Materials Shipping, use of high-containment facilities, and Biosafety Cabinet training among others. If you have a need for a specialized class, contact EHS.

Health and Medical Surveillance

Medical surveillance of personnel is essential to identify health factors that may increase one's risk for lab-acquired infections. Under specific circumstances, work with high-risk agents or diagnostic specimens that may contain high-risk agents may require consideration of vaccinations for some personnel or restricted access for others, and health monitoring as prescribed by MSU Occupational Health to facilitate recovery (Refer to Appendix F for exposure response procedures).

Principal Investigators are responsible for all lab and support personnel, and visitors. All entering lab are fully informed of the following:

- Risks associated with handling the biological materials in use, including routes of transmission and signs and symptoms.
- Restricted access policies for those at elevated risk of infection for all infectious agent in use.
- Conditions that can lead to one becoming immunocompromised or immunosuppressed, and the option to notify one's supervisor or MSU Occupational Health in that instance to assure one's health.

Lab personnel and visitors observe the following.

- Entry or work in **any** lab where biological materials are in use (regardless of the biosafety level) may pose an elevated risk of infection for individuals who are immunocompromised.
- Consultation with an occupational health provider before working in a lab is strongly advised if the person may be immunocompromised. Pregnancy, recent illnesses caused by an infectious agent (i.e., the flu), chemotherapy, etc. can result in an immunocompromised state of health. Each

health condition must be evaluated by the appropriate occupational health provider and consider all the recommendations.

Vaccinations

Specific projects may use infectious materials and techniques that warrant consideration of vaccines. In these instances, the Principal Investigator will notify the MSU Biosafety Officer and MSU Occupational Health to further assess this need. Restricted access entry or vaccination requirements are implemented for a study underway, and the information is clearly posted on the lab door to communicate elevated risk.

THE BIOSAFETY LEVEL 1 LABORATORY

Suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans and that present minimal potential hazard to laboratory personnel and the environment.

Standard Microbiological Practices

Controlled access

The lab supervisor ensures access to the laboratory is controlled. When procedures are in progress the lab doors are shut and when no one is present in the lab, the doors are locked. Anyone requesting access to the laboratory is questioned as to their purpose and identification provided.

Training

In addition to the completion of EHS required online training courses, the principal investigator ensures that all lab personnel receive site-specific training. This training includes information specific to their job duties, potential hazards, manipulation of infectious agents, precautions to prevent exposures, and exposure response procedures. Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are given information on potential hazards and appropriate safeguards, immune competence and conditions that could predispose them to infection, as appropriate. Individuals having conditions increasing the susceptibility to infection are encouraged to self-identify to the University Physician. See Appendix K for a checklist to assist with and document this training.

Laboratory specific biosafety manual

Each laboratory supplements this biosafety manual with information that is specific for the individual laboratory. Supplemental information found in risk assessment and standard operating procedures (SOPs), including specific PPE practices and location of supplies, laboratory specific training requirements, laboratory specific waste handling practices and autoclave procedures, safe operation and decontamination of lab specific equipment, proper use of disinfectants specific for the lab (appropriate concentration, contact time and shelf life), etc.

Door Sign

Lab doors have an “Admittance to Authorized Personnel Only” sign. This sign contains appropriate contact information for general and emergency entrance to the lab. Biosafety level, PPE requirements, and required procedures for entering and exiting lab if appropriate. Obtained from EHS.

PPE and Lab Attire

Basic laboratory attire when working with hazardous materials includes wearing closed-toe shoes, long pants or skirt which fully covers the legs (Refer to the MSU Chemical Hygiene Plan). All disinfectants have hazardous properties; therefore, the basic laboratory attire must be used in any laboratory working with BSL-1 or above materials and/or hazardous materials.

Personal protective equipment includes laboratory coats, gowns or uniforms are worn when actively working with or near someone actively working with BSL-1 materials and/or hazardous chemicals to prevent contamination of personal clothing. Flame retardant material is required when working with open flames or high heat sources.

Splash goggles are worn when there is the potential for splashes of microorganisms or other hazardous materials. Lab safety glasses are worn by personnel conducting procedures with impact hazard. Eye protection/face protection are disposed of with other contaminated lab waste or decontaminated after use appropriately. Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment.

Gloves are worn as protection from hazardous materials. Alternatives to latex are available when latex is used. Gloves are changed when contaminated, when the integrity has been compromised, or when necessary. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where those materials and/or animals are housed or manipulated. Disposable gloves are disposed of with other contaminated waste and are not to be washed or reused. Hand hygiene is required after removing gloves, and before leaving the laboratory.

Eating, drinking, handling contact lenses and applying cosmetics

Eating, drinking, contact lens handling and cosmetic application is done outside of the laboratory. Food and beverages for human consumption are stored outside of the laboratory area in refrigerators, freezers, or cabinets designated for that purpose.

Hand Hygiene

Hand hygiene is performed with soap and water after handling potentially infectious materials and after removing gloves. This process is important before leaving the laboratory and before touching common use surfaces (i.e, computers, telephones, and other personal items).

Pipetting

Mechanical pipetting devices are available and used. Mouth pipetting is prohibited.

Safe sharps practices

All policies regarding the safe use of sharps are followed. See the following section of this manual for additional information: *Recommended Work Practices- Sharps*.

Minimize splashes and aerosols

Essentially all laboratory procedures involve steps which create aerosols. All procedures are completed in a manner which minimizes the creation of both splashes and aerosols. This can be done by using centrifuges with safety features (i.e., sealed cups and rotors), mechanical pipettors, conducting work inside of a biological safety cabinet, etc.

Decontaminate work surfaces

Work surfaces are decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant with correct concentration and contact time is used. If bench paper or plastic backed absorbents are used, they are discarded and the space beneath decontaminated.

Proper decontamination and transport of waste

All cultures, stocks and other biohazardous materials are properly decontaminated before disposal. When transporting waste out of the laboratory (e.g., down the hall, to another floor of the building, etc.) to be decontaminated, waste is placed in a leak-proof container and is secured. Refer to the following section of this manual: *Biohazardous Waste*, and the MSU Biohazardous Waste Management Plan for additional information regarding to the proper decontamination of biohazardous waste.

Pest management program

A pest management program is managed through IPF. They are contacted at the first sign of a problem.

Non-research related animals and plants in the laboratory

Animals and plants not associated with the work being done are not allowed in the laboratory.

Special Practices

None required.

Safety Equipment

PPE including lab coats, gowns or uniforms worn to prevent personal clothing contamination.

Protective eyewear is worn when procedures require it.

Animal research requires an additional risk assessment regarding animal allergens and may require appropriate eye, face, and respiratory protection.

Laboratory Facilities

Doors

Doors are required for access control. They are kept locked when no one is present in the laboratory.

Sink

Sink is available and supplied for hand hygiene, stocked with soap and paper towels.

Eyewash station

Eyewash is readily available in the lab.

Easily cleaned

Lab is designed in a way to be cleaned easily. Carpets and rugs are not appropriate. Spaces between benches, cabinets and equipment must be accessible for cleaning.

Furniture

Furniture in the lab is appropriate for the anticipated use. Bench tops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

Chairs are covered with a non-porous material that can be easily cleaned and disinfected.

Windows

Windows that can be opened to the outdoors, are fitted with screens.

Lighting

Illumination is adequate for all activities and avoids reflections and glare that could impede vision.

THE BIOSAFETY LEVEL 2 LABORATORY

BSL-2 builds upon BSL-1 and is for work with agents associated with human disease that pose a moderate hazard to personnel and the environment. Lab personnel receive specific training in handling these pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures. Access to a BSL-2 lab is restricted when work is being conducted and all procedures involving infectious aerosol or splash producing procedures are conducted in a biosafety cabinet (BSC) and other physical containment equipment may apply.

Standard Microbiological Practices

Controlled access

The lab supervisor enforces controlled access to the laboratory. When procedures are in progress the lab door is shut and when no one is present in the lab, the doors are locked. Anyone requesting access to the laboratory is questioned as to their purpose and identification is provided.

Training

In addition to the completion of EHS required online training courses, the principal investigator ensures that all lab personnel receive site-specific training. This training includes information specific to their job duties, potential hazards, manipulation of infectious agents, precautions to prevent exposures, exposure

response procedures, emergency response. Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility are given information on potential hazards and appropriate safeguards, immune competence and conditions that could predispose them to infection, as appropriate. Individuals having conditions increasing the susceptibility to infection are encouraged to self-identify to the University Physician. See Appendix K for a checklist to assist with and document this training.

Laboratory specific biosafety manual

Each laboratory supplements this biosafety manual with information that is specific for the individual laboratory. Supplemental information found in standard operating procedures (SOPs) include specific PPE practices and location of supplies, laboratory specific training requirements, laboratory specific waste handling practices and autoclave procedures, safe operation and decontamination of lab specific equipment, proper use of disinfectants specific for the lab (appropriate concentration, contact time and shelf life), etc. In addition, references to protocols for emergency situations, including exposures, medical emergencies and other emergencies including facility malfunctions are included.

Door signage and hazard communication

Lab doors have an “Admittance to Authorized Personnel Only” sign. This sign contains appropriate contact information for general and emergency entrance to the lab. The universal biohazard symbol and biosafety level, PPE requirements, and required procedures for entering and exiting lab are also on this sign. Agent information including health requirements are included in accordance with MSU policy. Signs are obtained from EHS.

PPE and Lab Attire

Basic laboratory attire when working with hazardous materials includes wearing closed-toe shoes, long pants or skirt which fully covers the legs (Refer to the MSU Chemical Hygiene Plan). All disinfectants have hazardous properties; therefore, the basic laboratory attire must be used in any laboratory working with BSL-1 or above materials and/or hazardous materials.

Personal protective equipment includes laboratory coats, gowns or uniforms are worn when actively working with or near someone actively working with BSL-2 materials and/or hazardous materials to prevent contamination of personal clothing. Flame retardant material is required when working with open flames or high heat sources.

Splash goggles are worn when there is the potential for splashes of microorganisms or other hazardous materials. Lab safety glasses are worn by personnel conducting procedures with impact hazard. Eye protection/face protection are disposed of with other contaminated lab waste or decontaminated after use appropriately. Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment. Gloves are worn as protection from hazardous materials. Alternatives to latex are available when latex is used. Gloves are changed when contaminated, when the integrity has been compromised, or when necessary. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where those materials and/or animals are housed or manipulated. Disposable gloves are disposed of with other contaminated waste and are not to be washed or reused. Hands are washed after removing gloves, and before leaving the laboratory. Gloves are not worn outside of the laboratory especially in common areas.

Hand Hygiene

Hands are washed with soap and water after handling potentially infectious materials. Hands are washed before leaving the laboratory and before touching common use surfaces (i.e., computers, telephones, and other personal items.).

Eating, drinking, handling contact lenses and applying cosmetics

Eating, drinking, contact lens handling and cosmetic application is done outside of the laboratory. Food and beverages for human consumption are stored outside of the laboratory area in refrigerators, freezers, or cabinets designated for that purpose.

Pipetting

Mechanical pipetting devices are available and used. Mouth pipetting is prohibited.

Safe sharps practices

All policies regarding the safe use of sharps are followed. See the following section of this manual for additional information: *Recommended Work Practices- Sharps*.

Minimize splashes and aerosols

Essentially all laboratory procedures involve steps which create aerosols. All procedures are completed in a manner which minimizes the creation of both splashes and aerosols. This can be done by using centrifuges with safety features (i.e., sealed cups and rotors), mechanical pipettors, conducting work inside of a biological safety cabinet, etc.

Decontaminate work surfaces

Work surfaces are decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant is used with correct concentration and contact time. If bench paper or plastic backed absorbents are used, they are discarded and the space beneath decontaminated.

Proper decontamination and transport of waste

All cultures, stocks, and other biohazardous materials are decontaminated before disposal. When transporting waste out of the laboratory (e.g., down the hall, to another floor of the building, etc.) to be decontaminated, waste is placed in a leak-proof container and is secured. Refer to the following section of this manual: *Biohazardous Waste*, and the MSU Biohazardous Waste Management Plan for additional information regarding to the proper decontamination of biohazardous waste.

Pest management program

A pest management program is managed through IPF. They are contacted at the first sign of a problem.

Non-research related animals and plants in the laboratory

Animals and plants not associated with the work being done are not allowed in the laboratory.

Special Practices

Laboratory entrance

Access to lab is controlled. Before entering the laboratory, all people are made aware of the potential hazards. They also meet all entry and exit requirements (e.g., donning and doffing of personal protective equipment, immunization requirements, hand hygiene, etc.).

Training

Lab personnel are proficient in microbiological practices before handling BSL-2 agents. The PI is responsible and ensures that proficiency has been demonstrated.

Personal Electronic Device Use in the BSL-2 Laboratory

Personal Electronic Devices (PED) can include, but are not limited to headphones, ear buds, cell phones, smart phones, smart watches, MP3 players, personal computers, tablet computers, electronic book readers, recording/playback devices. The use of these devices while actively working with or near BSL-2 materials is prohibited. This includes while working in a biosafety cabinet, open bench, biohazardous waste or with equipment that may be contaminated. This is due to potential contamination of the PEDs leading to exposures, transfer of the contamination outside of the laboratory, as well as the device causing a distraction when conducting potentially dangerous procedures.

Laptops, Tablets and Computers

Personal computers are discouraged from being brought into the laboratory from home unless:

- The device will remain within the facility to be used while actively conducting research and covered/disinfected regularly
- The device is kept in an area far from experiments or hazardous materials, such as a laboratory desk designated for documentation and is disinfected regularly

Smart Phones

If they must be used in the laboratory while actively working with potentially infectious materials, they should be placed in a bag (i.e. Ziplock) and sealed.

The bagged item may not be placed inside personal clothing pockets or personal bags that are taken home. The bag is disinfected and the item can then be removed from the bag to be taken home.

Biological safety cabinets (BSC)

BSC, or a combination of PPE and other containment devices (as approved by the Biological Safety Officer) is used when there is the potential for the creation of infectious aerosols or splashes. This includes, but is not limited to pipetting, centrifuging, mixing, sonicating, blending, shaking, opening containers, intranasal inoculation of animals, and harvesting tissues. BSC is used when handling large volumes or high concentrations of potentially infectious materials. Centrifugation of these materials may be done outside of the BSC if sealed rotors or centrifuge safety cups are used. See the following section of this manual for additional information: *Safety Equipment- Biological Safety Cabinets*.

Decontamination of laboratory equipment

Lab equipment is decontaminated routinely and after spills, splashes or when potentially contaminated, and before repairs, maintenance, or removal from the lab. When maintenance, repair or removal are needed, lab personnel complete an Equipment Release Form and attach it to the piece of equipment. See Appendix E for an example of the form.

All BSL-2 labs have a biological spill kit available. See the following section of this manual for spill clean-up procedures and spill kit contents: *Biohazard Spill Cleanup Procedures.*

Exposure incidents

Exposure response procedures are posted in an easily accessible location in the laboratory. All lab personnel are aware of the proper procedures to follow in the event of a possible exposure to potentially infectious materials. See Appendix F for exposure response procedures.

Incidents resulting in exposure to infectious materials are immediately evaluated by following exposure procedure plan and reported to the supervisor and EHS as soon as possible.

Safety Equipment

Personal protective equipment (PPE)

Laboratory coats, gowns or uniforms designated for the lab are worn while working with hazardous materials and removed before leaving for non-lab areas (e.g., cafeteria, library, administrative offices, and bathroom). Flame retardant material is required when working with open flames or high heat sources. Protective clothing is disposed of appropriately or sent to laundry when soiled, torn, or no longer used. Lab clothing is not to be taken home.

Splash goggles are worn when there is the potential for splashes of microorganisms or other hazardous materials. Lab safety glasses are worn by personnel conducting procedures with impact hazard. Eye protection/face protection are disposed of with other contaminated lab waste or decontaminated after use appropriately.

Respiratory protection may be required based on risk assessment and personnel are enrolled in the MSU respiratory protection program.

Animal research requires an additional risk assessment regarding animal allergens and may require appropriate eye, face, and respiratory protection.

Gloves are worn as protection from hazardous materials. Alternatives to latex are available when latex is used. Gloves are changed when contaminated, when the integrity has been compromised, or when necessary. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where those materials and/or animals are housed or manipulated. Disposable gloves are disposed of with other contaminated waste and are not to be washed or reused. Hand hygiene is performed after removing gloves, and before leaving the laboratory. Gloves are not worn outside of lab in common areas.

Containers for potentially infectious materials

Containers used to collect, handle, process, store, or transport within a facility, potentially infectious materials are durable, leak-proof and have a lid. The containers are properly labeled with the contents and a biohazard symbol including lid.

Laboratory Design and Facilities

Doors

Self-closing doors with locks are required for access control. They are closed when work is in progress inside the lab and kept locked when no one is present in the laboratory. Do not prop doors open.

Sink

A sink is available and supplied for handwashing (i.e., stocked with soap and paper towels). It is located near the exit door.

Eyewash station

Eyewash station is readily available in the lab. The eyewash is kept in good working order and flushed at least once weekly until water runs clear.

Easily cleaned

The lab is designed in a way that allows it to be cleaned easily. Carpets and rugs are not appropriate. Spaces between benches, cabinets and equipment are accessible for cleaning.

Furniture

Bench tops are impervious to water and resistant to heat, organic solvents, acids, alkalis and other chemicals.

Chairs used in lab work are covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

Windows

Windows that open to the outside are not recommended. If windows are present, they are fitted with screens.

Lighting

Illumination is adequate for all activities and avoids reflections and glare that may impede vision.

Vacuum Lines

Vacuum lines are protected with liquid disinfectant traps and in-line HEPA filters. Filters are replaced, as needed, or are on a replacement schedule determined by a risk assessment. Dual flask system or single flask with overflow valve that stops vacuum when full is required. Flasks are stable and contained in case of tip over or spill. Appropriate fresh disinfectant is within the flask to decontaminate all waste collected and emptied at end of work or end of the day whichever comes first.

Ventilation

Ventilation systems allow for inward flow of air without recirculation to spaces outside of the laboratory. Contact IPF if ventilation is neutral or outward.

Biological safety cabinets

Biological safety cabinets (BSC) are installed in a manner so that changes in room air do not interfere with the operation of the cabinet. They are located away from doors, windows that can be opened, high traffic areas, and other areas that could cause disruptions in the airflow of the cabinet. They are tested and certified at least annually and whenever they are relocated or serviced. BSCs are operated in accordance with the manufacturer's recommendations. See the following section of this manual for additional information: *Safety Equipment- Biological Safety Cabinets*.

BIOLOGICAL SAFETY LEVEL 3 LABORATORIES

Biosafety Level 3 (BSL-3) is designed for work with agents that may cause serious or potentially lethal disease via inhalation. A BSL-3 lab may also be used when working with large volumes or high concentrations of Risk Group 2 microorganisms that pose an increased risk of aerosol spread. Lab personnel receive specific training in handling these agents and are supervised by scientists competent in handling infectious agents and associated procedures.

BSL-3 labs have special engineering and design features that are required.

All manipulation of agents is conducted within BSCs or other containment devices.

Standard Microbiological Practices

Controlled access

The lab supervisor enforces controlled access to the laboratory. When procedures are in progress the lab door are shut and when no one is present in the lab, the doors are locked. Anyone requesting access to the laboratory are questioned as to their purpose and identification is provided.

Training

In addition to the completion of EHS required online training courses, the principal investigator ensures that all lab personnel receive site-specific training. This training includes information specific to their job duties, potential hazards, manipulation of infectious agents, precautions to prevent exposures, exposure response procedures, emergency response. Personnel receive annual updates and additional training when equipment, procedures, or policies change. All persons entering the facility will be given information on potential hazards and appropriate safeguards, immune competence and conditions that could predispose them to infection, as appropriate. Individuals having conditions increasing the susceptibility to infection are encouraged to self-identify to the University Physician. See Appendix K for a checklist to assist with and document this training.

Laboratory specific biosafety manual

Each laboratory will supplement this biosafety manual with information that is specific for the individual laboratory. Supplemental information found in standard operating procedures (SOPs) include specific PPE practices and location of supplies, laboratory specific training requirements, laboratory specific

waste handling practices and autoclave procedures, safe operation and decontamination of lab specific equipment, proper use of disinfectants specific for the lab (appropriate concentration, contact time and shelf life), etc. In addition, references to protocols for emergency situations, including exposures, medical emergencies and other emergencies including facility malfunctions are included.

Door signage and hazard communication

Lab doors have an “Admittance to Authorized Personnel Only” sign. This sign contains appropriate contact information for general and emergency entrance to the lab. The universal biohazard symbol and biosafety level, PPE requirements, and required procedures for entering and exiting lab are also on this sign. Agent information including health requirements are included in accordance with MSU policy. Signs are obtained from EHS.

PPE and Lab Attire

Long hair is restrained so that it cannot contact hands, specimens, containers, or equipment. Hair bonnets or other covering may be required based on risk assessment.

Laboratory coats, gowns or uniforms are worn to prevent contamination of personal clothing. Flame retardant material is required when working with open flames or high heat sources.

Splash goggles are worn when there is the potential for splashes of microorganisms or other hazardous materials. Lab safety glasses are worn by personnel conducting procedures with impact hazard. Eye protection/face protection are disposed of with other contaminated lab waste or decontaminated after use appropriately.

Gloves are worn as protection from hazardous materials. Alternatives to latex are available when latex is used. Gloves are changed when contaminated, when the integrity has been compromised, or when necessary. Gloves and other PPE are removed in a manner that minimizes personal contamination and transfer of infectious materials outside of the areas where those materials and/or animals are housed or manipulated. Disposable gloves are disposed of with other contaminated waste and are not to be washed or reused. Hands are washed after removing gloves, and before leaving the laboratory.

Respiratory protection is evaluated based on risk assessment. When it is required, enrollment in the MSU respiratory protection program is also required.

Hand Hygiene

Hands are washed with soap and water after handling potentially infectious materials. Hands are washed before leaving the laboratory and before touching common use surfaces.

Eating, drinking, handling contact lenses and applying cosmetics

Eating, drinking, contact lens handling and cosmetic application is done outside of the laboratory. Food and beverages for human consumption are stored outside of the laboratory area in refrigerators, freezers or cabinets designated for that purpose.

Pipetting

Mechanical pipetting devices are available and used. Mouth pipetting is prohibited.

Safe sharps and glassware practices

Glassware is eliminated whenever possible and replaced with plastic. All policies regarding the safe use of sharps are followed. See the following section of this manual for additional information: *Recommended Work Practices- Sharps*.

Minimize splashes and aerosols

Essentially all laboratory procedures involve steps which create aerosols. All procedures are completed in a manner which minimizes the creation of both splashes and aerosols. This can be done by using centrifuges with safety features (i.e., sealed cups and rotors), mechanical pipettors, conducting work inside of a biological safety cabinet, etc.

Decontaminate work surfaces

Work surfaces are decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant is used with correct concentration and contact time. If bench paper or plastic backed absorbents are used, they are discarded and the space beneath decontaminated. Spills are cleaned up by staff who are properly trained and equipped to work with infectious material. Spill procedure is developed and posted in lab.

Proper decontamination of waste

All cultures, stocks, and other biohazardous materials are decontaminated before disposal or removal from lab. Refer to the following section of this manual: *Biohazardous Waste*, and the MSU Biohazardous Waste Management Plan for additional information regarding to the proper decontamination of biohazardous waste.

Pest management program

A pest management program is managed through IPF. They are contacted at the first sign of a problem.

Non-research related animals and plants in the laboratory

Animals and plants not associated with the work being done are not allowed in the laboratory.

Special Practices

Laboratory entrance

Access to lab is controlled. Before entering the laboratory, all people are made aware of the potential hazards. They also meet all entry and exit requirements (e.g., donning and doffing of personal protective equipment, immunization requirements, hand hygiene, etc.). Only persons whose presence in the lab areas is required for scientific or support purposes are authorized to enter.

Medical surveillance

All personnel entering the operational lab areas are provided information on signs and symptoms of disease and receive occupational medical services including medical evaluation, surveillance, and

appropriate vaccinations, and treatment as appropriate. The need for collection and storage of serum samples from at – risk personnel is considered and may be implemented based on risk assessment.

All laboratories using human-derived materials or cell lines must participate in the Bloodborne Pathogens Program. See the following section of this manual for additional information: Medical Surveillance. For the use of other agents, medical surveillance and immunizations will be provided as appropriate. Occupational Health as well as the EHS should be contacted for assistance.

Lab specific biosafety manual

Lab-specific biosafety manual contains standard operating procedures that are prepared and adopted as policy and are available and accessible.

Training

PI ensures lab personnel demonstrate proficiency in standard and special microbiological practices before handling BSL-3 agents.

Exposure incidents

Exposure response procedures are posted in an easily accessible location in the laboratory. All lab personnel are aware of the proper procedures to follow in the event of a possible exposure to potentially infectious materials. See Appendix F for exposure response procedures. Documentation and record retention policies apply.

Containers for potentially infectious materials

Prior plans are made when transporting biological agents being aware of all applicable regulations. Materials that require BSL-3 containment are placed in durable leak-proof sealed primary container and then enclosed in a non-breakable, sealed secondary container prior to removal from lab. The containers must be properly labeled with the contents and a biohazard symbol. Once removed the primary container is only opened once inside a BSC in a BSL-3 containment facility.

Manipulating infectious materials

All procedures that involve the manipulation of infectious materials are conducted within a biological safety cabinet or other approved containment device. Work involving open vessels cannot be conducted on the open bench. If a procedure cannot be conducted in a BSC, a combination of PPE and other containment devices are reviewed and approved by the Biological Safety Officer upon Institutional Biosafety Committee review.

Decontamination of laboratory equipment

Lab equipment is decontaminated routinely. It is decontaminated after spills, splashes or when potentially contaminated. All spills are cleaned by personnel who are properly trained and have the proper equipment to handle infectious materials. All BSL-3 labs have a biological spill kit available. See the following section of this manual for spill cleanup procedures and spill kit contents: *Biohazard Spill Cleanup Procedures*.

All equipment is decontaminated before being repaired, maintained, or removed from the laboratory. When any of these is to occur lab, personnel complete an Equipment Release Form and attach it to the piece of equipment. See Appendix E for an example of the form.

Decontamination of the entire lab is considered when gross contamination occurs in the space, significant changes in lab usage, major renovations, or maintenance shut – downs.

Decontamination processes are verified on a routine basis.

Proper decontamination and transport of waste

All cultures, stocks, and other biohazardous materials are decontaminated before disposal and removal from lab facility. When transporting waste out of the laboratory (e.g., down the hall, to another floor of the building, etc.) to be decontaminated, waste is placed in a leak-proof container and is secured. Refer to the following section of this manual: *Biohazardous Waste*, and the MSU Biohazardous Waste Management Plan for additional information regarding to the proper decontamination of biohazardous waste.

Personal Electronic Device Use in the BSL-3 Laboratory

Personal Electronic Devices (PED) can include, but are not limited to headphones, ear buds, cell phones, smart phones, MP3 players, personal computers, tablet computers, electronic book readers, recording/playback devices. Their use in the laboratory is prohibited unless approved for research by EHS. This is due to potential contamination of the PEDs, transfer of the contamination outside of the laboratory, as well as the device causing a distraction when doing critical work.

Safety Equipment

Biological safety cabinets (BSC)

A biological safety cabinet is used whenever working with infectious materials. Other physical containment devices may be used with the approval of the Biological Safety Officer. The BSCs used are certified and have received the appropriate maintenance.

Personal protective equipment (PPE)

Laboratory coats, gowns or uniforms with a solid front are required when in the laboratory. Work with certain agents may require that street clothes be removed, and dedicated lab clothing be worn. Long hair is to be secured or placed within a hair net to avoid contamination. Protective clothing cannot be worn outside of the lab. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.

Splash goggles and face protection must be used when there is the potential for splashes of microorganisms or other hazardous materials. Personnel who wear contact lenses will wear safety glasses or other eye protection while in the laboratory.

Gloves are worn as protection from hazardous materials. Two pairs should be worn as appropriate. If latex gloves are used, alternatives are available. Gloves are changed when contaminated, when the integrity has been compromised, or when necessary. Wash hands immediately after removing gloves. Disposable gloves must not be washed or reused. Hands are washed after removing gloves, and before leaving the laboratory. All protective equipment is removed before leaving the laboratory. Used disposable is disposed of with other contaminated waste. Reusable PPE (i.e., goggles or PAPR) are appropriately decontaminated before reuse.

Respiratory protection is considered including enrollment in respiratory protection program. Consideration of animal allergens when animals are present in the lab may require eye, face, and respiratory protection.

Laboratory Design and Facilities

Location

The laboratory should be separated from areas that have unrestricted traffic flow within a building.

Doors

Access is restricted. A series of two self-closing lockable doors is required for access control. Doors always remain closed, even when no work is being conducted in the lab. A clothing change room (ante room) is included in the passageway between the two self-closing doors.

Sink

A hands-free or automatic sink is available and supplied for hand hygiene (i.e., stocked with soap and paper towels). It should be located near the exit door. If the lab is separated into multiple labs, each area has a sink available and supplied for handwashing.

Eyewash

An eyewash station is readily available in the lab.

Easily cleaned and decontaminated

The lab must be designed in a way that allows it to be cleaned and decontaminated easily. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces are sealed. Spaces around doors and ventilation openings are capable of being sealed for whole room decontamination. Floors are slip resistant, impervious to liquids and resistant to chemicals. Flooring is seamless, sealed, or poured with integral cove bases. Walls and ceilings are smooth sealed finish to allow for decontamination. Whole lab decontamination is considered when gross contamination has occurred, when there is a change in lab usage, for renovations, for maintenance shutdowns, or decommissioning.

Furniture

Furniture in the lab must be appropriate for the anticipated use. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

Chairs used in lab work are covered with a non-porous material that can be easily cleaned and disinfected.

Windows

All windows in the lab are sealed.

Lighting

Illumination is adequate for all activities and avoids reflection and glare.

Aerosol producing equipment

Equipment that may produce infectious aerosols (e.g., centrifuges, blenders, sonicators, etc.) must be used in containment devices that HEPA filter the exhaust air before being released to the laboratory. The HEPA filters must be tested or changed at least annually.

Vacuum lines

Vacuum lines are protected by High Efficiency Particulate Air (HEPA) filters. Filters are replaced as needed. Liquid disinfectant trap is required and emptied after end of work.

Airflow

A ducted ventilation system that provides directional airflow from “clean” areas to “potentially contaminated” ones is required. The lab is designed so that under failure conditions that airflow will not be reversed. Lab personnel can verify directional airflow. A means of visual verification of airflow is available. Audible alarms are considered. Exhaust air is dispersed away from occupied building areas and from air intakes (or must be HEPA filtered) and cannot be recirculated to other areas of the building. HEPA filter housings have gas – tight isolation dampers, decontamination ports, and/or bag – in/bag – out capability. They also allow for leak testing of each filter and assembly and are certified at least annually.

Biological safety cabinet exhaust air

The HEPA filtered exhaust air from a Class II BSC can be re-circulated within the laboratory if the cabinet is certified annually and operated according to the manufacturer’s recommendations. The cabinet can also be connected to the building exhaust. Class III cabinets are directly connected to the building exhaust through the second exhaust HEPA filter of the cabinet. Air supply is provided in a way that does not allow for positive pressurization of the cabinet or the room.

Facility Decontamination

Facility is designed to allow decontamination of the entire lab space when gross contamination occurs, significant changes in usage, major renovations, or maintenance shutdowns. Facility design consideration is also given to means of decontaminating large pieces of equipment before removal from the lab.

Enhanced environmental and personal protection may be necessary based on risk assessment and regulations. Anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas-tight dampers to facilitate lab isolation; final HEPA filtration of the lab exhaust air; lab effluent decontamination; containment of other piped services; or advanced access control devices such as biometrics.

HEPA filter housings have gas-tight isolation dampers, decontamination ports, and/or bag-in/bag-out capability. Located near as practicable to the lab to minimize the length of potentially contaminated ductwork. Housing allows for leak testing of each filter and assembly and are certified at least annually.

Communication systems are provided between lab and the outside. Provisions for emergency communication and access or egress are developed and implemented.

Facility verification

The facility design, operational parameters and procedures are verified and documented before initial operation or as needed (i.e., when facilities are affected by natural disasters). Annual facility re-verification is required.

LABORATORY ANIMAL FACILITIES

Like laboratories, animal facilities, may be designated according to a risk assessment and the risk group of the microorganisms under investigation, as Animal facility Biosafety Level 1, 2, 3, or 4.

With respect to agents to be used in the animal laboratory, factors for consideration include:

- The normal route of transmission
- The volumes and concentrations to be used
- The route of inoculation
- Whether and by what route these agents may be excreted

With respect to animals to be used in the animal laboratory, factors for consideration include:

- The nature of the animals, i.e., their aggressiveness and tendency to bite and scratch
- Their natural ecto- and endoparasites
- The zoonotic diseases to which they are susceptible
- The possible dissemination of allergens

The requirements for design features, equipment and precautions increase according to the animal biosafety level. These are described below and summarized in Appendix G – Lab and Animal Biosafety Level Summaries.

Animal Facility – Biosafety Level 1 (ABSL-1)

Suitable for work in animals involving well-characterized agents not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and environment.

Facilities should be separated from the general traffic patterns of the building and restricted as appropriate. Special containment equipment or facility design may be required by risk assessment determination.

Personnel have the specific training in animal facility procedures and are supervised by an individual with adequate knowledge of potential hazards and experimental animal procedures.

Standard Microbiological Practices

Prior to initiation of work:

All procedures involving animals are approved by the Institutional Animal Care and Use Committee (IACUC) before initiation of work.

Facility specific biosafety manual

Each animal facility supplements this biosafety manual with information that is specific for the facility. Supplemental information may include specific PPE practices and location of supplies, laboratory specific training requirements, laboratory specific waste handling practices and autoclave procedures, safe operation and decontamination of lab specific equipment, proper use of disinfectants specific for the lab

(appropriate concentration, contact time and shelf life), etc. It is the responsibility of the facility director to ensure that all personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.

Training

All personnel complete required EHS training courses. See the following section of this manual for a description of courses: *General Laboratory Practices- Training*. The facility director ensures that all personnel receive site-specific training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel receive annual updates and additional training when procedures or policies change. Records are maintained.

Medical surveillance

All personnel involved in animal research complete an assessment through Occupational Health before work is initiated which includes an animal allergy portion. All personnel using human-derived materials or cell lines participate in the Bloodborne Pathogens Program. See the MSU Exposure Control Plan for additional information. For the use of other agents, medical surveillance and immunizations will be provided as appropriate. Those people using respirators participate in the Respiratory Protection Program. Occupational Health as well as the EHS are contacted for assistance.

Door signage

Entrances to all animal areas must have an “Admittance to Authorized Personnel Only” label. This label contains appropriate contact information for general and emergency entrance to the lab. Additionally, the lab entrance must be labeled with: Animal biosafety level, applicable occupational health requirements, personal protective equipment requirements, contact information for the person responsible, as well as any specific procedures for entering and exiting the area. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room. Security – sensitive agent information is posted in accordance with MSU policy.

Controlled access

The facility supervisor ensures that access to the animal areas is controlled. Only people necessary are allowed into the facility. When procedures are in progress the lab door is closed and when no one is present in the lab the doors are locked. Anyone requesting access to the facility is questioned as to their purpose and identification is provided. All people requesting access are advised of the potential hazards as well as appropriate safeguards.

Personal protective equipment (PPE)

The use of laboratory coats, gowns or uniforms is required to prevent contamination of street clothing. It is recommended to tie back or cover long hair to avoid contamination. Splash goggles and face protection must be used when there is the potential for splashes of microorganisms or other hazardous materials. Respirators must be worn as appropriate. Gloves are worn as protection from hazardous materials and when handling animals. Two pairs are worn as appropriate. If latex gloves are used, alternatives are made available. Gloves are changed when contaminated, when the integrity has been compromised, or when necessary. Disposable gloves are not to be washed or reused. All PPE is doffed so that the transfer of infectious materials to areas beyond where they or animals are being handled is minimized. Hand hygiene after removing gloves, and before leaving the animal room. Used disposable PPE is disposed of with other contaminated waste. Reusable PPE (i.e., goggles) is appropriately decontaminated before reuse. Reusable protective clothing is laundered through MSU Laundry. It is not

to be taken home. If visibly contaminated, laundry is placed in a biohazard bag before it is placed with other items to go to laundry. Hands are washed immediately after doffing gloves and before leaving.

Eating, drinking, handling contact lenses and applying cosmetics

Eating, drinking, contact lens handling and cosmetic application is done outside of animal and procedure rooms. Food and beverages for human consumption is stored outside of the animal and procedure areas in refrigerators or cabinets designated for that purpose.

Minimize splashes and aerosols

Essentially all laboratory procedures involve steps which create aerosols. All procedures are completed in a manner which minimizes the creation of both splashes and aerosols. This can be done by using centrifuges with safety features (i.e., sealed cups and rotors), mechanical pipettors, conducting work inside of a biological safety cabinet, etc.

Handwashing

Hands are washed with soap and water after handling potentially infectious materials. Hands are washed before leaving the laboratory and before touching common use surfaces (i.e, computers, telephones, etc.).

Pipetting

Mechanical pipetting devices are available and used. Mouth pipetting is prohibited.

Safe sharps practices

All policies regarding the safe use of sharps are followed. See the following section of this manual for additional information: *Recommended Work Practices- Sharps*.

Decontaminate work surfaces

Work surfaces are decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant is used.

Non-research related animals and plants in the laboratory

Animals and plants not associated with the work being done are not allowed in areas where work with infectious materials or animals is being done or where infectious materials are stored, or animals are housed.

Pest management program

A pest management program is managed through IPF. They are contacted at the first sign of a problem.

Proper decontamination and transport of waste

All cultures, stocks, wastes from animal rooms, and other biohazardous materials are decontaminated before disposal. If you will be transporting waste out of the laboratory (e.g., down the hall, to another floor of the building, etc.) to be decontaminated you ensure that the waste is placed in a leak-proof, covered container and is secured. Refer to the section of this manual: *Biohazardous Waste*, and the MSU Biohazardous Waste Management Plan for additional information regarding the proper decontamination of biohazardous waste.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

Containment equipment

A biological safety cabinet or other containment devices are not generally required. However, this must be determined by conducting a risk assessment.

Personal protective equipment (PPE)

The use of laboratory coats, gowns or uniforms is recommended. Protective clothing must not be worn outside of areas where infectious materials or animals are being handled. Uniforms must not be worn outside of the animal facility. It is recommended that long hair be tied back or covered to avoid contamination. Splash goggles must be worn when there is the potential for splashes of microorganisms or other hazardous materials. Personnel who wear contact lenses should wear safety glasses or other eye protection when in areas with a potential for high concentrations of airborne particles. Gloves must be worn as protection from hazardous materials. If latex gloves are used, alternatives should be made available. Gloves must be changed when contaminated, when the integrity has been compromised, or when necessary. Disposable gloves should be disposed of with other contaminated waste and must not be washed or reused. Hands must be washed after removing gloves, and before leaving the laboratory. Gloves cannot be worn outside of the animal room.

Laboratory Facilities (Secondary Barriers)

Location

The animal facility is in an area of the building that is not open to unrestricted foot traffic.

Doors

Self-closing and self-locking external doors are required for access control. Doors to animal rooms and areas where infectious materials are stored or used are self-closing. They are closed when animals are present inside the room and they are kept locked when no one is present in the room.

Sink

A sink is available and supplied for handwashing (i.e., stocked with soap and paper towels). Sink traps are filled with water or other appropriate liquid.

Easily cleaned

The lab is designed in a way that allows it to be cleaned easily. Spaces between benches, cabinets and equipment are accessible for cleaning. Interior surfaces are water resistant. Floors are slip resistant, impervious to liquids and resistant to chemicals. It is recommended that interior penetrations be sealed to allow for proper pest control and proper cleaning.

Furniture

Furniture is appropriate for the anticipated use. Bench tops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Chairs used in conjunction with lab work are covered with a non-porous material that can be easily cleaned and disinfected.

Windows

If the facility has windows, they are break resistant. If they can be opened to the outdoors, they are fitted with screens.

Airflow

Ventilation systems must allow for inward flow of air without recirculation of exhaust air. Ventilation must be in accordance with the *Guide for Care and Use of Laboratory Animals.* The system design considers the heat and high moisture load produced during the cleaning of animal rooms and the cage wash process.

Appurtenances

Internal appurtenances (e.g., light fixtures, air ducts, etc.) are installed to minimize horizontal surfaces. This facilitates cleaning and minimizes debris and fomite accumulation.

Floor drains

Traps are filled with water or disinfectant as appropriate.

Cage washers

Cages are preferentially washed with an automatic cage washer. The cage washer has a final rise temperature of 180ºF.

Lighting

Lighting must be adequate for all activities. Reflections and glare are avoided.

Eyewash stations and showers

An eyewash station and shower are readily available. Location is determined by risk assessment.

Animal Facility – Biosafety Level 2 (ABSL-2)

This is suitable for work involving animals that are infected with agents assigned to Risk Group 2 and builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1.

Standard Microbiological Practices

Prior to initiation of work

All procedures involving animals are approved by the Institutional Animal Care and Use Committee (IACUC) before initiation of work.

Facility specific biosafety manual

Each animal facility supplements this biosafety manual with information that is specific for the facility. Supplemental information may include specific PPE practices and location of supplies, laboratory specific training requirements, laboratory specific waste handling practices and autoclave procedures, safe operation and decontamination of lab specific equipment, proper use of disinfectants specific for the lab (appropriate concentration, contact time and shelf life), etc. It is the responsibility of the facility director to

ensure that all personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.

Training

All personnel complete required EHS training courses. See the following section of this manual for a description of courses: *General Laboratory Practices- Training*. The facility director ensures that all personnel receive site-specific training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel receive annual updates and additional training when procedures or policies change. Records are maintained.

Medical surveillance

All personnel involved in animal research complete an assessment through Occupational Health before work is initiated. All personnel using human-derived materials or cell lines participate in the Bloodborne Pathogens Program. See the MSU Exposure Control Plan for additional information. For the use of other agents, medical surveillance and immunizations are provided as appropriate. Those people using respirators participate in the Respiratory Protection Program. Occupational Health as well as the EHS are contacted for assistance.

Door signage

Entrances to all animal areas have an “Admittance to Authorized Personnel Only” label. This label contains appropriate contact information for general and emergency entrance to the lab. Additionally, the lab entrance is labeled with an Animal Biosafety Level 2 door sign and a signed Animal Hazard Control Form. These signs include applicable occupational health requirements, personal protective equipment requirements, contact information for the person responsible, as well as any specific procedures for entering and exiting the area. Identification of specific infectious agents is recommended when more than one agent is being used within an animal room. Security – sensitive agent information is posted in accordance with MSU policy.

Controlled access

The facility supervisor ensures that access to the animal areas is controlled. Only those people necessary are allowed into the facility. When procedures are in progress the lab door is shut and when no one is present in the lab the doors are locked. Anyone requesting access to the facility is questioned as to their purpose and identification is provided. All people requesting access are advised of the potential hazards as well as appropriate safeguards.

Personal protective equipment (PPE)

The use of laboratory coats, gowns or uniforms is required to prevent contamination of street clothing. Splash goggles and face protection is used when there is the potential for splashes of microorganisms or other hazardous materials. Respirators are worn as appropriate. It is recommended that long hair be secured or placed within a hair net to avoid contamination. Gloves are worn as protection from hazardous materials and when handling animals. Two pairs are worn as appropriate. If latex gloves are used, alternatives are made available. Gloves are changed when contaminated, when the integrity has been compromised, or when necessary. Disposable gloves are not to be washed or reused. All PPE is doffed so that the transfer of infectious materials to areas beyond where they or animals are being handled is minimized. Hand hygiene is performed after removing gloves, and before leaving the animal room. Used disposable PPE is disposed of with other contaminated waste. Reusable PPE (i.e., goggles) is appropriately decontaminated before reuse. Reusable protective clothing is laundered through MSU

Laundry. It is not to be taken home. If visibly contaminated, laundry is placed in a biohazard bag before placed with other items to go to laundry.

Eating, drinking, handling contact lenses and applying cosmetics

Eating, drinking, contact lens handling and cosmetic application is done outside of animal and procedure rooms. Food and beverages for human consumption are stored outside of the animal and procedure areas in refrigerators, freezers, or cabinets designated for that purpose.

Minimize splashes and aerosols

Essentially all laboratory procedures involve steps which create aerosols. All procedures are completed in a manner which minimizes the creation of both splashes and aerosols. This can be done by using centrifuges with safety features (i.e., sealed cups and rotors), mechanical pipettors, conducting work inside of a biological safety cabinet, etc.

Hand Hygiene

Hands are washed with soap and water after handling potentially infectious materials. Hands are washed before leaving the laboratory and before touching common use surfaces (i.e, computers, telephones, etc.).

Pipetting

Mechanical pipetting devices are available and used. Mouth pipetting is prohibited.

Safe sharps practices

All policies regarding the safe use of sharps are followed. See the following section of this manual for additional information: *Recommended Work Practices- Sharps*.

Decontaminate work surfaces

Work surfaces are decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant is used.

Non-research related animals and plants in the laboratory

Animals and plants not associated with the work being done are not allowed in areas where work with infectious materials or animals is being done or where infectious materials are stored or animals are housed.

Pest management program

A pest management program is managed through IPF. They are contacted at the first sign of a problem.

Proper decontamination and transport of waste

All cultures, stocks, wastes from animal rooms, and other biohazardous materials are decontaminated before disposal. If you will be transporting waste out of the laboratory (e.g., down the hall, to another floor of the building, etc.) to be decontaminated ensure that the waste is placed in a leak-proof, covered container and is secured. Refer to the following section of this manual: *Biohazardous Waste*, and the MSU Biohazardous Waste Management Plan for additional information regarding to the proper decontamination of biohazardous waste.

Special Practices

Medical surveillance

A medical surveillance program is implemented as indicated by risk assessment. It will apply to animal caretakers, laboratory and support personnel. All personnel using human-derived materials or cell lines participate in the Bloodborne Pathogens Program. See the following section of this manual for additional information: *Medical Surveillance*. For the use of other agents, medical surveillance and immunizations will be provided as appropriate. Occupational Health as well as the EHS are contacted for assistance.

Aerosol generating procedures

A biological safety cabinet, or a combination of PPE and other containment devices (as approved by the Biological Safety Officer) is used when there is the potential for the creation of infectious aerosols. This includes, but is not limited to: pipetting, centrifuging, mixing, sonicating, blending, shaking, opening containers, intranasal inoculation of animals, and harvesting tissues. Centrifugation of these materials may be done outside of the BSC if sealed rotors or centrifuge safety cups are used.

Restraint devices

Restraint devices and practices that reduce risk of exposure while handing animals are used as appropriate.

Proper decontamination and transport of waste

All cultures, stocks, wastes from animal rooms, and other biohazardous materials are decontaminated before disposal. This includes potentially infectious animal tissues, carcasses, bedding, feed, sharps, etc. If you will be transporting waste materials outside of the areas where infectious materials or animals are housed or manipulated (e.g., down the hall, to another floor of the building, etc.) to be decontaminated ensure that the waste is placed in a leak-proof, covered container and is secured. The container is surface disinfected before transport and should bear a biohazard label. Refer to the following section of this manual: *Biohazardous Waste*, and the MSU Biohazardous Waste Management Plan for additional information regarding to the proper decontamination of biohazardous waste.

Decontamination of equipment

Lab equipment is decontaminated routinely. All equipment is decontaminated before being repaired, maintained, or removed from the laboratory. When any of these is to occur lab, personnel complete an Equipment Release Form and attach it to the piece of equipment. See Appendix E for an example of the form. It is decontaminated after spills, splashes or when potentially contaminated. All spills are cleaned by personnel who are properly trained and have the proper equipment to handle infectious materials. All ABSL-2 labs have a biological spill kit available. See the following section of this manual for spill cleanup procedures and spill kit contents: *Biohazard Spill Cleanup Procedures*.

Exposure incidents

Exposure response procedures is posted in an easily accessible location in the laboratory. All lab personnel are made aware of the proper procedures to follow in the event of a possible exposure to potentially infectious materials. See Appendix F for exposure response procedures.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

Containment equipment

A biological safety cabinet (BSC), or a combination of PPE and other containment devices (as approved by the Biological Safety Officer) must be used when there is the potential for the creation of infectious aerosols or splashes. This includes, but is not limited to pipetting, centrifuging, mixing, sonicating, blending, shaking, opening containers, intranasal inoculation of animals, and harvesting tissues. A BSC must also be used when handling large volumes or high concentrations of potentially infectious materials. The BSC must be properly maintained as per the manufacturer's recommendations. This includes certification of the cabinet at least annually, when moved and when serviced. Animals are housed in primary containment equipment when it is indicated by the risk assessment.

Personal protective equipment (PPE)

Appropriate personal protective equipment is determined by the risk assessment. The use of laboratory coats, gowns or uniforms and other required PPE are worn while in areas where infectious materials or animals are manipulated or housed. Protective clothing is worn outside of areas where infectious materials or animals are being handled. Uniforms are not to be worn outside of the animal facility. It is recommended that long hair be secured or placed within a hair net to avoid contamination. Splash goggles are worn when there is the potential for splashes of microorganisms or other hazardous materials. Personnel who wear contact lenses, wear safety glasses or other eye protection when in areas with a potential for high concentrations of airborne particles. Gloves are worn as protection from hazardous materials. If latex gloves are used, alternatives are made available. Gloves are changed when contaminated, when the integrity has been compromised, or when necessary. Disposable gloves are disposed of with other contaminated waste and are not to be washed or reused. Hands are washed after removing gloves, and before leaving the laboratory. Gloves cannot be worn outside of the animal room. All reusable protective clothing is laundered by MSU laundry or at the animal facility. It cannot be taken home.

Laboratory Facilities (Secondary Barriers)

Location

The animal facility is in an area of the building that is not open to unrestricted foot traffic.

Doors

Self-closing and self-locking external doors are required for access control. Doors to animal rooms and areas where infectious materials are stored or used open inward and be self-closing. They are closed when animals are present inside the room and they are kept locked when no one is present in the room.

Sink

A sink is available and supplied for handwashing (i.e., stocked with soap and paper towels). The sink is located near the exit. Sink traps are filled with water or other appropriate liquid.

Easily cleaned

The lab is designed in a way that allows it to be cleaned easily. Spaces between benches, cabinets and equipment are accessible for cleaning. Interior surfaces are water resistant. Floors are slip resistant, impervious to liquids and resistant to chemicals. It is recommended that interior penetrations be sealed to allow for proper pest control and proper cleaning.

Furniture

Furniture is appropriate for the anticipated use. Bench tops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Chairs used in conjunction with lab work are covered with a non-porous material that can be easily cleaned and disinfected.

Windows

If the facility has windows, they are break resistant and sealed.

Airflow

Ventilation systems allows for inward flow of air without recirculation of exhaust air. Ventilation is in accordance with the *Guide for Care and Use of Laboratory Animals.*

Appurtenances

Internal appurtenances (e.g., light fixtures, air ducts, etc.) are installed to minimize horizontal surfaces. This facilitates cleaning and minimizes debris and fomite accumulation.

Floor drains

Traps are filled with water or disinfectant as appropriate.

Cages

Cages are decontaminated before being washed. The cage washer has a final rinse temperature of 180ºF.

Lighting

Lighting must be adequate for all activities. Reflections and glare is avoided.

Biological safety cabinets (BSCs)

Biological safety cabinets (BSC) are installed in a manner so that changes in room air do not interfere with the operation of the cabinet. They are located away from doors, windows that can be opened, high traffic areas, and other areas that could cause disruptions in the airflow of the cabinet. They are tested and certified at least annually and operated in accordance with the manufacturer's recommendations.

Autoclave

It is recommended that an autoclave be available in the facility.

Eyewash stations and showers

An eyewash station and safety shower is readily available.

Animal Facility – Biosafety Level 3 (ABSL-3)

This is suitable for work involving animals that are infected with agents assigned to Risk Group 3.

Standard Microbiological Practices

Prior to initiation of work

All procedures involving animals are approved by the Institutional Animal Care and Use Committee (IACUC) before initiation of work.

Facility specific biosafety manual

Each animal facility supplements this biosafety manual with information that is specific for the facility. Supplemental information may include specific PPE practices and location of supplies, laboratory specific training requirements, laboratory specific waste handling practices and autoclave procedures, safe operation and decontamination of lab specific equipment, proper use of disinfectants specific for the lab (appropriate concentration, contact time and shelf life), etc. It is the responsibility of the facility director to ensure that all personnel are advised of potential hazards and are required to read and follow instructions on practices and procedures.

Training

All personnel complete required EHS training courses. See the following section of this manual for a description of courses: *General Laboratory Practices- Training*. The facility director ensures that all personnel receive site-specific training regarding their duties, animal husbandry procedure, potential hazards, manipulations of infectious agents, necessary precautions to prevent hazard or exposures, and hazard/exposure evaluation procedures (physical hazards, splashes, aerosolization, etc.). Personnel receive annual updates and additional training when procedures or policies change. Records are maintained.

Medical surveillance

All personnel involved in animal research complete an assessment through Occupational Health before work is initiated. All personnel using human-derived materials or cell lines must participate in the Bloodborne Pathogens Program. See the MSU Exposure Control Plan for additional information. For the use of other agents, medical surveillance and immunizations will be provided as appropriate. Those people using respirators participate in the Respiratory Protection Program. Occupational Health as well as the EHS should be contacted for assistance.

Door signage

Entrances to all animal areas have an “Admittance to Authorized Personnel Only” label. This label contains appropriate contact information for general and emergency entrance to the lab. Additionally, the lab entrance is labeled with an Animal Biosafety Level 3 door sign and a signed Animal Hazard Control Form. These signs include applicable occupational health requirements, personal protective equipment requirements, contact information for the person responsible, as well as any specific procedures for entering and exiting the area.

Controlled access

The facility supervisor ensures that access to the animal areas is controlled. The fewest number of individuals possible are allowed access. Only those people necessary are allowed into the facility. When procedures are in progress the lab door is shut and when no one is present in the lab the doors are locked. Anyone requesting access to the facility is questioned as to their purpose and identification is provided. All people requesting access are advised of the potential hazards as well as appropriate safeguards.

Personal protective equipment (PPE)

The use of laboratory coats, gowns or uniforms is required to prevent contamination of street clothing. Splash goggles and face protection are used when there is the potential for splashes of microorganisms or other hazardous materials. Respirators are worn as appropriate. It is recommended that long hair be secured or placed within a hair net to avoid contamination. Gloves are worn as protection from hazardous materials and when handling animals. Two pairs are worn as appropriate. If latex gloves are used, alternatives should be made available. Gloves are changed when contaminated, when the integrity has been compromised, or when necessary. Disposable gloves are not to be washed or reused. All PPE are doffed so that the transfer of infectious materials to areas beyond where they or animals are being handled is minimized. Hand hygiene is performed after removing gloves, and before leaving the animal room. Used disposable PPE is disposed of with other contaminated waste. Reusable PPE (i.e., goggles) is appropriately decontaminated before reuse. Reusable protective clothing is laundered through MSU Laundry. It is not to be taken home. If visibly contaminated, laundry is placed in a biohazard bag before being placed with other items to go to laundry.

Eating, drinking, handling contact lenses and applying cosmetics

Eating, drinking, contact lens handling and cosmetic application is done outside of animal and procedure rooms. Food and beverages for human consumption is stored outside of the animal and procedure areas in refrigerators or cabinets designated for that purpose.

Minimize splashes and aerosols

Essentially all laboratory procedures involve steps which create aerosols. All procedures are completed in a manner which minimizes the creation of both splashes and aerosols. This can be done by using centrifuges with safety features (i.e., sealed cups and rotors), mechanical pipettors, conducting work inside of a biological safety cabinet, etc.

Hand Hygiene

Hands are washed with soap and water after handling potentially infectious materials. Hands are washed before leaving the laboratory and before touching common use surfaces (i.e, computers, telephones, etc.).

Pipetting

Mechanical pipetting devices are available and used. Mouth pipetting is prohibited.

Safe sharps practices

All policies regarding the safe use of sharps are followed. See the following section of this manual for additional information: *Recommended Work Practices- Sharps*.

Decontaminate work surfaces

Work surfaces are decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant is used.

Non-research related animals and plants in the laboratory

Animals and plants not associated with the work being done are not allowed in areas where work with infectious materials or animals is being done or where infectious materials are stored, or animals are housed.

Pest management program

A pest management program is managed through IPF. They are contacted at the first sign of a problem.

Proper decontamination and transport of waste

All cultures, stocks, wastes from animal rooms, and other biohazardous materials is decontaminated before disposal. If you will be transporting waste out of the laboratory (e.g., down the hall, to another floor of the building, etc.) to be decontaminated ensure that the waste is placed in a leak-proof, covered container and is secured. Refer to the following section of this manual: *Biohazardous Waste* and the MSU Biohazardous Waste Management Plan for additional information regarding to the proper decontamination of biohazardous waste.

Special Practices

Medical surveillance

A medical surveillance program is implemented as indicated by risk assessment. It applies to animal caretakers, laboratory, and support personnel. All personnel using human-derived materials or cell lines participate in the Bloodborne Pathogens Program. See the MSU Exposure Control Plan for additional information. For the use of other agents, medical surveillance and immunizations is provided as appropriate. Occupational Health as well as the EHS should be contacted for assistance.

Work conducted inside of a biological safety cabinet

A biological safety cabinet, or a combination of PPE and other containment devices (as approved by the Biological Safety Officer) is used when working with infectious materials, infected animals or there is the potential for the creation of infectious aerosols. This includes, but is not limited to: pipetting, centrifuging, mixing, sonicating, blending, shaking, opening containers, intranasal inoculation of animals, and harvesting tissues. Restraint devices and practices that reduce risk of exposure while handing animals is considered as appropriate.

Containment caging systems

Consideration is given to the use of containment caging systems to reduce the risk of infectious aerosols from animals and bedding.

Ventilated caging systems

Caging systems are ventilated to prevent escape of microbes from the cage. Exhaust plenums are sealed, and the exhaust HEPA filtered. The system is alarmed to indicate when malfunctions occur.

Proper decontamination and transport of waste

All cultures, stocks, wastes from animal rooms, and other biohazardous materials are decontaminated before disposal. This includes potentially infectious animal tissues, carcasses, bedding, feed, sharps, etc. An approved method of decontamination is available in the facility. If you will be transporting waste materials outside of the areas where infectious materials or animals are housed or manipulated (e.g., down the hall) to be decontaminated ensure that the waste is placed in a leak-proof, covered container and is secured. The container is surface disinfected before transport and bears a biohazard label. Please refer to the Biohazardous Waste section of this manual and the MSU Biohazardous Waste Management Plan for additional information regarding to the proper decontamination of biohazardous waste.

Decontamination of equipment

Lab equipment is decontaminated routinely. All equipment is decontaminated before being repaired, maintained, or removed from the laboratory. When any of these is to occur, lab personnel complete an Equipment Release Form and attach it to the piece of equipment. See Appendix E for an example of the form. It is decontaminated after spills, splashes or when potentially contaminated. All spills are cleaned by personnel who are properly trained and have the proper equipment to handle infectious materials. All ABSL-3 labs have a biological spill kit available. See the following section of this manual for spill cleanup procedures and spill kit contents: *Biohazard Spill Cleanup Procedures*.

Exposure incidents

Exposure response procedures is posted in an easily accessible location in the laboratory. All lab personnel are made aware of the proper procedures to follow in the event of a possible exposure to potentially infectious materials. See Appendix F for exposure response procedures.

Safety Equipment (Primary Barriers and Personal Protective Equipment)

Containment equipment

A biological safety cabinet (BSC), or a combination of PPE and other containment devices (as approved by the Biological Safety Officer) are used for all procedures involving infectious materials and animals (when possible). The BSC is properly maintained as per the manufacturer's recommendations. This includes certification of the cabinet at least annually, when moved and when serviced. Housing animals in primary containment equipment can reduce the risk of infectious aerosols from the animals and their bedding.

Personal protective equipment (PPE)

Appropriate personal protective equipment should be determined by the risk assessment. Uniforms, scrub suits or other protective clothing are worn while in the animal facility. Disposable PPE (e.g., wrap-around, or solid front gowns, non-woven olefin cover-all suit, etc.) and other required protective equipment is worn while in areas where infectious materials or animals are manipulated or housed. Front button lab coats are not appropriate. Disposable protective clothing is not to be worn outside of areas where infectious materials or animals are being handled. Uniforms are not worn outside of the animal facility. It is recommended that long hair be secured or placed within a hair net to avoid contamination. Splash goggles are worn when there is the potential for splashes of microorganisms or other hazardous materials. Personnel who wear contact lenses wear safety glasses or other eye protection when in areas with a potential for high concentrations of airborne particles. Gloves are worn as protection from hazardous materials. If latex gloves are used, alternatives are made available. Gloves are changed when contaminated, when the integrity has been compromised, or when necessary. Disposable gloves are disposed of with other contaminated waste and are not to be washed or reused. Hands are washed after removing gloves, and before leaving the laboratory. Gloves are not worn outside of the animal room. Appropriate respiratory protection and foot protection are worn when entering areas where infectious materials or animals are housed. All reusable protective clothing is decontaminated before being laundered by MSU laundry or at the animal facility. It cannot be taken home.

Laboratory Facilities (Secondary Barriers)

Location

The animal facility is in an area of the building that is not open to unrestricted foot traffic.

Doors

Self-closing and self-locking external doors are required for access control. Doors to animal rooms and areas where infectious materials are stored or used, open inward and be self-closing. They are closed when animals are present inside the room, and they are kept locked when no one is present in the room. Entry into the area is through a double-door entry.

Showers

Showers are considered based on the risk assessment.

Sink

A hands-free or automatically operated sink is available and supplied for handwashing (i.e., stocked with soap and paper towels). The sink is located near the exit. Additional sinks are located as appropriate throughout the containment area. If the facility has segregated areas where infectious materials or animals are housed or manipulated, each area has a sink available at the exit. Sink traps must be filled with water or other appropriate liquid.

Easily cleaned

The lab is designed in a way that allows it to be cleaned and decontaminated easily. Spaces between benches, cabinets and equipment are accessible for cleaning. Interior surfaces are water resistant. Floors are slip resistant, impervious to liquids and resistant to chemicals. It is recommended that interior penetrations be sealed to allow for proper pest control and proper cleaning.

Furniture

Furniture is appropriate for the anticipated use and minimized. Cabinets and bench tops are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals. Chairs used in animal areas are covered with a non-porous material that can be easily cleaned and disinfected.

Windows

If the facility has windows, they are break resistant and sealed. External windows are not recommended.

Airflow

Ventilation systems allow for inward flow of air without recirculation of exhaust air. Exhaust is dispersed away from air intakes and occupied areas, or it is HEPA filtered. Ventilation is in accordance with the *Guide for Care and Use of Laboratory Animals.* The direction of airflow is verified before entering the area. Audible alarms and visual monitoring devices are considered.

Appurtenances

Internal appurtenances (e.g., light fixtures, air ducts, etc.) are installed to minimize horizontal surfaces. This facilitates cleaning and minimizes debris and fomite accumulation.

Floor drains

Traps are filled with water or disinfectant as appropriate.

Cages

Cages are decontaminated before being removed from the ABSL-3 space. They are washed using an automatic cage washer. The cage washer has a final rise temperature of 180ºF.

Lighting

Lighting is adequate for all activities. Reflections and glare are avoided.

Biological safety cabinets (BSCs)

Biological safety cabinets (BSC) are installed in a manner so that changes in room air do not interfere with the operation of the cabinet. They are located away from doors, windows that can be opened, high traffic areas, and other areas that could cause disruptions in the airflow of the cabinet. They are tested and certified at least annually and operated in accordance with the manufacturer's recommendations.

Autoclave

An autoclave is conveniently available to the areas where the biohazard is contained.

Eyewash stations and showers

An eyewash station and safety shower are readily available.

Design and operational procedures

Design and operational procedures are documented. The facility is tested prior to use and at least annually to verify that design and operational parameters have been met.

Additional environmental protection

Additional protective measures are considered as determined by the risk assessment and applicable regulations. These measures may include personnel showers, HEPA filtration of exhaust, effluent decontamination, etc.

Invertebrates

As with vertebrates, the animal facility biosafety level is determined by the risk groups of the agents under investigation. Even when arthropods are not infected with human pathogens, they can become a risk to the environment outside of the lab if, by escaping, they complete a transmission cycle for a disease that they vector. For that reason, handling practices, safety equipment and containment facilities are taken into consideration before handling arthropods. For additional information on arthropod containment guidelines, contact the Biosafety Office (355-0153). Guideline document is available on EHS website.

PLANT BIOLOGICAL SAFETY LEVELS

Plant biological safety levels specify physical and biological containment conditions and practices suitable for conducting greenhouse experiments involving recombinant DNA-containing plants, plant-associated microorganisms, and small animals. The primary intent of plant containment is to avoid the unintentional transmission of plant pathogens, noxious native weeds, a recombinant DNA-containing plant genome or the release of recombinant DNA-derived organisms associated with plants.

The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility (e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem).

For experiments in which plants are grown in the laboratory setting, laboratory containment practices are followed as described previously. These containment practices include the use of plant tissue culture rooms, growth chambers within laboratory facilities, or experiments performed on open benches. Additional biological containment practices are added as necessary, if botanical reproductive structures are produced that have the potential of being released.

Plant Biosafety Level 1 (PBSL-1)

Standard Practices

Greenhouse access

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

Records

A record is kept of experiments currently in progress in the greenhouse facility.

Decontamination and inactivation

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

Control of undesired species and motile macroorganisms

A program is implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and Federal laws. Arthropods and other motile macroorganisms are housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions are taken to minimize escape from the greenhouse facility.

Concurrent experiments conducted in the greenhouse

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

Facilities

Definitions

The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.

Greenhouse design

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

Plant Biosafety Level 2 (PBSL-2)

Standard Practices

Greenhouse access

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

Records

A record is kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility. A record is kept of experiments currently in progress in the greenhouse facility. The Principal Investigator reports any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director and the Biological Safety Officer. Documentation of any such accident is prepared and maintained.

Decontamination and inactivation

Experimental organisms are rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility. Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatments are made periodically, to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

Control of undesired species and motile macroorganisms

A program is implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws. Arthropods and other motile macroorganisms are housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions are taken to minimize escape from the greenhouse facility.

Concurrent experiments conducted in the greenhouse

Experiments involving other organisms that require a containment level lower than PBSL-2 may be conducted in the greenhouse concurrently with experiments that require PBSL-2 containment provided that all work is conducted in accordance with PBSL-2 greenhouse practices.

Signs

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

Transfer of materials

Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, are transferred in a closed non-breakable container.

Greenhouse practices manual

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

Facilities

Definitions

The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.

Greenhouse design

A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil. Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds).

Autoclaves

An autoclave is available for the treatment of contaminated greenhouse materials.

Supply and exhaust air ventilation systems

If intake fans are used, measures are taken to minimize the ingress of arthropods. Louvers or fans are constructed such that they can only be opened when the fan is in operation.

Other

BL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses.

For more information refer to NIH Guidelines Appendix P and A Practical Guide to Containment – Plant Biosafety in Research Greenhouses. (Links on EHS website)

LABORATORY BIOSECURITY

Events have brought to the forefront the necessity of having a comprehensive laboratory security program. However, before outlining the biosecurity requirements that have been implemented by the University it is important to understand the distinction between “biosafety” and “biosecurity.”

“Biosafety” is the application of knowledge, techniques, and equipment to prevent personal, laboratory and environmental exposure to potentially infectious agents or other biohazards. “Biosecurity” refers to measures designed to protect microbiological agents from loss, theft, misuse, or intentional release, and to protect research-related information from loss, theft, or misuse. This can be accomplished by limiting access to facilities, biological materials, and research-related information. Sufficient security for the biological materials in use may already be in place for laboratories that do not handle Select Agents, permissible levels of toxins on the Select Agents list or excluded strains of Select Agents. These security measures include access controls and training requirements outlined for BSL-1 and BSL-2 laboratories previously. If you wish to handle Select Agents, permissible levels of Select Agent toxins, excluded strains of Select Agents, other agents of public health or agricultural concern, or agents of high commercial value contact the Biosafety Team for required additional biosecurity requirements.

Elements of the biosecurity program at MSU include:

1. **Physical security:** Access control and monitoring are intended to prevent the removal of materials for unauthorized purposes. Access is limited to authorized personnel based on the necessity of entering sensitive areas. At a minimum, laboratory doors are locked when no one is present in the lab, all storage units housed in shared space (i.e., hallway, storage room, etc.) are locked, and all persons entering the laboratory are asked for identification and questioned as to their purpose for being there. Emergencies requiring emergency responders or public safety entry are also considered. Laboratory emergency response plans also consider bomb threats, natural disasters and sever weather, power outages, or other facility emergencies that may become a security threat.
2. **Personnel Management:** Identifying responsibilities and duties for employees who handle, use, store, and transport dangerous pathogens or other important assets. Employee screening policies are developed for personnel and visitor identification, visitor management, access procedures and reporting incidents.
3. **Inventory and accountability:** It is the responsibility of each laboratory to establish material accountability procedures. These are designed to track the inventory, storage, use, transfer and destruction of biological materials. The purpose is to know what agents are housed in a lab, where they are located and if they are all accounted for. See Appendix H for an example of an inventory log.
4. **Information security:** Policies are maintained for handling sensitive data or information related to the security of pathogens, toxins, biological materials, or other important assets. Some examples are security codes to equipment housing pathogens or access granted to computer data regarding inventories. Include policies for identifying and securing sensitive information on

electronic files or removable electronic media. Material Transfer Agreements are in place when a transfer of data or material will take place outside of the university and has been required by MSU Technologies office.

5. **Transport of biological materials:** Material transport policies are in place that outline requirements for transporting locally on campus and outside of campus. Documentation and material accountability and control procedures for pathogens in transit are included. Measures are instituted ensuring appropriate authorizations have been received and adequate communication between facilities has occurred prior, during, and after transport. See the following section of this manual for additional information: *Introduction to the Transport of Biological Materials*.
6. **Reporting and communication:** In addition to following departmental reporting requirements when a security breach occurs, the laboratory notifies MSU Police and the Biological Safety Officer. Investigation into the breach will occur as appropriate. The "chain – of – notification" is established in advance of an actual event and reviewed periodically.
7. **Training:** Laboratory security awareness training is required for anyone who has access to a laboratory. This training is available online at www.EHS.msu.edu. This training must be completed as soon as possible after hire. In addition, practice drills may be used to address a variety of scenarios and are considered part of site-specific training.
8. **Security Updates and Re-evaluations**
   Biosecurity risk assessments are reviewed and updated routinely and especially following a security incident. Records are maintained.
9. **Select Agents**
   The Select Agent program at MSU will be followed under the regulation requirements of the National Select Agent Program.

SAFETY EQUIPMENT

As aerosols are important sources of infection, care should be taken to reduce the extent of their formation and dispersion. Hazardous aerosols can be generated by many laboratory operations, e.g., blending, mixing, grinding, shaking, stirring, sonicating, and centrifuging of infectious materials. Even when safe equipment is used, it is best to carry out these operations in an approved biological safety cabinet whenever possible. The use of safety equipment is no assurance of protection unless the user is trained and uses proper techniques. Equipment should be tested regularly to ensure its continued safe performance. Table 2 provides a list of safety equipment designed to eliminate or reduce certain hazards and briefly outlines the safety features. Further details of much of this equipment are given in subsequent pages.

Table 2: Safety Equipment

Equipment	Hazard Corrected	Safety Features
Biological Safety Cabinet --Class I	Aerosol and spatter	Minimum inward airflow (face velocity) at work access opening. Adequate filtration of exhaust air. Does not provide product protection
Biological Safety Cabinet --Class II	Aerosol and spatter	Minimum inward airflow (face velocity) at work access opening. Adequate filtration of exhaust air. Provides product, personnel, and environmental protection
Biological Safety Cabinet --Class III	Aerosol and spatter	Maximum containment. Provides product, personnel, and environmental protection if laminar flow air is included.

Equipment	Hazard Corrected	Safety Features
Pipetting aids	Hazards from pipetting by mouth, e.g., ingestion of pathogens, inhalation of aerosols produced by mouth suction on the pipette, blowing out of liquid or dripping from pipet, contamination of suction end of pipette	Ease of use Controls contamination of suction end of pipette, protecting pipetting aid, user, and vacuum line Can be sterilized Controls leakage from pipette tip
Loop microincinerators, disposable loops	Spatter from transfer loops	Shielded in open-ended glass or ceramic tube. Heated by gas or electricity. Disposable, no heating necessary
Leakproof vessels for collection and transport of infectious materials	Aerosols, spillage, and leakage	Leakproof construction with lid of cover Durable Autoclavable
Sharps disposal containers	Puncture wounds	Robust, puncture-proof
Transport containers between laboratories, buildings	Release of biological materials that can be potentially contaminated with several microorganisms	Robust Watertight primary and secondary containers to contain spills Absorbent materials to contain spills and cushioning materials
Autoclaves, manual or automatic	Infectious material (made safe for disposal or reuse)	Approved design Effective heat sterilization
Screw-capped bottles	Aerosols and spillage	Effective containment
Vacuum line protection	Contamination of laboratory vacuum system with aerosols and overflow fluids	Cartridge-type filter prevents passage of aerosols (particle size 0.45 µm) Overflow flask contains appropriate disinfectant. Rubber bulb may be used to close off vacuum automatically when storage flask is full. Entire unit is autoclavable.

Biological Safety Cabinets (BSCs)

Biological safety cabinets (BSCs) are designed to protect the operator, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating materials containing infectious agents. Aerosol particles are created by any activity that imparts energy into a liquid, such as shaking, pouring, stirring, or dropping liquid onto a surface or into another liquid. Other laboratory activities, such as streaking agar plates, inoculating cell culture flasks with a pipette, using a multichannel pipette to dispense liquid suspensions of infectious agent into microculture plates, homogenizing and vortexing infectious materials, transportation of biological materials, and centrifugation of infectious liquids, or working with animals, can generate infectious aerosols. Aerosol particles of less than 5 µm in diameter and small droplets of 5-100 µm in diameter are not visible to the naked eye. These particles may be inhaled or may cross contaminate work surface materials. BSCs, when properly used, have been shown to be highly effective in reducing laboratory-acquired infections and cross-contaminations of cultures due to aerosol exposures.

BSCs are designed to provide personnel, environmental and product protection when appropriate practices and procedures are followed. Three kinds of biological safety cabinets, designated as Class I, II and III are available. Biological safety cabinets use high efficiency particulate air (HEPA) filters in their exhaust and/or supply systems. The HEPA filter traps 99.97% of particles of 0.3 µm in diameter and 99.99% of particles of greater or smaller size. Biological safety cabinets must not be confused with other laminar flow devices or “clean benches.” Horizontal flow cabinets direct air towards the operator and should never be used for handling infectious or toxic materials.

For more information on biological safety cabinets, please refer to the CDC/NIH publication: *Primary Containment for Biohazards Selection, Installation and Use of Biological Safety Cabinets.*

Class I Biological Safety Cabinet

This is a ventilated cabinet for personnel protection with an un-recirculated inward airflow away from the operator. The air from the cabinet is exhausted through a HEPA filter: (a) into the laboratory and then to the outside of the building exhaust; (b) to the outside through the building exhaust; or (c) directly to the outside. The HEPA filter may be in the exhaust plenum of the BSC or in the building exhaust. Some Class I BSCs are equipped with an integral exhaust fan, whereas others rely on the exhaust fan in the building exhaust system.

The Class I BSC was the first recognized BSC and, because of its simple design, is still in wide use throughout the world. It has the advantage of providing personnel and environmental protection and can also be used for work with radionuclides and volatile toxic chemicals. Because unsterilized room air is drawn over the work surface through the front opening, it does not provide product protection.

Class II Biological Safety Cabinet

This is a ventilated cabinet for personnel, product and environmental protection which provides inward airflow and HEPA-filtered supply and exhaust air. The Class II cabinet has four designs depending on how much air is recirculated and/or exhausted and if the BSC is hard ducted to the ventilation system or not. Class II cabinets may be of use with low to moderate risk biological agents, minute quantities of toxic chemicals, and trace quantities of radionuclides; however, care must be exercised in selecting the correct Class II cabinet design for these purposes.

Class II Type A1 Biological Safety Cabinet

An internal fan draws room air (supply air) into the cabinet through the front opening and into the front intake grill. The supply air then passes through a supply HEPA filter before flowing downwards over the work surface. As the air flows downwards it “splits” about 6-18 cm from the work surface, one half of the downwards flowing air passing through the front exhaust grill, and the other half passing through the rear exhaust grill. Any aerosol particles generated at the work surface are immediately captured in this downward airflow and passed through the front or rear exhaust grills, thereby providing the highest level of product protection. The air is then discharged through the rear plenum into the space between the supply and exhaust filters located at the top of the cabinet. About 70% of the air recirculates through the supply HEPA filter back into the work zone; the remaining 30% passes through the exhaust filter into the room or to the outside.

Air from the Class IIA1 BSC exhaust can be recirculated to the room or discharged to the outside of the building through a thimble connection to a dedicated duct or through the building exhaust system. A connection to a ducted exhaust system also allows some BSCs to be used for work with limited volatile radionuclides and volatile toxic chemicals (Table 3).

Class II Type A2 Vented to the Outside, B1 and B2 Biological Safety Cabinets

Class IIA2 vented to the outside, IIB1 and IIB2 BSCs are variations of the Class IIA1. Each variation allows the BSC to be used for specialized purposes (see Table 3). These BSCs differ from one another in several aspects: the air intake velocity through the front opening; the amount of air recirculated over the work surface and exhausted from the cabinet’s exhaust system, which determines whether air from the cabinet is exhausted to the room, or to the outside, through a dedicated exhaust system or through the building exhaust and the pressure arrangements (whether cabinets have biologically contaminated ducts and plenums are surrounded by negative-pressure ducts and plenums).

Class II Type C Biological Safety Cabinets

Class II type C is equipment that can be used in a recirculating type (i.e., type A BSC) when working with biological agents, or can be connected to an exhaust system to work as type B BSC for handling hazardous chemical vapors and radionuclides. These BSCs differ from the other types as provides personnel, product, and environmental protection by filtering hazardous particles such as biological agents and also to other hazards such as antineoplastic drugs, genetic material, carcinogens, allergens, or other airborne hazards.

Class III Biological Safety Cabinet

This type provides the highest level of personnel protection and in used for Risk Group 4 agents. All penetrations are sealed "gas tight." Supply air is HEPA-filtered and exhaust air passes through two HEPA filters. Airflow is maintained by a dedicated exhaust system exterior to the cabinet, which keeps the cabinet interior under negative pressure. Access to the work surface is by means of heavy-duty rubber gloves, which are attached to ports in the cabinet. The Class III BSC should have an attached pass-through box that can be sterilized and is equipped with a HEPA-filtered exhaust. The Class III cabinet may be connected to a double-door autoclave used to decontaminate all materials entering to exiting the cabinet. Several glove boxes can be joined together to extend the work surface. Class III BSCs are suitable for work in Biosafety Level 3 and 4 laboratories. There are also some devices that use ULPA (Ultra Low Particulate Air) filters which will remove 99.999% of particulates that are greater than or equal to 0.1um in size.

Table 3: Selection of a biological safety cabinet (BSC), by type of protection needed

Type of Protection	BSC Selection
Personnel protection, microorgansims in Risk Groups 1-3	Class I, Class II, Class III
Personnel protection, microorganisms in Risk Group 4, glove box laboratory	Class III
Personnel protection, microorganisms in Risk Group 4, Suit Laboratory	Class I, Class II
Product protection	Class II, Class III only if laminar flow included
Volatile radionuclide/chemical protection, minute amounts	Class IIB1, Class IIA2 vented to the outside, Class IIC1
Volatile radionuclide/chemical protection	Class I, Class IIB2, Class III
Antineoplastic drugs, genetic material, carcinogens, allergens	Class IIC1

Selection of a biological safety cabinet

A BSC should be selected primarily in accordance with the type of protection needed: product protection; personnel protection against biohazards; personnel protection against exposure to radionuclides and volatile toxic chemicals; or a combination of these. Table 3 shows which BSCs are recommended for each type of protection.

Volatile or toxic chemicals should not be used in BSCs that recirculate exhaust air to the room, i.e. Class I BSCs that are not ducted to building exhaust systems, or Class IIA1 or Class IIA2 cabinets. Class IIB1 BSCs are acceptable for work with minute amounts of volatile chemicals and radionuclides. A Class IIB2 or a Class IIC1 BSC, also called a total exhaust cabinet, is necessary when significant amounts of radionuclides and volatile chemicals are expected to be used.

Using Biological Safety Cabinets in the Laboratory

Location

The velocity of air flowing through the front opening into a BSC is about 0.45 m/s. At this velocity the integrity of the directional air inflow can be easily disrupted by air currents generated by people walking close to the BSC, open windows, air supply registers, and opening and shutting doors. Ideally, BSCs should be situated in a location away from traffic and potentially disturbing air currents. Whenever possible a 30-cm clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance. A clearance of 30-35 cm above the cabinet may be required to provide for accurate air velocity measurement across the exhaust filter and for exhaust filter changes.

Operators

If BSCs are not used properly, their protective benefits are reduced. Operators need to be careful not to disrupt the air inflow when moving their arms into and out of cabinets. Arms should be moved in and out slowly, perpendicular to the front opening. Operators should not begin work until one minute after placing hands and arms inside. This will allow the cabinet to adjust and to “air sweep” the surface of the hands and arms. The number of movements across the front opening should be minimized by placing all necessary items inside the cabinet before beginning procedures.

Material Placement

The front intake grill of Class II BSCs must not be blocked with paper, equipment, or other items. Materials to be placed inside the cabinet should be surface decontaminated with 70% alcohol. Work may be performed on disinfectant-soaked absorbent towels to capture splatters and splashes. All materials should be placed as far back in the cabinet, towards the rear edge of the work surface, as practical without blocking the rear grill. Aerosol-generating equipment (e.g., mixers, centrifuges, etc.) should be placed towards the rear of the cabinet. Bulky items, such as biohazard bags, discard pipette trays and suction collection flasks should be placed to one side of the inside of the cabinet. Active work should flow from clean to contaminated areas across the work surface.

The autoclavable biohazard collection bag and pipette collection tray should not be placed outside the cabinet. The frequent in-and-out movement needed to use these containers is disruptive to the integrity of the cabinet’s air barrier and can compromise both personnel and product protection.

Operation and Maintenance

Most BSCs are designed to permit operation 24 h/day, and investigators find that continuous operation helps to control the levels of dust and particulate materials in the laboratory. Class IIA1 and IIA2 BSCs exhausting to the room or connected by thimble connections to dedicated exhaust ducts can be turned off when not in use. Other types such as IIB1, IIB2, or IIC1 BSCs, which have hard-duct installations, must always have airflow through them to help maintain room air balance. Cabinets should be turned on at least 5 min before beginning work and after completion of work to allow the cabinet to “purge” (i.e., to allow time for contaminated air to be removed from the cabinet environment).

All repairs made of BSCs should be made by a qualified technician. Any malfunction in the operation of the BSC should be reported and repaired before the BSC is used again.

Ultraviolet Lights

Ultraviolet lights are not required in BSCs. If they are used, they must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the light. Ultraviolet light intensity should be

checked when the cabinet is recertified to ensure that light emission is appropriate. Ultraviolet lights must be turned off while the room is occupied, to protect eyes and skin from inadvertent exposure.

Open Flames

Open flames are not allowed inside the BSC. The Centers for Disease Control and Prevention (CDC) reports that “open-flames are not required in the near microbe-free environment of a biological safety cabinet” and create “turbulence which disrupts the pattern of air supplied to the work surface” jeopardizing the sterility of the work area. This is also the recommendation of the World Health Organization (WHO) as well as the major Biosafety cabinet manufacturers.

Flames compromise the protection of the worker and the work by disrupting the airflow patterns and causing excessive heat buildup damaging the HEPA filter and its components. Recirculation of cabinet air can create flammable atmospheres that directly result in a fire or explosion. The use of flames in the cabinet inactivates the manufacturer’s warranties on the cabinet: cabinet manufacturers will assume no liability in the event of fire, explosion, or worker exposure due to the use of a flammable gas in the cabinet. Additionally, the UL approval will automatically be void.

Sterile, disposable inoculating loops, needles and cell spreaders are available as an alternative to using open flames in the BSC for sterilizing equipment. Electric “furnaces” are also available. If it is deemed necessary for the work being done, use a pilotless burner or touch-plate microburner to provide a flame on demand.

Spills

When a spill of biohazardous material occurs within a BSC, clean-up should begin immediately, while the cabinet continues to operate. An effective disinfectant should be used and applied in a manner that minimizes the generation of aerosols. All materials that meet the spilled agent should be disinfected or autoclaved. See the following section of this manual for additional information on spill cleanup procedures: *Biohazard Spill Cleanup Procedures*.

Certification

The functional operation and integrity of each BSC should be certified to NSF Standard 49 at the time of installation and annually thereafter by qualified technicians. Certification includes tests for cabinet integrity, HEPA filter leaks, downflow velocity profile, face velocity, negative pressure/ventilation rate, air-flow smoke pattern, and alarms and interlocks. Optional tests for electrical leaks, lighting intensity, ultraviolet light intensity, noise level and vibration may also be conducted. Special training, skills and equipment are required to perform these tests. Annual certification is required for BSCs that are used for work with human pathogens, recombinant DNA, or human derived materials (e.g., cell lines, blood, etc.). To request service or certification contact the EHS at 355-0153.

Cleaning and Disinfection

All items within BSCs, including equipment, should be surface-decontaminated and removed from the cabinet when work is completed, since residual culture media may provide an opportunity for microbial growth.

The interior surfaces of BSCs should be decontaminated before and after each use. The work surfaces and interior walls should be wiped with a disinfectant that will kill any microorganisms that might be found inside the cabinet. At the end of the workday, the final surface decontamination should include a wipe-down of the work surface, the sides, back and interior of the glass. A solution of bleach or 70% alcohol should be used when effective for target organisms. A second wiping with sterile water is needed when a corrosive disinfectant, such as bleach, is used.

It is recommended that the cabinet is left running. If not, it should be run for 5 min to purge the atmosphere inside before it is switched off.

Decontamination

BSCs must be decontaminated before filter changes and before being moved. The most common decontamination method is by fumigation with various gases such as hydrogen peroxide, formaldehyde, or chlorine dioxide gas. BSC decontamination should performed by a qualified professional or company.

Personal Protective Equipment

Personal protective clothing should be worn whenever using a BSC. Laboratory coats are acceptable for work being performed at biosafety levels 1 and 2. A solid front, back-closing laboratory gown provides better protection and should be used at biosafety level 3. Gloves should be pulled over the wrists of the gown rather than worn inside. Elasticized sleeves can be worn to protect the investigator's wrists. Masks and safety glasses may be required for some procedures.

Alarms

BSCs can be equipped with one of two kinds of alarm. Sash alarms are found only on cabinets with sliding sashes. The alarm signifies that the sash has been moved to an improper position. Airflow alarms indicate a disruption in the cabinet's normal airflow pattern. This represents an immediate danger to the operator or product. When an airflow alarm sounds, work should cease immediately, and the laboratory supervisor should be notified. Manufacturer's instruction manuals should provide further details.

Pipetting aids

A pipetting aid must always be used for pipetting procedures. Mouth pipetting must be strictly forbidden. The most common hazards associated with pipetting procedures are the result of mouth suction. Oral aspiration and ingestion of hazards associated with pipetting procedures are the result of mouth suction. Oral aspiration and ingestion of hazardous materials have been responsible for many laboratory-associated infections.

Aerosols can be generated when a liquid is dropped from a pipette onto a work surface, when cultures are mixed by alternate sucking and blowing, and when the last drop is blown out of a pipette. The inhalation of aerosols unavoidably generated during pipetting operations can be prevented by working in a biological safety cabinet.

Pipetting aides should be selected with care and based on the activities to be performed. Their design and use should not create an additional infectious hazard and they should be easy to sterilize and clean. Plugged (aerosol resistant) pipette tips should be used when manipulating microorganisms and cell cultures.

Pipettes with cracked or chipped suction ends should not be used as they damage the seating seals of pipetting aids.

Homogenizers, shakers, blenders, and sonicators

Domestic (kitchen) homogenizers are not sealed and release aerosols. Only equipment designed for laboratory use should be used. Their construction minimizes or prevents such release. Homogenizers used for Risk Group 3 microorganisms should always be loaded and reopened in biological safety cabinets. Sonicators may release aerosols. They should be operated in biological safety cabinets or covered with shields during use. The shields and outsides of sonicators should be decontaminated after use.

Disposable transfer loops, needles, and cell spreaders

The advantage of disposable transfer loops, needles and cell spreaders is that they do not have to be sterilized and can therefore be used in biological safety cabinets where Bunsen burners and microincinerators would disturb the airflow. These loops should be placed in disinfectant after use and discarded as contaminated waste.

RECOMMENDED WORK PRACTICES

Autoclaves

The following procedure is recommended for the decontamination of biohazardous waste:

- Items should be autoclaved in approved autoclave bags and in a rigid, autoclavable secondary container.
- Follow the guidelines set by the posted autoclave parameter signs when setting the cycle time.
- Add one cup of water to each bag of solid waste and keep the bags open. Steam cannot penetrate closed bags.
- To prevent spills and accidents, be sure that the exhaust setting is appropriate for the type of material you are autoclaving. Fast exhaust should be used for solid items and solid waste and slow exhaust for liquids and liquid waste.
- After the cycle is complete, let the bag cool before removing it from the autoclave.
- Securely close the orange autoclave bag.
- Place treated autoclave bags into opaque black bags and close them securely before disposing.

The following PPE should be worn when operating an autoclave:

- Heat resistant autoclave gloves- for loading and unloading the autoclave.
- Fluid resistant gloves- to eliminate contact with contaminated wastes.
- Lab coat- to protect your personal clothing; and
- Splash goggles- if a splash hazard is present.

Flow Cytometers

Cells should be sorted under the same containment conditions (e.g., BSL-2 for human cells) in which they are handled for other manipulations. When sorting potentially infectious unfixed cells, it is important to keep in mind that potentially infectious aerosols are generated. When the cell sorter fails to operate properly (e.g., a clogged sort nozzle) there can be an increased production of aerosols. High speed sorters also produce an increased number of aerosols. Because of this risk it is recommended that the aerosol containment of the cell be verified. The following precautions should also be taken:

- Universal precautions should be followed (see the MSU Exposure Control Plan for details).
- Appropriate PPE should be worn (i.e., lab coat, gloves, N-95 respirator, splash goggles, face shield if desired).
- If possible, the cell sorter should be in a separate room.
- The sorter should be operated according to the manufacturer's recommendations; and
- Decontaminate the sorter after each run using an appropriate disinfectant. The disinfectant should be run through the machine for at least 10 minutes.

Additional biosafety features can be installed to the sorter as appropriate.

Pipettes and Pipetting Aids

Mouth pipetting is strictly prohibited. Mechanical pipetting aids must be used. Confine pipetting of biohazardous or toxic fluids to a biosafety cabinet if possible. Use the following precautions:

- Always use cotton-plugged pipettes when pipetting biohazardous or toxic fluids.
- Never prepare any kind of biohazardous mixtures by suction and expulsion through a pipette.
- Biohazardous materials should not be forcibly discharged from pipettes. Use “to deliver” pipettes rather than those requiring “blowout.”
- Do not discharge biohazardous material from a pipette at a height. Whenever possible allow the discharge to run down the container wall.
- Place contaminated, reusable pipettes horizontally in a pan containing enough liquid disinfectant to completely cover them.
- Autoclave the pan and pipettes as a unit before processing them for reuse.
- Discard contaminated Pasteur pipettes in an appropriate size sharps container.
- When work is performed inside a biosafety cabinet, all pans or sharps containers for contaminated glassware should be placed inside the cabinet as well while in use.

Sharps

Generally, the use of sharps should be restricted to procedures for which there is no alternative. Situations where the use of sharps may be appropriate include parenteral injection, phlebotomy, and aspiration of fluids. Plastic alternatives should be substituted for glassware whenever possible to prevent the unnecessary potential for sharps related exposure incidents.

If it has been determined that the use of sharps is unavoidable, the following practices should be adhered to:

1. All personnel should be trained in safe sharps handling procedures.
2. Use disposable sharps devices (i.e., scalpels, biopsy punches, needles) if possible.
3. Procedures should be organized in a manner that limits personnel exposure to the sharp device. For example:
    - Do not expose/unsheath sharp devices until the procedure requires the use of these items
    - Do not leave exposed sharp items unattended
    - If feasible, place an MSU-approved sharps container within arm’s reach of the point of use for the sharp item to allow for immediate disposal (For reusable sharps, use a hard-walled container that encloses the sharp end of the device)
4. Do not bend or break sharps.
5. Do not recap sharps if possible. If recapping is required, use a one-handed scoop technique. Note: The need for recapping can be eliminated using safer sharps devices.
6. Do not handle sharps with two hands.
7. Dispose of waste sharps in a properly labeled MSU-approved sharps container.
8. Permanently close and dispose of sharps containers when they are ¾ full or within 18 months of the date of first use, whichever comes first. Do NOT overfill or shake containers because these actions can result in accidental sharps exposure.
9. Reusable sharps should be placed in a hard walled container for storage until processing for reuse.
10. Broken glassware should be handled with a mechanical device, such as tongs, forceps, or a broom and dustpan rather than directly by hand.

Safer Sharps Program

Laboratories that use human derived materials or work with bloodborne pathogens are subject to the requirements of the Bloodborne Infectious Diseases Standard. This standard requires that available safer sharps devices be used and that those devices be reviewed annually in consideration of newly marketed ones. For additional information on safer sharps refer to the MSU Bloodborne Pathogens Exposure Control Plan or contact the Biosafety Office at 355-0153.

Cryostats

Frozen sections of 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 (i.e., Hepatitis C Virus). 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:

1. Consider the contents of the cryostat to be contaminated and decontaminate it frequently with a disinfectant suitable for the agent(s) in use.
2. Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.
3. Decontaminate the cryostat with a tuberculocidal type disinfectant regularly and immediately after tissue known to contain bloodborne pathogens, *M. tuberculosis* or other infectious agents is cut.
4. Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
5. Consider solutions for staining potentially infected frozen sections to be contaminated.

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.

Aerosols are created by practices such as filling centrifuge tubes, 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:

1. 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.
2. 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.
3. Add disinfectant to the space between the tube and the bucket to disinfect material in the event of breakage during centrifugation.
4. Always balance buckets, tubes, and rotors properly before centrifugation.
5. Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters.
6. 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.
7. Small low - speed centrifuges may be placed in a BSC during use to reduce the aerosol escape.

Safety Blenders

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. Blenders should be loaded, operated, and unloaded in a biosafety cabinet when used in conjunction with potentially infectious materials. The use of glass blender jars is not recommended because of the breakage potential. A towel moistened with disinfectant should be placed over the top of the blender during use. Blender jars should be allowed to rest for at least one minute to allow the aerosol to settle before opening them. 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 infectious 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 container. Discard the towel and ampoule top and bottom as biohazardous waste.

Ampoules used to store biohazardous material in liquid nitrogen have exploded causing eye injuries and exposure to the infectious agent. 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.

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 or hot bead sterilizers minimizes aerosol production during loop sterilization. Alternatively, disposable plastic loops and needles may be used for culture work.

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.

GUIDELINES FOR WORKING WITH TISSUE CULTURE/CELL LINES

When cell cultures are known to contain an etiologic agent or an oncogenic virus, the cell line can be classified at the same RG 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 BSL-2. All personnel working with or handling these materials need to be included in MSU's Bloodborne Pathogen Program (Refer to the MSU Exposure Control Plan for additional information).

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 Class I cell lines and handled at a Biosafety Level 1. Appropriate tests should confirm this assessment.

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 shown to be free of all adventitious agents) and all virus and mycoplasma-containing primate cell lines are classified as RG-2 and should be handled at a Biosafety Level 2. Studies involving suspensions of HIV prepared from T-cell lines must be handled at BSL-3.

Product recalls for bovine serum have raised the awareness of potential Bovine Spongiform Encephalopathy (BSE) or TSE (Transmissible Spongiform Encephalopathy) contamination of those sera. For more information on testing and purity of bovine serum used in your laboratory, contact your supplier.

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. Several healthcare workers have died.

In October 1994, CDC first published its “Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health-Care Facilities.” These guidelines were reviewed and updated by the CDC in 2005. 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 the EHS at 355-0153.

Investigators intending to work with *Mycobacterium sp*. in the laboratory must contact the EHS well in advance. Propagation and/or manipulation of *Mycobacterium tuberculosis* and *M. bovis* cultures in the laboratory or animal room must be performed at BSL-3.

GUIDELINES FOR 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 BSL-2.

A primary barrier, such as a biological safety cabinet, should be used:

- when it is anticipated that splashing, spraying, or splattering of clinical materials may occur,
- 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 included in the Bloodborne Pathogens Program as outlined in MSU’s Exposure Control Plan. “Universal Precautions” need to 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. A copy of

the Exposure Control Plan must be available in all laboratories. Additional recommendations specific for clinical laboratories may be obtained from the National Committee for Clinical Laboratory Standards (NCCLS).

GUIDELINES FOR PRION USE

Research-related activities involving prions or tissues containing prions have been on the rise at MSU in both the animal health and human health arenas. Because the infectious nature of prions is not well characterized and destruction of these particles goes beyond the techniques typically required for biohazard inactivation, work with these agents requires special considerations for biocontainment to minimize both occupational and environmental exposure risk.

At this time, work with prion-risk materials at MSU is limited to research and diagnostic laboratory applications. A guidance document has been prepared that applies to these procedures only (See Appendix I). Guidelines for use of prion-risk materials in conjunction with live animals will be developed if needed. Therefore, if future project plans call for use of live animals and prion-risk materials, please notify the MSU Biosafety Officer at the proposal-writing stage to perform a risk assessment and identify containment requirements.

GUIDELINES REGARDING SELECT AGENTS

The Centers for Disease Control and Prevention (CDC) and United States Department of Agriculture (USDA) regulate the possession, use, and transfer of Select Agents and toxins that have the potential to pose a severe threat to public health and safety. The CDC/USDA Select Agent Program oversees these activities and registers all laboratories and other entities in the United States of America that possess, use, or transfer a Select Agent or toxin.

The U.S. Departments of Health and Human Services (HHS) and Agriculture (USDA) published final rules for the possession, use, and transfer of Select Agents and toxins (42 C.F.R. Part 73, 7 C.F.R. Part 331, and 9 C.F.R. Part 121) in the Federal Register on March 18, 2005. All provisions of these final rules supersede those contained in the interim final rules and became effective on April 18, 2005.

The purpose of the CDC/USDA Select Agents regulation (42CFR72) is to provide a means of accountability for the use of Select Agents - biological agents that could pose a severe threat to public health and safety.

On June 10, 2002, President George W. Bush signed into law the "Public Health Security and Bioterrorism Preparedness and Response Act of 2002." This Act expands current regulations governing listed biological agents or toxins to require that all persons who possess, use, and/or transfer these materials register with the Department of Health and Human Services and the U.S. Department of Agriculture. All such persons are subject to safety and security requirements and inspections.

As a result of the bioterrorism events of 2001 and 2002, federal legislation (USA Patriot Act) has been passed that restricts specific groups of people from handling or accessing Select Agents. Therefore, anyone who plans to work with these materials may be asked to complete an affidavit to verify that he/she is not a restricted person in addition to registering with the CDC via the EHS.

President Bush signed Executive Order 13486 “Strengthening Laboratory Biosecurity in the United States”, to review the effectiveness of biosecurity policies regarding Select Agents in 2009.

President Obama signed an Executive Order 13546 in 2010, “Optimizing the Security of Biological Select Agents and Toxins in the United States” that directed HHS and USDA to consider reduction of the Select Agent list and establish physical security standards with highest risk of misuse.

Final rule published 2012 designated Tier1 Select Agents, reduced the number, and established physical security and information security standards for Tier 1 Select Agents, which is a subset designated to

present the greatest risk of deliberate misuse with significant potential for mass casualties or devastating effect to the economy, critical infrastructure, or public confidence.

Registration of Select Agent & Toxin Possession is MANDATORY.

Under Select Agent regulations, all individuals who possess Select Agents must register with the CDC and/or APHIS through the designated institutional responsible official (RO). At MSU, the EHS staff and Biosafety Officer serve in this capacity. The registration process is rigorous and includes many provisions such as:

- Description of research space including HVAC details, safety equipment and security features
- Research summary outlining use of agent
- Agent-specific safety and biocontainment procedures
- Safety and technical training of lab personnel
- Security & emergency response plans
- Security risk assessment, including U.S. Attorney General background check of personnel with access to agent

Once the registration document is prepared and submitted to the appropriate federal authorities, the turnaround time for approval is expected to be at least 2 months. For new registrations, the agent cannot be transferred to MSU facilities until approval is granted by the CDC and/or APHIS.

Considerations for Colleges & Departments

It is critical for departments to identify any potential for use or possession of Select Agents by research personnel to protect both the university and the researcher from unknowingly violating a regulatory requirement that bears both civil and criminal penalties. University policies are likely to be developed to address this potential. In the meantime, the following actions can be taken to prevent this from happening:

- Screen all research materials received to assure that no items on the Select Agent list have been inadvertently sent to campus. This is especially true for items received from foreign countries because the Select Agents regulations apply to the United States. International colleagues may not be aware of these new restrictions.
- Query all visiting research personnel, or newly recruited faculty before they come to campus to assure that they are not planning to bring any materials that are restricted under the Select Agent regulations. Again, international colleagues may not be aware of these new restrictions.
- Consult the EHS if any researcher plans to pursue grant money for research involving Select Agents.

GUIDELINES FOR HANDLING EXCLUDED STRAINS OF SELECT AGENTS

The United States Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA) have established regulations for the possession, use and transfer of Select Agents and toxins (see 42 CFR Part 73, 7 CFR Part 331 and 9 CFR Part 121). These regulations have also established a procedure by which an attenuated strain of a Select Agent that does not pose a severe threat to public health and safety, animal health, or animal products may be excluded from the requirements of the regulations when used for specific purposes. Please note that if an excluded attenuated strain is manipulated in such a way that virulence is restored or enhanced, or if factors associated with virulence are reintroduced, it will then be subject to the regulations. Because of the nature of these excluded strains and the potential for them to be manipulated for use as a biological weapon, the Office of Environmental Health and Safety EHS has implemented the containment and security requirements outlined in Appendix J for handling excluded strains of Select Agents.

Nontoxic HHS toxins, Section 73.3 (d)(2)

- Botulinum neurotoxins
- Conotoxins
- Staphylococcal Enterotoxins (SE)

Excluded Toxins Modified to be Less Potent or Toxic, Section 73.3 (e)

- Tetrodotoxin

Excluded Attenuated Strains of HHS Select Agents, Section 73.3 (e)

- Botulinum neurotoxin producing species of *Clostridium*
- *Coxiella burnetii*
- Eastern Equine Encephalitis virus
- Ebola virus
- *Francisella tularensis*
- Junin virus
- Lassa fever virus
- Monkeypox virus
- SARS-Coronavirus
- *Yersinia pestis*

Excluded Attenuated Strains of Overlap Select Agents, Section 73.4 (e) and 121.4 (e)

- *Bacillus anthracis*
- *Brucella abortus*
- *Brucella melitensis*
- *Burkholderia mallei*
- *Burkholderia pseudomallei*
- Rift Valley Fever Virus
- Venezuelan Equine Encephalitis virus

Attenuated Strains of USDA-only Select Agents Excluded, Section 121.3 (e)

- African swine fever viruses
- Avian influenza virus (low pathogenic)
- Avian influenza virus (highly pathogenic)
- Foot-and-mouth disease virus

GUIDELINES FOR THE USE OF PERMISSIBLE TOXIN AMOUNTS

Several toxins that appear on the NIH/CDC Select Agent list may be used in reduced quantities without completing the rigorous CDC registration. A list of such toxins can be found in **Appendix B**. Registration with the EHS is required, and Standard Operating Procedures (SOPs) regarding storage, disposal, and handling must be implemented before toxins are used in the laboratory.

DECONTAMINATION

Decontamination is defined as the reduction of microorganisms to an acceptable level. Methods applied to reach this goal can vary and most often include disinfection or sterilization. Disinfection is used when the acceptable level of microorganisms is defined as being below the level necessary to cause disease. This means that viable microorganisms are still present. In contrast, sterilization is defined as the complete killing of all organisms present. Depending on the circumstances and tasks, decontamination of a surface (e.g., lab bench) is accomplished with a disinfectant, while decontamination of biomedical waste is done by sterilization in an autoclave.

Many different terms are used for disinfection and sterilization. The following are among the more common in biosafety:

- *Antimicrobial* – An agent that kills microorganisms or suppresses their growth and multiplication.
- *Antiseptic* – A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.
- *Biocide* – A general term for any agent that kills organisms.
- *Chemical germicide* – A chemical or a mixture of chemicals used to kill microorganisms.
- *Disinfectant* – A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.
- *Microbicide* – A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of “biocide”, “chemical germicide” or “antimicrobial.”
- *Sporicidal* – A chemical or mixture of chemicals used to kill microorganisms and spores.

When choosing a method of decontamination, it is important to consider the following aspects:

- Type of biohazardous agents, concentration, and potential for exposure.
- Physical and chemical hazards to products, materials, environment, and personnel.

Cleaning Laboratory Materials

Cleaning is the removal of dirt, organic matter, and stains. Cleaning includes brushing, vacuuming, dry dusting, washing or damp mopping with water containing a soap or detergent. Dirt, soil and organic matter and shield microorganisms and can interfere with the killing action of decontaminants (antiseptics, chemical germicides, and disinfectants).

Precleaning is essential to achieve proper disinfection or sterilization. Many germicidal products claim activity only on precleaned items. Precleaning mush be carried out with care to avoid exposure to infections agents.

Materials chemically compatible with the germicides to be applied later must be used. It is quite common to use the same chemical germicide for precleaning and disinfection.

Ways to Decontaminate

Physical and chemical means of decontamination fall into four main categories:

- Heat
- Liquid chemicals
- Vapors and gases, and
- Radiation

Disinfection is normally accomplished by applying liquid chemicals or wet heat during boiling or pasteurization. To sterilize, vapors and gases (e.g., ethylene oxide), radiation, and wet heat (steam

sterilization in an autoclave) are used. Some liquid chemicals are also applied for sterilization, if used in the right concentration and contact time.

Heat

To kill microbial agents, heat can be applied in dry or wet form. The advantage of wet heat is a better heat transfer to and into the cell resulting in shorter exposure time and lower temperature. Steam sterilization uses pressurized steam at 121-132°C (250-270°F) for 30 or 40 minutes. This type of heat kills all microbial cells including spores, which are normally heat resistant. To accomplish the same effect with dry heat in an oven, the temperature needs to be increased to 160-170°C (320-338°F) for periods of 2 to 4 hours.

Decontamination of Biohazardous Waste by Autoclaving

Autoclaving is accepted as a safe and effective procedure for sterilization. There are currently over one hundred fifty operating autoclaves on the MSU campus. To ensure that any biohazardous waste created by the MSU community is properly decontaminated, the EHS tests each autoclave on an annual basis. Biological and chemical tests are used to monitor the autoclave cycle inside the chamber. Ampoules with heat resistant spores (*Bacillus stearothermophilus*) and steam sterilization integrator strips are used to indicate that adequate sterilization conditions are reached.

Procedures for MSU Autoclaves:

- All autoclaves used for decontamination of biohazardous waste need to be registered with EHS and tested on at least an annual basis.
- Strong oxidizing material (chemicals) must not be autoclaved with organic material: Oxidizer + Organic Material + Heat = Possible Explosion
- All biohazardous waste must be placed in orange biohazard bags with a heat sensitive "Autoclaved" indicator.
- Prior to autoclaving, a biohazard bag containing waste must be kept closed to prevent airborne contamination and nuisance odors. However, when autoclaving, the bag must be open to allow the steam to penetrate. Upon removal of the bag from the autoclave, it should be closed and disposed of in an opaque (black) waste bag.
- It is recommended to add water to each bag before autoclaving.
- Autoclave biohazardous materials using the recommended parameters posted on the autoclave.

Liquid Chemicals Used as Disinfectants

The appropriate liquid disinfectant should be chosen after carefully assessing the biohazardous agent and the type of material to be decontaminated. Liquid disinfectants are preferably used for solid surfaces and equipment. They vary greatly in their efficiency, depending on the chemical constituents and the agents involved. Variables to remember when disinfecting:

- **Nature of surface being disinfected** - Porous or smooth; the more porous and rougher the surface, the longer a disinfectant will need to be effective.
- **Number of microorganisms present** - Higher concentrations require a longer application time and/or higher concentration of disinfectant.
- **Resistance of microorganisms** - Microbial agents can be classified according to increasing resistance to disinfectants and heat (see Table 4).
- **Presence of organic material** - The proteins in organic materials such as blood, bodily fluids, and tissue can prevent or slow the activity of certain disinfectants.
- **Duration of exposure and temperature** - Increased exposure time increases the effectiveness of disinfectants. Low temperatures may slow down the activity requiring more exposure time.

EPA regulation of disinfectants

The Environmental Protection Agency (EPA) regulates pesticides, including chemical disinfectants, under the Federal Insecticide, Fungicide, and Rodenticide Act. They are required to be registered with the EPA. It is important to follow the directions on the manufacturer's label, including those for concentration and contact time, when using disinfectants to ensure compliance with the EPA requirements.

Increasing Resistance to Chemical Disinfectants

Least Resistant to Most Resistant

- Lipid or Medium – Size Viruses
    - Herpes Simplex Virus
    - Cytomegalovirus
    - Hepatitis B Virus
- Vegetative Bacteria
    - *Psuedomonas aeruginosa*
    - *Staphylococcus aureus*
    - *Salmonella choleraesuis*
- Fungi
    - *Trichophyton sp.*
    - *Cryptococcus sp.*
    - *Candida sp.*
- Non-lipid or Small Viruses
    - Poliovirus
    - Coxsackievirus
    - Rhinovirus
- Mycobacteria
    - *Mycobacterium tuberculosis*
    - *M. bovis*
- Bacterial Spores
    - *Bacillus subtilis*
    - *Clostridium sporogenes*
- Prions
    - *Creutzfeldt-Jacob disease*

There are many different liquid disinfectants available under a variety of trade names. In general, these can be categorized as halogens, acids or alkalines, heavy metal salts, quaternary ammonium compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the most effective disinfectants are often corrosive and toxic.

Alcohols

Ethyl or isopropyl alcohol in concentration of 70% are good general-use disinfectants. However, they evaporate fast and therefore have limited exposure time. They are less active against non-lipid viruses and ineffective against bacterial spores.

Formalin

Formalin is 37% solution of formaldehyde in water. Dilution of formalin to 5% results in an effective disinfectant. Formaldehyde is a suspected human carcinogen and creates respiratory problems at low levels of concentration.

Glutaraldehyde

This compound although chemically related to formaldehyde, is more effective against all types of bacteria, fungi, and viruses. Vapors of glutaraldehyde is irritating to the eyes, nasal passages, and upper respiratory tract. They should always be used in accordance with the instructions on the label and the appropriate personal protective equipment.

Phenol and Phenol Derivatives

Phenol based disinfectants come in various concentrations ranging primarily from 5% to 10 %. These derivatives, including phenol, have an odor which can be somewhat unpleasant. Phenol itself is toxic and appropriate personal protective equipment is necessary during application. The phenolic disinfectants are used frequently for disinfection of contaminated surfaces (e.g., walls, floors, bench tops). They effectively kill bacteria including *Mycobacterium tuberculosis*, fungi, and lipid-containing viruses. They are not active against spores or non-lipid viruses.

Quaternary Ammonium Compounds (Quats)

Quats are cationic detergents with strong surface activity. They are acceptable for general-use disinfectants and are active against Gram-positive bacteria and lipid-containing viruses. They are less active against Gram-negative bacteria and are not active against non-lipid-containing viruses. Quats are easily inactivated by organic materials, anionic detergents or salts of metals found in water. If Quats are mixed with phenols, they are very effective disinfectants as well as cleaners. Quats are relatively nontoxic and can be used for decontamination of food equipment and for general cleaning.

Halogens (Chlorine and Iodine)

Chlorine-containing solutions have broad spectrum activity. Sodium hypochlorite is the most common base for chlorine disinfectants. Common household bleach (5% available chlorine) can be diluted 1/10 to 1/100 with water to yield a satisfactory disinfectant solution. Chlorine containing disinfectants are inactivated by excess organic materials. They are also strong oxidizers and very corrosive. Always use appropriate personal protective equipment when using these compounds. At high concentrations and extended contact time, hypochlorite solutions are considered a cold sterilant since they inactivate bacterial spores. Calcium hypochlorite is more stable than sodium hypochlorite and this must be considered when preparing these solutions. Iodine has similar properties to chlorine. Iodophors (organically bound iodine) are recommended disinfectants. They are most often used as antiseptics and in surgical soaps and are relatively nontoxic to humans.

Vapors and Gases

A variety of vapors and gases possess germicidal properties. The most used are formaldehyde and ethylene oxide. Applied in closed systems under controlled conditions (e.g., humidity) these gases achieve sterility.

Formaldehyde gas is primarily used in the decontamination of spaces or biological containment equipment like biological safety cabinets. Formaldehyde is a toxic substance and a suspected human carcinogen. Considerable caution must be exercised in handling, storing, and using formaldehyde. Ethylene oxide is used in gas sterilizers under controlled conditions. Ethylene oxide is also a human carcinogen and monitoring is necessary during its use.

Radiation

Gamma and X-ray are two principal types of ionizing radiation used in sterilization. Their application is mainly centered on the sterilization of prepackaged medical devices. Ultraviolet (UV) radiation is a practical method for inactivating viruses, mycoplasma, bacteria, and fungi. UV radiation is successfully used in the destruction of airborne microorganisms. The sterilizing capabilities of UV light, such as that found in biosafety cabinets, are limited on surfaces because of its lack of penetrating power.

Incineration

Incineration is useful for disposing of animal carcasses as well as anatomical and other laboratory waste, with or without prior decontamination. Refer to the MSU Biohazardous Waste Management Plan for additional information on MSU's incineration procedures.

BIOHAZARDOUS WASTE

Definition

Refer to **MSU Biohazardous Waste Management Plan** for more specific information on how to dispose of biohazard materials.

At MSU, the term **biohazardous waste** is used to describe different types of waste that might include infectious agents. Currently, the following waste categories are all considered to be biohazardous waste:

1. Medical waste

Medical waste, defined as any solid waste which is generated in the diagnosis, treatment (e.g., provision of medical services), or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biologicals, as well as all categories defined by the Michigan Medical Waste Regulatory Act (MMWRA).

According to the MMWRA, Medical waste includes:

- Cultures and stocks of infectious agents and associated biologicals, including laboratory waste, biological production waste, discarded live and attenuated vaccines, culture dishes, and related devices.
- Liquid human and animal waste, including blood and blood products and body fluids, but not including urine or materials stained with blood or body fluids.
- Pathological waste: defined as human organs, tissues, body parts other than teeth, products of conception, and fluids removed by trauma or during surgery or autopsy or other medical procedure, and not fixed in formaldehyde.
- Sharps: Defined as needles, syringes, scalpels, and intravenous tubing with needles attached regardless of whether they are contaminated or not.
- Contaminated wastes from animals that have been exposed to agents infectious to humans, these being primarily research animals.

2. Regulated waste

Regulated waste as defined by the *Michigan Occupational Safety and Health Act on Bloodborne Infectious Diseases* (MIOSHA) including:

- Liquid or semi-liquid blood or other potentially infectious materials.

- Contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state if compressed.
- Items that are caked with dried blood or other potentially infectious materials and can release these materials during handling.
- Contaminated sharps which include any contaminated object that can penetrate the skin.
- Pathological and microbiological wastes containing blood or other potentially infectious materials.

3. Laboratory waste and regulated waste

Laboratory waste and regulated waste as defined in the Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines).

The CDC/NIH Biosafety Guidelines cover contaminated waste that is potentially infectious or hazardous for humans and animals. The same is true for the NIH Guidelines on recombinant DNA which also cover contaminated waste potentially infectious or hazardous for plants.

BIOHAZARD SPILL CLEAN-UP PROCEDURES

Since spills of biological materials will happen, it is important to be prepared prior to dealing with the problem. Laboratories working with biohazards have a basic biological spill kit ready to use. A basic kit can be assembled with materials already used in the laboratory. All labs operating at BSL-2 or higher have an assembled spill kit available in the lab. In BSL-1 labs, although it is preferable to have the contents of the spill kit in one location, the materials are easily accessible to everyone in the lab, prior assembly might not be necessary. Ready assembled spill kits are available for a fee through the EHS.

The following is a list of items that should go into a basic biological spill kit. Additional materials can be added to meet the needs of your unique situation.

Basic Biological Spill Kit Contents:

- Disinfectant bottle (e.g., bleach 1:10 dilution, prepared fresh)
- Absorbent material (e.g., paper towels, absorbent powder)
- Waste container (e.g., biohazard bags, sharps containers)
- Personal protective equipment (e.g., gloves, eye, and face protection)
- Mechanical tools (e.g., tongs, dustpan, and broom)
- Appropriate PPE
- Spill clean-up procedures
- Barrier tape

The following procedures are provided as a guideline to biohazardous spill clean-up and will need to be modified for specific situations. As with any emergency, stay calm, call 911 if necessary, and proceed with common sense. Call the EHS at 355-0153 if further assistance is required, especially if the spill outgrows the resources in the laboratory.

Spills Inside the Laboratory

Clear out personnel from spill area. Wait for any aerosols to settle before entering spill area. Remove any contaminated clothing and place in biohazard bag for further processing by laundry (MSU or department). Have a complete biological spill kit ready to go before you start the clean-up.

Spills with NO broken glass/sharps:

1. Remove spill supplies from container and line the container with a biohazard bag.
2. Put on two layers of gloves. Put on splash goggles.
3. Prepare the disinfectant solution, following the manufacturer's recommendations for concentration.
4. Cover the spill area with absorbent material (i.e., Superfine or paper towels).
5. Using the broom and dustpan, remove absorbent powder and deposit it in the biohazard bag, or if using paper towels, place them in the biohazard bag for disposal.
6. Spray the contaminated area with disinfectant and wait the appropriate contact time. Remove disinfectant with paper towels and place the paper towels in the biohazard bag for disposal.
7. Repeat step 6 to allow for sufficient disinfection of contaminated surfaces.
8. Remove outer pair of gloves only and dispose of them in the biohazard bag.
9. Remove goggles with inner gloves still on and clean the goggles with an antimicrobial towelette. Also wipe down contact surfaces of disinfectant container.
10. Remove inner gloves and dispose of them in biohazard bag.
11. Place the biohazard bag in a biohazardous waste container for treatment and disposal as soon as possible.
12. Wash your hands with soap and water as soon as possible.
13. Restock the kit for next use.

Spills involving broken glass/sharps:

1. Remove spill supplies from container and line the container with a biohazard bag. Retrieve a sharps container for disposal of glass/sharps.
2. Put on two layers of gloves. Put on splash goggles.
3. Prepare the disinfectant solution, following the manufacturer's recommendations for concentration.
4. Using tongs or forceps, place broken glass/sharps in sharps container.
5. Cover the spill area with absorbent powder.
6. Using the broom and dustpan, remove absorbent powder and deposit it in the biohazard bag.
7. Spray the contaminated area with disinfectant and wait the appropriate contact time. Remove disinfectant with paper towels and place the paper towels in the biohazard bag for disposal.
8. Repeat step 7 to allow for sufficient disinfection of contaminated surfaces.
9. Remove outer pair of gloves only and dispose of them in the biohazard bag.
10. Remove goggles with inner gloves still on and clean the goggles with an antimicrobial towelette. Also wipe down contact surfaces of disinfectant container.
11. Remove inner gloves and dispose of them in biohazard bag.
12. Place the biohazard bag in a biohazardous waste container for treatment and disposal as soon as possible.
13. Wash your hands with soap and water as soon as possible.
14. Restock the kit for next use.

Spills Inside the Biological Safety Cabinet

Have a complete biological spill kit ready to go before you start the clean-up.

- Wear lab coat, safety goggles and gloves during clean-up.
- Allow cabinet to run during clean-up.
- Soak up spilled material with paper towels (work surface and drain basin) and apply disinfectant using the manufacturer's recommended concentration and contact time.
- Wipe up spillage and disinfectant with disposable paper towels.

- Wipe the walls, work surface and any equipment in the cabinet with a disinfectant soaked paper towel.
- Discard contaminated disposable materials in biohazard bag(s) and autoclave before discarding as waste.
- Place contaminated reusable items in biohazard bags or heat resistant pans or containers with lids before autoclaving and further clean-up.
- Expose non-autoclavable materials to disinfectant, 10 minutes contact time, before removal from the BSC.
- Remove protective clothing used during cleanup and place in a biohazard bag for further processing by laundry (MSU or department).
- Run cabinet at least 10 minutes after clean-up and before resuming work.
- Inform all users of the BSC as well as the laboratory supervisor about the spill and successful clean-up as soon as possible.

Spills Inside a Centrifuge

Have a complete biological spill kit ready to go before you start the clean-up.

- Clear area of all personnel. Wait 30 minutes for aerosols to settle before attempting to clean up the spill.
- Wear a lab coat, safety goggles and gloves during clean-up.
- Remove rotors and buckets to the nearest biological safety cabinet. · Thoroughly disinfect inside of centrifuge.

Remove contaminated debris after disinfection, place in appropriate biohazardous waste container(s) and autoclave before disposal.

Spills During Transport

If a spill occurs in a public area:

- Do not attempt cleanup without the proper supplies.
- Contact the EHS (355-0153) for assistance.

If a spill occurs in a vehicle:

- Leave the vehicle with closed windows and locked doors.
- Contact the EHS (355-0153) for assistance.

Spill Kit Maintenance

Your biological spill kit is restocked after each use. It is checked for completeness on an annual basis or more frequently. The following maintenance activities should be done:

- Check expiration on disinfectant and replace as needed (e.g., bleach should be replaced every 6 months and after 24 hours when diluted, it is stored out of direct sunlight and below 77 degrees Fahrenheit);
- Replace gloves.
- Replace antimicrobial towelettes; and
- Check straps on splash goggles for deterioration.

HAND HYGIENE AND HAND DECONTAMINATION

Whenever possible, suitable gloves should be worn when handling biohazardous materials. However, this does not replace the need for regular and proper hand hygiene by laboratory personnel. Hands must be washed after handling biohazardous materials and animals, and before leaving the laboratory.

In most situations, thorough washing of hands with ordinary soap and water is sufficient to decontaminate them, but the use of germicidal soaps is recommended in high-risk situations. Hands should be thoroughly lathered with soap, using friction, for at least 20 seconds, rinsed in clean water and dried.

Foot- or elbow-operated faucets are recommended. Where not available, a paper towel should be used to turn off the faucet handles to avoid re-contaminating washed hands.

Alcohol-based hand-rubs may be used to decontaminate lightly soiled hand when proper hand hygiene facilities are not available. The use of hand-rubs should be followed up with a soap and water wash as soon as possible.

INTRODUCTION TO THE TRANSPORT OF BIOLOGICAL MATERIALS

Transporting Biological Materials on Campus

Biological materials can be safely transported between buildings on campus when they are appropriately packaged, labeled, and transported in a manner that minimizes the potential for environmental release.

The following procedure for preparing and transporting biological materials between university buildings should be used:

1. Use primary containers that are designed to contain the material to be stored. Do not use food containers or other containers not originally designed for laboratory storage purposes.
2. Place primary sample containers into an appropriate secondary container for transport. If sample material is liquid or may release liquids, use a leakproof secondary container with a secure lid (i.e., cooler with a latchable lid). Additionally, place enough absorbent material (i.e., paper towels) in the secondary container to absorb all free liquids if primary containers rupture or break during transport.
3. Package primary containers in the secondary container in a manner that will reduce shock, rupture, and/or breakage. Bubble wrap or similar shock-absorbing materials may also be used to minimize the potential for primary container rupture.
4. Label all secondary containers with a brief description of the contents and an emergency contact name and phone number. Containers used for transporting blood specimens (regardless of source) or specimens known or suspected to contain a pathogen should be additionally labeled with the biohazard symbol.
5. Use a university-owned vehicle whenever possible for transport. Store and secure the transport container in a location in the vehicle whereby if an accident were to occur, the container or its contents will not be an exposure risk to the driver or to the environment. For example, in transporting materials by car or van, store the container in the back seat or cargo bay. Secure the container with bungee cords or belts to keep the container upright and stable.

Shipping of Biological Materials to an Off Campus Destination

Transportation of biological materials is an activity that affects all research and diagnostic service entities. In some instances, these materials may be regulated for transportation and will require specific packaging, labeling and documentation. Additionally, the shipper must have documented training relative

to his or her tasks associated with the shipment. This is the case for shipment of diagnostic specimens (from humans or animals), cultures of infectious substances (infectious to humans and/or animals), genetically modified organisms and any biological materials shipped on dry ice. Recently there has been an increased level of surveillance on the part of federal and international authorities for all hazardous materials/dangerous goods shipments that may include diagnostic specimens and infectious substances. As a shipper, it is essential to assure that materials are properly classified and that all applicable regulatory provisions for shipment are met.

EHS offers consultation for campus personnel who plan to ship biological materials including diagnostic specimens, infectious substances, genetically modified organisms, and biological materials on dry ice frequently, more than once a month. Contact University Logistics for all shipments off campus. Their office is trained and has resources required for shipping.

Impact of non-compliance:

- Increased risk of material release during the shipping process.
- May result in refusal or return of packages during the shipping process. This could be critical if materials are temperature sensitive.
- May result in fines from the Federal Aviation Administration (FAA).
- Can impact the institution's reputation and ability to obtain funding in the future.

Preparing to Ship Biological Materials

Before you package and ship materials to an off-campus destination there are several items that should be taken care of. These paperwork requirements can take several weeks to complete; therefore, you should prepare well in advance for them.

1. *Material Transfer Agreements*

The Office of Intellectual Property requires that a Material Transfer Agreement be completed for materials entering or leaving campus. Before you send your shipment, it is important that you contact the Office of Intellectual Property to ensure that the appropriate agreements are completed and processed.

You can contact the Office of Intellectual Property at 355-2186, or you can view their website at MSU Technologies

2. Export Controls and Trade Sanctions

Export controls and trade sanctions are regulatory areas that may apply to you, depending on your activity. Exports are any items (commodities, software, technology, select biological agents) sent from the United States to a foreign destination.

Export control laws may apply when one or more of the following concerns pertain to your research project:

- It has actual or potential military applications, including dual use items (i.e., commercial items with potential military application)
- The destination country, organization, or individual is restricted by Federal law
- The declared or suspected end use or the end user of the export compromises national security
- Economic protection issues are associated with the destination country

If you have questions about whether there are export controls issues associated with your activity, contact the Office of Export Controls and Trade Sanctions (432-4500)

3. Permits

The CDC, USDA, U.S. Fish and Wildlife Service and Department of Commerce require permits for shipping certain etiological agents and other materials.

FAQ: Can I take my materials on the airplane with me (either in carried-on or checked baggage)?

The answer to this question is, it depends. It depends on the materials that you wish to take and if you have the proper paperwork in place. You **CANNOT** carry on or check biological materials if any of the following apply:

- The materials that have been classified as "Dangerous Goods;"
- Carriage of the materials is against rules established by the Transportation Security Administration (TSA);
- You do not have a completed material transfer agreement in place for the materials.
- Transport of the materials does not comply with export control and trade sanctions regulations; or
- Transport of the materials does not comply with Department of Transportation regulations.

A Customs Broker is required to enter the country with biological materials. Shipping through companies such as Fed Ex may be contracted for this service. Contact University Procurement and Logistics regarding the MSU contracted Customs Broker.

When in doubt, ASK!
For more information on biological materials shipping requirements, please contact the Biological Safety Office at 355-0153.

USE OF ANIMALS IN RESEARCH

The use of animals in research, teaching, and outreach activities is subject to state and Federal laws and guidelines. University policy specifies that:

- All animals under university care will be treated humanely.
- Prior to their inception, all animal projects receive approval by the Institutional Animal Care and Use Committee (IACUC).
- MSU will comply with state and federal regulations regarding animal use and care.

Project directors are responsible for the humane treatment of animals under their supervision, and for adherence to applicable University, state, and Federal regulations. Faculty members planning to use live vertebrate animals for any University-related activity must submit an animal use form (AUF) to the IACUC for review or request an exemption from the Committee Chairperson and receive approval, prior to the start of the project, regardless of the source of funding for the project.

For additional information contact the IACUC at 432-4151.

USE OF HUMAN SUBJECTS AND MATERIALS IN RESEARCH

Federal and University regulations and policies require that all research involving human subjects or materials be reviewed and approved before initiation by the University's Institutional Review Board (IRB) to protect the rights and welfare of human subjects.

Michigan State University's IRB is the Human Research Protection Program. Prescribed by the National Research Act of 1974 (PL 93-348) and endorsed by the Academic Council, the IRB reviews applications for research involving human subjects. Reviews are performed in accordance with the U.S. Department of Health and Human Services (HHS) regulations for the Protection of Human Research Subjects (45 CFR 46, as amended) as codified and extended by the University's formal Assurance to HHS: M-1239.

It is the responsibility of the Project Investigator to assure that all research involving human subjects is reviewed and approved by the IRB prior to initiation. All personnel with a reasonable anticipated risk of exposure to bloodborne pathogens through the contact with human blood or other human materials must be included in MSU's Bloodborne Pathogen Program.

For more information, contact the IRB office at 355-2180.

NONHUMAN PRIMATE (NHP) MATERIAL BIOSAFETY HAZARDS

Definition of Nonhuman Primate (NHP)

Refers to the species ranging from apes to monkeys, more specifically old-world macaque monkeys. These include rhesus macaques, pig-tailed macaques, and cynomolgus monkeys.

Biosafety Hazards – Zoonotic Agents

Humans and nonhuman primates have similar genetics and are therefore susceptible to many of the same diseases. Working with NHP materials including cell culture and tissues can put workers at risk for these.

Viruses

- Hepatitis A and B virus
- **B virus***
- Poxviruses
- Rotavirus
- Simian hemorrhagic fever virus
- Simian immunodeficiency viruses
- Simian retrovirus type D
- Simian T-cell leukemia virus
- Simian virus 40

Bacteria

- *Campylobacter spp*
- *Mycobacterium tuberculosis*
- *Shigella flexneri*

Parasites

- *Balantidium coli*
- *Entamoeba histolytica*
- *Strongyloides spp*

* B Virus is also known as: Cercopithecine herpesvirus 1, Herpes B Virus, Monkey B Virus, herpesvirus simiae, herpesvirus B, and Macacine herpesvirus 1.

B Virus

The virus is known to have caused 40 fatalities. Lab related infections occur because of bite or scratches from an infected macaque, handling infected tissues, handling infected kidney cell lines, person to person and splash to the eye. Transmission is also possible through ingestion breathing aerosolized contaminants like when pipetting or accidental inoculation with a sharp contaminated object. 70% mortality rate in humans when not immediately treated. Virus may be present in materials from macaques including saliva, feces, urine, tissues, or fluids, which included cell cultures derived from infected animals.

NHP Material Handling

NHP materials should be used for research purposes only when clearly indicated, access is limited to areas where the NHP is stored and utilized, and mandatory training is required prior to working with them. The work must be presented to the Biological Safety Officer prior to research initiation.

Handle all NHP materials as though they are infected, secured appropriately within lab, transport containment between labs, wear proper PPE (i.e.: long pants, closed toes shoes, lab coat, nitrile gloves, eye protection, face shield with mask), and use correct decontamination processes including waste. Engineering controls including eye wash, autoclave, biosafety cabinet, and centrifuges with safety features preventing aerosols are required.

Exposure to NHP Materials

Immediately after exposure medical treatment and surveillance must be obtained. Flush the area for 15 minutes and go immediately to an emergency facility. If possible, carefully transport the sample from the exposure in a labeled leak proof container to be tested for the presence of virus. It is extremely urgent to be seen immediately by a physician. Initiates follow up with physician to monitor for symptoms for upwards of 30 days.

Symptoms of B Virus

Immediate symptoms may be fever, numbness or tingling, increased sensitivity to pain or touch, and muscle weakness or even paralysis. Within 6 days to 3 weeks after exposure the following symptoms may occur: Fluid filled blisters or sores near the site of exposure and swelling or irritation of the lymph nodes around the exposure. Later symptoms can be sinus infection, neck stiffness, headache lasting more than 24 hours, nausea, vomiting, change in level of consciousness, double vision, difficulty speaking or swallowing, dizziness, loss of sensation, inability to move or control movement, seizures, difficulty breathing, inability to urinate.

Reference Material for B Virus

"Guidelines for the Prevention of Herpesvirus simiae (B Virus) Infection in Monkey Handlers", MMWR (Morbidity and Mortality Weekly Report - Centers for Disease Control) October 23, 1987.

"Fatal Cercopithecine herpesvirus 1 (B Virus) Infection Following a Mucotaneous Exposure and Interim Recommendations for Worker Protection", MMWR December 18, 1998/47(49); 1073 – 6, 1083.

National Institute for Occupational Safety and Health – Hazard Evaluation and Hazard ID Documents (1997).

Occupational Health and Safety in the Care and Use of Nonhuman Primates. National Research Council (US) Committee on Occupational Health and Safety in the Care and Use of Nonhuman Primates. Washington (DC): National Academies Press (US); 2003.

BIOSAFETY AND RECOMBINANT DNA TECHNOLOGY

In the past several years, recombinant DNA has become widely used in many fields of research. The National Institutes of Health (NIH) has established regulations on the use and containment of recombinant DNA materials in the laboratory. Regulations require persons conducting such research to file a registration form with the Institutional Biosafety Committee (IBC) which must approve the protocols related to recombinant DNA molecules.

The recombinant DNA research registration system is set up so that registration forms are filled out online and submitted directly into a database. The system allows for principal investigators to access their form and make any necessary changes easily and quickly. The information in the database may only be accessed by authorized individuals and is secured using a name/password system with two factor authentication.

As a condition for funding of recombinant DNA research, MSU must ensure that research conducted at or sponsored by MSU, irrespective of the source of funding, complies with the most current NIH *Guidelines for Research Involving Recombinant DNA Molecules.* At MSU, the responsibility for ensuring that recombinant DNA activities comply with all applicable guidelines rests with the institution and the Institutional Biosafety Committee (IBC) acting on its behalf.

Before experiments involving recombinant DNA begin, the Principal Investigator (PI) must submit a *Registration Document for Recombinant DNA Research* to the IBC.

Guidelines for Working with Genetically Modified Animals

The Environmental Protection Agency (EPA) has specific guidelines for containment measures for transgenic animals including but not limited to *mice, rats, invertebrates, and fruit flies*. Regulations: S. I. No 73 of 2001.

NIH recombinant DNA review categories

All recombinant DNA research proposals require the PI to make an initial determination of the required level of physical and biological containment. For that reason, the NIH has developed six categories (**III-A** to **III-F**) addressing different types of rDNA research.

If the proposed research falls within section **III-A** of the NIH Guidelines, the experiment is considered a "Major Action". This includes experiments involving human gene transfer experiments. As a result, the experiment cannot be initiated without submission of relevant information to the Office of Recombinant DNA Activities at NIH. In addition, the proposal must be published in the Federal Register for 15 days, it needs to be reviewed by the NIH Recombinant DNA Advisory Committee (RAC), and specific approval by the NIH is obtained. The containment conditions for such an experiment will be recommended by the RAC and set by the NIH at the time of approval. The proposal requires IBC approval before initiation.

If the proposed research falls within section **III-B**, the research cannot be initiated without submission of relevant information on the proposed experiment to NIH/ Office of Biotechnology Activities (OBA) (For exceptions see the guidelines). Experiments covered in III-B include the cloning of toxic molecules. The containment conditions for such experiments will be determined by NIH/OBA in consultation with ad hoc experts. Such experiments require Institutional Biosafety Committee approval before initiation. Please refer to the guidelines for more specifics.

In section **III-C**, experiments with human subjects are covered. These experiments require IBC and IRB (Institutional Review Board) approval and NIH/OBA registration before initiation.

Section **III-D**, the next category, covers whole animal or plant experiments as well as projects involving DNA from Risk Group 2, 3 or 4 agents. Prior to the initiation of an experiment that falls into Section **III-D**, the PI must submit a *Registration Document for Recombinant DNA Research* to the Institutional Biosafety Committee. The IBC reviews and approves all experiments in this category prior to initiation.

Section **III-E** experiments require the filing of a *Registration Document for Recombinant DNA Research* with the IBC at the time the experiment is initiated. The IBC reviews and approves all such proposals, but Institutional Biosafety Committee review and approval prior to initiation of the experiment is not required.

Section **III-F** experiments are exempt from the NIH Guidelines however, they must still be registered with the IBC who will verify the exempt status of the registration.

Review Process Overview

Once your registration document has been submitted, a representative from the Biosafety Office will screen it and may contact you for more information about your research or for fine-tuning of your registration document before it is turned over to the committee. Members of the Biosafety Team at the EHS will meet with you as necessary to conduct a brief inspection of the proposed laboratory location for the research and discuss a risk assessment specific to your project. The registration document is then distributed to the IBC for review. Since the committee normally meets towards the end of each month to review projects, registrations must be submitted by mid-month at the latest, to be considered that month. The committee will then review it and report back to you. They may request additional information or changes to the registration before approval. The entire review process usually takes 6 to 8 weeks. Meetings may be scheduled depending on needs.

Responsibilities of the Principal Investigator (PI) for Recombinant DNA Research

The Principal Investigator is responsible for full compliance with the NIH Guidelines in the conduct of recombinant DNA research. Please refer to the most recent edition of the NIH *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* for more information.

General Responsibilities

As part of this general responsibility, the Principal Investigator shall:

1. Initiate or modify no recombinant DNA research which requires IBC approval prior to initiation until that research or the proposed modification thereof has been approved by the IBC and has met all other requirements of the NIH Guidelines.
2. Determine whether experiments are covered by Section III-E, Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation, and that the appropriate procedures are followed.
3. Report any significant problems, violations of the NIH Guidelines, or any significant research-related accidents and illnesses to the Biological Safety Officer, the Institutional Biosafety Committee, NIH, and other appropriate authorities (if applicable) within 30 days.
4. Report any new information bearing on the NIH Guidelines to the Institutional Biosafety Committee and to NIH.
5. Be adequately trained in good microbiological techniques.
6. Adhere to IBC-approved emergency plans for handling accidental spills and personnel contamination; and
7. Comply with shipping requirements for recombinant DNA molecules. Contact the EHS for more information.

Submissions by the Principal Investigator to the NIH/OBA

The Principal Investigator shall:

1. Submit information to NIH/OBA for certification of new host-vector systems.
2. Petition NIH/OBA, with notice to the IBC, for proposed exemptions to the NIH Guidelines.
3. Petition NIH/OBA, with concurrence of the IBC, for approval to conduct experiments specified in Sections III-A-1, Major Actions Under the NIH Guidelines, and III-B, Experiments that Require NIH/OBA and IBC Approval Before initiation.
4. Petition NIH/OBA for determination of containment for experiments requiring case-by-case review; and

5. Petition NIH/OBA for determination of containment for experiments not covered by the NIH Guidelines.

Submissions by the Principal Investigator to the Institutional Biosafety Committee

The Principal Investigator shall:

1. Make an initial determination of the required levels of physical and biological containment in accordance with the NIH Guidelines.
2. Select appropriate microbiological practices and laboratory techniques to be used for the research.
3. Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system) to the IBC for review and approval or disapproval; and
4. Remain in communication with the IBC throughout the duration of the project.

Responsibilities of the Principal Investigator Prior to Initiating Research

The Principal Investigator shall:

1. Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken.
2. Instruct and train laboratory staff in the:
    a. Practices and techniques required to ensure safety, and
    b. Procedures for dealing with accidents; and
3. Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

Responsibilities of the Principal Investigator During the Conduct of the Research

The Principal Investigator shall:

1. Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed.
2. Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer, the IBC, NIH/OBA, and other appropriate authorities (if applicable);
3. Correct work errors and conditions that may result in the release of recombinant DNA materials.
4. Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).
5. Comply with annual data reporting and adverse event reporting requirements for NIH- and FDA-approved human gene transfer experiments.

APPENDIX A – BIOSAFETY RESOURCES

MSU Safety Manuals

Biohazardous Waste Management Plan
Chemical Hygiene Plan
Exposure Control Plan for Bloodborne Pathogens
Radiation Safety Manual

References for Biological Safety and Biosecurity

- *CDC/NIH Biosafety in Microbiological and Biomedical Laboratories 6th Edition (BMBL-6); 2020.*
- *World Health Organization. Laboratory Biosafety Manual – 4th ed. Geneva: WHO Press; 2021.*
- *World Health Organization. Biorisk management: Laboratory biosecurity guidance. Geneva: WHO Press; 2006.*
- *World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2021-2022; Geneva: WHO Press; 2021.*
- *ISO 3500: 2019, Biorisk management for laboratories and other related organisations*
- *Fleming DO & Hunt DL. Biological Safety: Principles and Practices. 4th ed. ASM Press; 2006.*
- *Salerno RM & Gaudioso J. Laboratory Biosecurity Handbook. CRC Press; 2007.*

- *Anthology of Biosafety 1: Perspectives in Laboratory Design. American Biological Safety Association (ABSA), 1999.*
- *Anthology of Biosafety 2: Facility Design Considerations. ABSA), 2000.*
- *Anthology of Biosafety 3: Applications of Principles. ABSA, 2000.*
- *Anthology of Biosafety 4: Issues in Public Health. ABSA, 2002.*
- *Anthology of Biosafety 5: BSL-4 Laboratories. ABSA, 2002.*
- *Anthology of Biosafety 6: Arthropod Borne Diseases. ABSA, 2003.*
- *Anthology of Biosafety 7: Biosafety Level 3. ABSA, 2004.*
- *Anthology of Biosafety 8: Involving Issues in Containment. ABSA, 2003.*
- *Anthology of Biosafety 9: Exploring the Performance Envelope for BSL-3 and BSL-4 Laboratories. ABSA, 2006.*
- *Anthology of Biosafety 10: Animal Biosafety. ABSA, 2007.*
- *Anthology of Biosafety 11: Worker Health and Safety Issues. ABSA, 2008.*
- *Anthology of Biosafety 12: Managing Challenges for Safe Operation of BSL-3/ABSL-3 Facilities. ABSA, 2011.*
- *Anthology of Biosafety 13: Animal Production and Protection. ABSA, 2012.*
- *Anthology of Biosafety 14: Sustainability. ABSA, 2015.*

Biosafety-related Products
Products

Items like sharps containers, biohazard bags, splash goggles, gloves, disposable spreaders, and loops, can be purchased through the following:

University Stores 517-355-1700

Biochemistry Research Store 517-353-0813

Spartan Marketplace 517-355-1700

Chemistry Research Stockroom 517-353-1195

Physics and Astronomy Machine Shop 517- 884-5540

Core Facility Research Support

Investigative HistoPathology Laboratory 517-884-5026

Mass Spectrometry Core (MMD-CMSC) 517-355-1119

Metabolic Core (MMD-CIVMC) 517-884-5172

Cognitive Imaging Research Center

Genomics (Research Technology Support Facility – RTSF)

Mass Spectrometry and Metabolomics Core (RTSF)

Proteomics (RTSF)

Flow Cytometry Core Facility

Center for Advanced Microscopy

Max T. Rogers NMR Facility

Center for Statistical Training and Consulting

Biomedical Research Informatics Core

Animal Care Program

Laundry Services

Lab coats cannot be taken home to be washed, they are laundered by a facility that is certified to handle biological contaminated items.

MSU's laundry facility which services research buildings on campus through a pickup and drop off service.

Spartan Linen Services 517-355-8520

APPENDIX B – HHS AND USDA SELECT AGENTS AND TOXINS

7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

(November 17, 2021 latest list on selectagents.gov website)

HHS Select Agents and Toxins

1. Abrin [6]
2. *Bacillus cereus* Biovar *anthracis* [1]
3. Botulinum neurotoxins [1][6]
4. Botulinum neurotoxin producing species of *Clostridium* [1]
5. Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$) [6]
6. *Coxiella burnetii*
7. Crimean-Congo haemorrhagic fever virus
8. Diacetoxyscirpenol [6]
9. Eastern Equine Encephalitis virus [4][5]
10. Ebola virus [1]
11. *Francisella tularensis* [1]
12. Lassa fever virus
13. Lujo virus
14. Marburg virus [1]
15. Monkeypox virus [4]
16. Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
17. Ricin [6]
18. *Rickettsia prowazekii*
19. SARS-associated coronavirus (SARS-CoV) [5]
20. SARS-CoV/SARS-CoV-2 chimeric viruses resulting from any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors
21. Saxitoxin [6]

South American Haemorrhagic Fever viruses:

22. Chapare
23. Guanarito
24. Junin
25. Machupo
26. Sabia
27. Staphylococcal enterotoxins (subtypes A,B,C,D,E) [6]
28. T-2 toxin [6]
29. Tetrodotoxin [6]

Tick-borne encephalitis complex (flavi) viruses:

30. Far Eastern subtype [5]

31. Siberian subtype [5]
32. Kyasanur Forest disease virus [5]
33. Omsk hemorrhagic fever virus [5]
34. Variola major virus (Smallpox virus) [1]
35. Variola minor virus (Alastrim) [1]
36. *Yersinia pestis* [1]

Overlap Select Agents and Toxins

37. *Bacillus anthracis* [1]
38. *Bacillus anthracis* Pasteur strain
39. *Brucella abortus*
40. *Brucella melitensis*
41. *Brucella suis*
42. *Burkholderia mallei* [1]
43. *Burkholderia pseudomallei* [1]
44. Hendra virus
45. Nipah virus
46. Rift Valley fever virus
47. Venezuelan equine encephalitis virus [4][5]

USDA Veterinary Services (VS) Select Agents and Toxins

48. African horse sickness virus
49. African swine fever virus
50. Avian influenza virus [4]
51. Classical swine fever virus [5]
52. Foot-and-mouth disease virus [1][5]
53. Goat pox virus
54. Lumpy skin disease virus
55. *Mycoplasma capricolum* [4]
56. *Mycoplasma mycoides* [4]
57. Newcastle disease virus [3][4]
58. Peste des petits ruminants virus
59. Rinderpest virus [1]
60. Sheep pox virus
61. Swine vesicular disease virus [5]

USDA Plant Protection And Quarantine (PPQ) Select Agents and Toxins

62. *Coniothyrium glycines*
    (formerly *Phoma glycinicola* and *Pyrenochaeta glycines*)
63. *Peronosclerospora philippinensis*
    (Peronosclerospora sacchari)
64. *Ralstonia solanacearum* [7]

65. *Rathayibacter toxicus*
66. *Sclerophthora rayssiae* [7]
67. *Synchytrium endobioticum*
68. *Xanthomonas oryzae*

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[1] Denotes Tier 1 Agent

[2] C = Cysteine residues are all present as disulfides, with the 1st and 3rd Cysteine, and the 2nd and 4th Cysteine forming specific disulfide bridges; The consensus sequence includes known toxins a-MI and a-GI (shown above) as well as a-GIA, Ac1.1a, a-CnIA, a-CnIB; X1 = any amino acid(s) or Des-X; X2 = Asparagine or Histidine; P = Proline; A = Alanine; G = Glycine; X3 = Arginine or Lysine; X4 = Asparagine, Histidine, Lysine, Arginine, Tyrosine, Phenylalanine or Tryptophan; X5 = Tyrosine, Phenylalanine, or Tryptophan; X6 = Serine, Threonine, Glutamate, Aspartate, Glutamine, or Asparagine; X7 = Any amino acid(s) or Des X and; "Des X" = "an amino acid does not have to be present at this position." For example if a peptide sequence were XCCHPA then the related peptide CCHPA would be designated as Des-X.

[3] A virulent Newcastle disease virus (avian paramyxovirus serotype 1) has an intracerebral pathogenicity index in day-old chicks (Gallus gallus) of 0.7 or greater or has an amino acid sequence at the fusion (F) protein cleavage site that is consistent with virulent strains of Newcastle disease virus. A failure to detect a cleavage site that is consistent with virulent strains does not confirm the absence of a virulent virus.

[4] Select agents that meet any of the following criteria are excluded from the requirements of this part: Any low pathogenic strains of avian influenza virus, South American genotype of eastern equine encephalitis virus, west African clade of Monkeypox viruses, any strain of Newcastle disease virus which does not meet the criteria for virulent Newcastle disease virus, all subspecies Mycoplasma capricolum except subspecies capripneumoniae (contagious caprine pleuropneumonia), all subspecies Mycoplasma mycoides except subspecies mycoides small colony (Mmm SC) (contagious bovine pleuropneumonia), and any subtypes of Venezuelan equine encephalitis virus except for Subtypes IAB or IC, provided that the individual or entity can verify that the agent is within the exclusion category.

[5] For determining the regulatory status of nucleic acids that are capable of producing infectious forms of select agent viruses, please reference guidance here.

[6] For determining the regulatory status of Recombinant and/or Synthetic nucleic acids that encode for the toxic form(s) of any select toxins if the nucleic acids (i) can be expressed in vivo or in vitro, or (ii) are in a vector or recombinant host genome and can be expressed in vivo or in vitro; please reference guidance here.

[7] Select agents or toxins that meet any of the following criteria are excluded from the requirements of this part: Any subspecies of *Ralstonia solanacearum* except race 3, biovar 2 and all subspecies of *Sclerophthora rayssiae* except var. *zeae,* provided that the individual or entity can identify that the agent is within the exclusion category.

APPENDIX C – RISK ASSESSMENT FORM

To address all realistic, perceivable risks to protect personnel, the community, and the environment. Reviewed regularly with active participation from all relevant stakeholders.

Biological Safety Risk Assessment for Proposed Procedures

Date:_____ Principal Investigator:_____

Description of Materials & Procedures:_____

This form consists of 3 sections. Please complete this form in conjunction with the MSU Biosafety Officer.

SECTION 1
Material Source Information
Use this space to identify:

- Types of materials to be used including quantities and biological activation status
- Source, and any known infectious disease considerations associated with either the source species or the geographic location of the source species
- Procedural steps for the analysis, from material preparation through waste disposal

SECTION 2 **Infectious Disease Considerations** Complete this section for each agent identified as an infectious disease consideration in the previous section. Make additional copies of this section if needed.		
Agent		
Pathogenicity of the organism & Routes of transmission	Infectious Dose	
	Routes of Transmission	
	Host Range	
	Disease Severity	
	Previous history of Lab-Associated Infection	
Medical Surveillance	Pre-exposure recommendations (vaccines availability, indications, etc.)	
	Post-exposure recommendations (therapy or post-exposure prophylaxis availability, indications, etc.)	
	Personnel considerations (identify any health status conditions that would make a person more susceptible to infection or for who exposure to this agent is contraindicated.)	
Agent Stability & Specific Features	Means of chemical or physical inactivation	
	Any specific qualities of the agent that will hinder inactivation or medical treatment (i.e., antibiotic-resistance, genetic modification, etc.)	

Biosafety Level & Containment Practices Assignment (*Consult with the Biosafety Office as needed)*)

Use this space to summarize:

- Regulatory recommendation or restriction factors (USDA, CDC, etc.)
- Factors associated with the process that impact biosafety level assignment
- Biosafety level assignment along with any additional procedural considerations

Date of implementation: Date due for review:

Note that any biological exposure incident associated with the outlined procedure may be indicative of a need for procedural change. In this instance, a review of the procedure and the risk assessment document must be conducted within 30 days of a biological exposure incident.

APPENDIX D – RISK GROUPS

Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in previous healthy adult humans or animals. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis* (see Appendix C-IV-A, *Bacillus subtilis* or *Bacillus licheniformis* Host-Vector Systems, Exceptions); adeno- associated virus (AAV – all serotypes); and recombinant or synthetic AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus. A strain of *Escherichia coli* (see Appendix C-II-A, *Escherichia coli* K-12 Host Vector Systems, Exceptions) is an RG1 agent if it (1) does not possess a complete lipopolysaccharide (*i.e.*, lacks the O antigen); and (2) does not carry any active virulence factor (*e.g.*, toxins) or colonization factors and does not carry any genes encoding these factors.

Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.

Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

- *Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)
- *Actinobacillus*
- *Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
- *Aeromonas hydrophila*
- *Amycolata autotrophica*
- *Archanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
- *Arizona hinshawii* - all serotypes
- *Bacillus anthracis*
- *Bartonella henselae, B. quintana, B. vinsonii*
- *Bordetella* including *B. pertussis*
- *Borrelia recurrentis, B. burgdorferi*
- *Burkholderia* (formerly *Pseudomonas* species) except those listed in Appendix B-III-A (RG3))
- *Campylobacter coli, C. fetus, C. jejuni*
- *Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*
- *Clostridium botulinum*, *C. chauvoei, C. haemolyticum, C. histolyticum, C. novyi, C. septicum, C. tetani*
- *Coxiella burnetii* – specifically the Phase II, Nine Mile strain, plaque purified, clone 4
- *Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale*
- *Dermatophilus congolensis*
- *Edwardsiella tarda*
- *Erysipelothrix rhusiopathiae*
- *Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
- **Francisella tularensis* specifically **F. tularensis* subspecies *novicida* [aka *F. novicida*], strain Utah 112; **F. tularensis* subspecies *holarctica* LVS; **F. tularensis* biovar *tularensis* strain ATCC 6223 (aka strain B38)

*For research involving high concentrations, BL3 practices should be considered (see Appendix G-II-C-2. Special Practices (BL3)).

- *Haemophilus ducreyi, H. influenzae*
- *Helicobacter pylori*
- *Klebsiella* - all species except *K. oxytoca* (RG1)
- *Legionella* including *L. pneumophila*
- *Leptospira interrogans* - all serotypes
- *Listeria*
- *Moraxella*
- *Mycobacterium* (except those listed in Appendix B-III-A (RG3)) including *M. avium* complex, *M. asiaticum, M. bovis* BCG vaccine strain, *M. chelonae, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi*
- *Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
- *Neisseria gonorrhoeae*, *N. meningitidis*
- *Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis*
- *Pseudomonas aeruginosa*
- *Rhodococcus equi*
- *Salmonella* including *S. arizonae, S. choleraesuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi,* A, B, C, *S. typhi*, *S. typhimurium*
- *Shigella* including *S. boydii, S. dysenteriae,* type 1, *S. flexneri, S. sonnei*
- *Sphaerophorus necrophorus*
- *Staphylococcus aureus*
- *Streptobacillus moniliformis*
- *Streptococcus* including *S. pneumoniae, S. pyogenes*
- *Treponema pallidum, T. carateum*
- *Vibrio cholerae, V. parahaemolyticus, V. vulnificus*
- *Yersinia enterocolitica*
- *Yersinia pestis* specifically *pgm*$^{(-)}$ strains (lacking the 102 kb pigmentation locus) and *lcr*$^{(-)}$ strains (lacking the LCR plasmid)

Risk Group 2 (RG2) - Fungal Agents

- *Blastomyces dermatitidis*
- *Cladosporium bantianum, C. (Xylohypha) trichoides*
- *Cryptococcus neoformans*
- *Dactylaria galopava (Ochroconis gallopavum)*
- *Epidermophyton*
- *Exophiala (Wangiella) dermatitidis*
- *Fonsecaea pedrosoi*
- *Microsporum*
- *Paracoccidioides braziliensis*
- *Penicillium marneffei*
- *Sporothrix schenckii*
- *Trichophyton*

Risk Group 2 (RG2) - Parasitic Agents

- *Ancylostoma* human hookworms including *A. duodenale, A. ceylanicum*
- *Ascaris* including *Ascaris lumbricoides suum*
- *Babesia* including *B. divergens, B. microti*

- *Brugia* filaria worms including *B. malayi, B. timori*
- *Coccidia*
- *Cryptosporidium* including *C. parvum*
- *Cysticercus cellulosae* (hydatid cyst, larva of *T. solium*)
- *Echinococcus* including *E. granulosis, E. multilocularis, E. vogeli*
- *Entamoeba histolytica*
- *Enterobius*
- *Fasciola* including *F. gigantica, F. hepatica*
- *Giardia* including *G. lamblia*
- *Heterophyes*
- *Hymenolepis* including *H. diminuta, H. nana*
- *Isospora*
- *Leishmania* including *L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruviana, L. tropica*
- *Loa loa* filaria worms
- *Microsporidium*
- *Naegleria fowleri*
- *Necator* human hookworms including *N. americanus*
- *Onchocerca* filaria worms including, *O. volvulus*
- *Plasmodium* including simian species, *P. cynomolgi, P. falciparum, P. malariae, P. ovale, P. vivax*
- *Sarcocystis* including *S. sui hominis*
- *Schistosoma* including *S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi*
- *Strongyloides* including *S. stercoralis*
- *Taenia solium*
- *Toxocara* including *T. canis*
- *Toxoplasma* including *T. gondii*
- *Trichinella spiralis*
- *Trypanosoma* including *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi*
- *Wuchereria bancrofti* filaria worms

Risk Group 2 (RG2) - Viruses

- Adenoviruses, human - all types
- Alphaviruses (Togaviruses) - Group A Arboviruses
    - Chikungunya vaccine strain 181/25
    - Eastern equine encephalomyelitis virus
    - Venezuelan equine encephalomyelitis vaccine strains TC-83 and V3526
    - Western equine encephalomyelitis virus
- Arenaviruses
    - Junin virus candid #1 vaccine strain
    - Lymphocytic choriomeningitis virus (non-neurotropic strains)
    - Tacaribe virus complex
    - Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)
- Bunyaviruses
    - Bunyamwera virus
    - Rift Valley fever virus vaccine strain MP-12
    - Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)
- Caliciviruses
- Coronaviruses
- Flaviviruses - Group B Arboviruses

    - Dengue virus serotypes 1, 2, 3, and 4
    - Japanese encephalitis virus strain SA 14-14-2
    - Yellow fever virus vaccine strain 17D
    - Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)
- Hepatitis A, B, C, D, and E viruses
- Herpesviruses - except Herpesvirus simiae (Monkey B virus) (see Appendix B-IV-D, *Risk Group 4 (RG4) - Viral Agents*)
    - Cytomegalovirus
    - Epstein Barr virus
    - *Herpes simplex* types 1 and 2
    - *Herpes zoster*
    - Human herpesvirus types 6 and 7
- Orthomyxoviruses
    - Influenza viruses types A, B, and C (except those listed in Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*)
    - Tick-borne orthomyxoviruses
- Papilloma viruses
    - All human papilloma viruses
- Paramyxoviruses
    - Newcastle disease virus
    - Measles virus
    - Mumps virus
    - Parainfluenza viruses types 1, 2, 3, and 4
    - Respiratory syncytial virus
- Parvoviruses
    - Human parvovirus (B19)
- Picornaviruses
    - Coxsackie viruses types A and B
    - Echoviruses - all types
    - Polioviruses - all types, wild and attenuated
    - Rhinoviruses - all types
- Poxviruses - all types except Monkeypox virus (see Appendix B-III-D, *Risk Group 3 (RG3) - Viruses and Prions*) and restricted poxviruses including Alastrim, Smallpox, and Whitepox (see Section V-L, *Footnotes and References of Sections I through IV*)
- Reoviruses - all types including Coltivirus, human Rotavirus, and Orbivirus (Colorado tick fever virus)
- Rhabdoviruses
    - Rabies virus - all strains
    - Vesicular stomatitis virus non exotic strains: VSV-Indiana 1 serotype strains (*e.g.* Glasgow, Mudd-Summers, Orsay, San Juan) and VSV-New Jersey serotype strains (*e.g.* Ogden, Hazelhurst)
- Rubivirus (Togaviruses)
    - Rubella virus

Risk Group 3 (RG3) Agents

RG3 agents are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available.

Risk Group 3 (RG3) - Bacterial Agents Including Rickettsia

- *Bartonella*

- *Brucella* including *B. abortus, B. canis*, *B. suis*
- *Burkholderia (Pseudomonas) mallei, B. pseudomallei*
- *Coxiella burnetii (*except the Phase II, Nine Mile strain listed in Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)*
- *Francisella tularensis (*except those strains listed in Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)*
- *Mycobacterium bovis* (except BCG strain, see Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia*), *M. tuberculosis*
- *Pasteurella multocida* type B -"buffalo" and other virulent strains
- Rickettsia akari, R. australis, R. canada, R. conorii, R. prowazekii, R. rickettsii, R, siberica, R. tsutsugamushi, R. typhi (R. mooseri)
- *Yersinia pestis (*except those strains listed in Appendix B-II-A, *Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia)*

Risk Group 3 (RG3) - Fungal Agents

- *Coccidioides immitis* (sporulating cultures; contaminated soil)
- *Histoplasma capsulatum, H. capsulatum var. duboisii*

Risk Group 3 (RG3) - Parasitic Agents

None

Risk Group 3 (RG3) - Viruses and Prions

- Alphaviruses (Togaviruses) - Group A Arboviruses
    - Chikungunya virus (except the vaccine strain 181/25 listed in Appendix B-II-D Risk Group2 (RG2) – Viruses)
    - Semliki Forest virus
    - St. Louis encephalitis virus
    - Venezuelan equine encephalomyelitis virus (except the vaccine strains TC-83 and V3526, see Appendix B-II-D (RG2) – Viruses)
    - Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)
- Arenaviruses
    - Flexal
    - Lymphocytic choriomeningitis virus (LCM) (neurotropic strains)
- Bunyaviruses
    - Hantaviruses including Hantaan virus
    - Rift Valley fever virus
- Coronaviruses
    - SARS-CoV-2 viruses (SARS associated viruses)
    - Middle East respiratory syndrome coronavirus (MERS-CoV)
- Flaviviruses - Group B Arboviruses
    - Japanese encephalitis virus (except those strains listed in Appendix B-II-D Risk Group2 (RG2) - Viruses)
    - West Nile virus (WNV)
    - Yellow fever virus
    - Other viruses as listed in the reference source (see Section V-C, *Footnotes and References of Sections I through IV*)
- Orthomyxoviruses

    - Influenza viruses 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968), and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1).
- Poxviruses
    - Monkeypox virus
- Prions
    - Transmissible spongiform encephalopathies (TSE) agents (Creutzfeldt-Jacob disease and kuru agents)(see Section V-C, *Footnotes and References of Sections I through IV*, for containment instruction)
- Retroviruses
    - Human immunodeficiency virus (HIV) types 1 and 2
    - Human T cell lymphotropic virus (HTLV) types 1 and 2
    - Simian immunodeficiency virus (SIV)
- Rhabdoviruses
    - Vesicular stomatitis virus (except those strains listed in Appendix B-II-D Risk Group2 (RG2) - Viruses)

Risk Group 4 (RG4) Agents

RG4 agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available.

Risk Group 4 (RG4) - Bacterial Agents

None

Risk Group 4 (RG4) - Fungal Agents

None

Risk Group 4 (RG4) - Parasitic Agents

None

Risk Group 4 (RG4) - Viral Agents

- Arenaviruses
    - Guanarito virus
    - Lassa virus
    - Junin virus (except the candid #1 vaccine strain listed in Appendix B-II-D Risk Group2 (RG2) – Viruses)
    - Machupo virus
    - Sabia
- Bunyaviruses (Nairovirus)
    - Crimean-Congo hemorrhagic fever virus
- Filoviruses
    - Ebola virus
    - Marburg virus
- Flaviruses - Group B Arboviruses
    - Tick-borne encephalitis virus complex including Absetterov, Central European encephalitis, Hanzalova, Hypr, Kumlinge, Kyasanur Forest disease, Omsk hemorrhagic fever, and Russian spring-summer encephalitis viruses

    - Herpesviruses (alpha)
    - Herpesvirus simiae (Herpes B or Monkey B virus)
- Paramyxoviruses
    - Equine Morbillivirus (Hendra virus)
    - Hemorrhagic fever agents and viruses as yet undefined

APPENDIX E – EQUIPMENT RELEASE FORM

Please see the MSU EHS website for the latest version:

https://ehs.msu.edu/_assets/docs/lab/equipment-release-form.pdf

APPENDIX F- EXPOSURE RESPONSE PROCEDURE

Potentially Infectious Materials and Toxins

Potentially infectious materials and biological toxins in the lab include items such as: Cell culture, serum, environmental specimens that may contain pathogens or biological toxins, or any items contaminated with the material.

A potentially infectious material or biological toxin exposure incident occurs when these materials:

- Come into contact with a worker's mucous membranes (eye, nose, or mouth)
  Example: Cell culture waste splash into the eye
- Enter the body through breaks in the skin (cut, rash, hangnail)
  Example: human blood contaminated piece of glass puncturing a finger
- Are accidentally ingested
  Example: Eating with toxin contaminated hands

WHAT TO DO IN THE EVENT OF AN EXPOSURE

Immediate response will reduce your risk of getting a laboratory acquired infection.

1. **Flush the exposed area with water:** flush eyes, nose, or mouth for 15 minutes. If skin was exposed, wash thoroughly with soap and water. Bandage area if needed to control bleeding.
2. **Notify your supervisor if he or she is available.** If you can let someone responsible know what has occurred and where you will be.
3. **Print and take an "Authorization to Invoice MSU" form with you.** Available at www.hr.msu.edu
4. **Report to Lansing Urgent Care** (Frandor location is open 24 hrs) for post-exposure follow-up as soon as possible or immediately if exposed to human derived materials like blood.
5. **Follow-up by completing the "Report of Claimed Occupational Injury or Illness"** form with your supervisor. Available at www.hr.msu.edu.

APPENDIX G – LAB AND ANIMAL BIOSAFETY LEVEL SUMMARIES

CDC/NIH Biosafety in Microbiological and Biomedical Laboratories; 5th Edition

Summary of Recommended Biosafety Levels for Infectious Agents

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices (Culture waste decontaminated/autoclaved)	None required	Open bench and sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of lab clothing before laundering • Baseline serum	Primary barriers = Class I or II BSCs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhausted air not recirculated • Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decon systems • Other requirements outlined in the text

Summary of Recommended Biosafety Levels for Activities in Which Experimentally or Naturally Infected Vertebrate Animals Are Used

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species.	Standard animal facility • No recirculation of exhaust air • Directional air flow recommended • Handwashing sink recommended
2	Associated with human disease. Hazard = percutaneous injury, ingestion, mucous membrane exposure	ABSL-1 practice plus: • Limited access • Biohazard warning signs • Sharps precautions • Biosafety manual • Decontamination of all infectious wastes and of animal cages prior to washing	ABSL-1 equipment plus primary barriers: containment equipment appropriate for animal species; PPEs: laboratory coats, gloves, face and respiratory protection as needed	ABSL-1 facility plus: • Autoclave available • Handwashing sink available • Mechanical cage washer used
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious health effects	ABSL-2 practice plus: • Controlled access • Decontamination of clothing before laundering • Cages decontaminated before bedding removed • Disinfectant foot bath as needed	ABSL-2 equipment plus: • Containment equipment for housing animals and cage dumping activities • BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols. PPEs: appropriate respiratory protection	ABSL-2 facility plus: • Physical separation from access corridors • Self-closing, double-door access • Sealed penetrations • Sealed windows • Autoclave available in facility
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	ABSL-3 practice plus: • Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting • All wastes are decontaminated before removal from the facility	ABSL-3 equipment plus: • Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure personnel suit) used for all procedures and activities	ABSL-3 facility plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum and decontamination systems • Other requirements outlines in the text

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APPENDIX H – EXAMPLE INVENTORY LOG

ID #	Organism Name	Characteristics	Source	Quantity Received	Rec'd From	Date Rec'd	Storage Location	Logged by	Date of last activity	Activity Reference
Example 204	*E.coli BL21*	*BSL-1, for recombinant research*	*ATCC*	*2 vials 100ul each*	*Smith Lab MSU*	*6/5/2014*	*Freezer #1*	*K. Smith*	*9/2/2014*	*IBC Registered Research*

APPENDIX I – BIOSAFETY PRACTICES FOR HANDLING PRIONS

Recommended Biosafety Practices for Handling Prions and Prion-Infected Tissues
Updated May 2007

Introduction

Research-related activities involving prions or tissues containing prions have been on the rise at MSU in both the animal health and human health arenas. Because the infectious nature of prions is not well characterized and destruction of these particles goes beyond the techniques typically required for biohazard inactivation, work with these agents requires special considerations for biocontainment to minimize both occupational and environmental exposure risk.

Prions & General Biosafety Recommendations

Prions (proteinaceous infectious particles, an abnormal isoform of a normal cellular protein) cause Creutzfeldt-Jakob disease (CJD), scrapie and other related human and animal neurodegenerative diseases. Human prions are manipulated at Biosafety Level (BSL) 2 or 3, depending on the activity, with most human prions treated as BSL-3 under most experimental conditions. In many instances, BSE prions can also be manipulated at BSL-2, however due to the high probability that BSE prions have been transmitted to humans, certain circumstances may require the use of BSL-3 facilities. All other animal prions are considered BSL-2 pathogens. However, when a prion from one species is inoculated into another the resultant infected animal should be treated according to the guidelines applying to the source of the inoculum. Please see the following table adapted from the BMBL for a list of common mammalian prions and general BSL recommendation.

Note: *Biosafety level assignment should be established using a risk assessment that accounts for the nature and host range of the agent, as well as the nature of the procedures and concentration and quantity of the agent.*

Table: The Prion Diseases *(BMBL, 6th ed., 2020)*

Disease (abbreviation)	**Natural Host**	**Prion**	**Pathogenic PrP Isoform**	**Biosafety Level**
Scrapie	sheep, goats and mouflon	scrapie prion	$OvPrP^{Sc}$	2
Transmissible mink encephalopathy (TME)	mink (farmed raised)	TME prion	$MkPrP^{Sc}$	2
Chronic wasting disease (CWD)	mule deer, Rocky Mountain elk, moose and white tail deer	CWD prion	$MdePrP^{Sc}$	2
Bovine spongiform encephalopathy (BSE)	cattle	BSE prion	$BoPrP^{Sc}$	2/3
Feline spongiform encephalopathy (FSE)	Domestic cats, wild cats in capitivity	FSE prion	$FePrP^{Sc}$	2
Exotic ungulate encephalopathy (EUE)	nyala, greater kudu and oryx	EUE prion	$UngPrP^{Sc}$	2
Kuru	humans	kuru prion	$HuPrP^{Sc}$	2/3

Disease (abbreviation)	Natural Host	Prion	Pathogenic PrP Isoform	Biosafety Level
Creutzfeldt-Jakob disease (CJD)	humans	CJD prion	$HuPrP^{Sc}$	2/3
Gerstmann-Sträussler-Scheinker syndrome (GSS)	humans	GSS prion	$HuPrP^{Sc}$	2/3
Fatal familial insomnia (FFI)	humans	FFI prion	$HuPrP^{Sc}$	2/3

The highest concentration of prions is found in the central nervous system (CNS), and extreme caution must be exerted when handling CNS samples. However, prions can also be found in the CSF, lung, liver, kidney, spleen/lymph nodes, and placenta. Unfixed samples of brain or spinal cord, as well as other tissues known to contain human prions should be handled at BSL-3. With regards to BSE prions, it is also recommended that animal tissue samples (e.g., brain, spinal cord) known or strongly suspected to contain prions be handled at BSL-3 (BMBL 2020). For other samples, the level of containment will depend on the type of tissue handled, the nature of the manipulation and the amount of material handled (MSDS 1997).

Formaldehyde or formalin-fixed, glutaraldehyde-fixed, and paraffin-embedded tissues, particularly of the brain, remain infectious for long periods, if not indefinitely (BMBL 2020, WHO 2000). They should be handled cautiously as fresh materials from fixation through embedding, sectioning, staining, and mounting on slides, unless treated with 95% formic acid (WHO 2000).

Laboratory-acquired prion infections, the primary hazard is from accidental parenteral inoculation or ingestion (ABSA 2021 LAI Database). Cuts and punctures should be avoided, and the use of sharp knives, scalpels, blades, and needles should be minimized. If the use of sharps cannot be avoided, cut-resistant gloves should be worn (CFIA 2005).

Wherever possible, the laboratory and equipment used for work with prions should be dedicated to that task alone. All employees should be informed and aware that prion research is being conducted in the lab. The entrance to the lab should allow for the separation of PPE/lab clothing and staff clothing. An exposure protocol should be developed, posted, and communicated to all employees (CFIA 2005, UCSD 2002). Procedures should be in place for the effective decontamination of all waste, re-usable equipment, surfaces, and other lab space (CFIA 2005, UCSD 2002).

Working with Prion-Risk Materials at MSU

At this time, work with prion-risk materials at MSU is limited to research and diagnostic laboratory applications. Therefore, this guidance document applies to these procedures only. Guidelines for use of prion-risk materials in conjunction with live animals will be developed if needed. Therefore, if future project plans call for use of live animals and prion-risk materials, please notify the MSU Biosafety Officer at the proposal-writing stage to perform a risk assessment and identify containment requirements.

Procedures involving the manipulation of animal tissues that are from known or suspected scrapie or CWD cases must be handled under BSL-2 conditions as a minimum standard. Procedures involving manipulation of human tissues that are known or suspected cases of CJD must typically be handled at BSL-3 conditions, unless a risk assessment completed in conjunction with an EHS Biosafety Professional allows for BSL-2 facilities and procedures. In general, procedures that involve aerosolization or vigorous disruption of the material (i.e., centrifugation, sonication, laser dissection) bear the greatest risk to personnel and the environment and will require special consideration for containment at both biosafety levels.

Summary

A summary of BSL-2 and BSL-3 facility and procedural requirements as outlined in the BMBL is attached at the end of this document. Additionally, the following specific measures should be implemented for all work with prion-risk materials:

1. Access to the laboratory must be restricted to trained personnel when work is being conducted on tissue.
2. Personnel working with prion-risk materials must complete Biosafety Principles for Animal Users through the EHS, as well as complete on-site training relative to the nature of the prion in use, routes of transmission, and specific hazards of the tissue handling process. Written procedures and training records should be kept as outlined in the BMBL.
3. Personnel must wear gloves and gowns while handling tissues that are potentially contaminated. All protective clothing must be removed before leaving the laboratory.
4. All fixed, non-fixed, or frozen tissues must be contained within watertight containers. Containers must be individually labeled with the universal biohazard symbol or placed in a secondary container (i.e., a tray with sides) that is labeled with the universal biohazard symbol.
5. Sonication or homogenization of tissues must be performed in a properly certified Class II biosafety cabinet.
6. Microtome blades and knives used for cutting tissue must be cleaned with an instrument that does not put the hand or finger of the operator in or near contact with the blade.
7. Disposable, absorbent pads or disposable trays should be used whenever possible to help confine contamination and to facilitate cleanup and disinfection.
8. The following practices should be followed when using reusable instruments:
    - Instruments should be kept wet until cleaned and decontaminated.
    - Instruments should be cleaned as soon as possible to prevent drying of material.
    - Do not mix instruments used on materials potentially infected with prions with those instruments used for other purposes.
    - Instruments that will be cleaned in a dishwasher must be decontaminated first and the washer must be run through an empty cycle before being used for other instruments
9. The following provisions for decontamination of wastes, reusable instruments and contaminated surfaces must be followed to assure effective inactivation of prions:

    - **Liquid waste**

    Liquid waste may be treated in the following ways:

    - Mix with NaOH for a final concentration of 1.0 N NaOH and hold at room temperature for 1 hour: or
    - Mix with bleach for a final concentration of 20,000 ppm available chlorine and hold at room temperature for 1 hour

    This waste should be stored in a chemical fume hood for the duration of the treatment period. After the treatment period, liquid waste may be neutralized and discharged to the sewer by way of the lab sink or disposed of through the EHS as liquid chemical waste.

    - **Contaminated surfaces**

    Contaminated surfaces may be treated in the following ways:

    - Bleach solution (20,000 ppm available chlorine) for 1 hour; or

- 1N NaOH for 1 hour

After treatment, surfaces should be thoroughly rinsed with clear water.

- **Contaminated reusable instruments**

Contaminated reusable instruments may be treated in the following ways:

- Immerse in 1N NaOH or sodium hypochlorite (20,000 ppm available chlorine) for 1 hour, transfer to water, autoclave (gravity displacement) at 121°C for 1 hour (BMBL 2020, WHO 2000);
- Immerse in 1N NaOH or sodium hypochlorite (20,000 ppm available chlorine) 1 hour, rinse with water, autoclave at 121°C for 1 hour (gravity displacement) or at 134 °C for I hour (porous load) (BMBL 2020, WHO 2000); or
- Immerse in sodium hypochlorite solution with 20,000 ppm available chlorine (preferred) or 1N NaOH (alternative) for 1 hour (WHO 2000)

- **Contaminated dry waste**

All contaminated dry waste should be picked up for incineration. Prion-contaminated sharps waste must be identified as “prion contaminated sharps- for incineration only” on the hazardous waste pickup request to assure incineration of these materials. Contact the EHS Biosafety Staff for further assistance regarding treatment and disposal.

10. Intact skin exposure to prion-risk materials should be followed by washing with 1N NaOH or 10% bleach for two to three minutes, followed by extensive washing with water. For needle sticks or lacerations, gently encourage bleeding, wash with warm soapy water, rinse, dry and cover with a waterproof dressing. In the event of a splash to the eye, rinse the affected eye with copious amounts of water or saline **only**. In the instance of a splash or puncture, the exposed individual should then report to Olin Urgent Care for follow-up through MSU Occupational Health.
11. The Principal Investigator (PI) must assure that all spills or exposures involving prion-risk materials are managed with the proper procedures. Additionally, these events should be reported to the MSU Biosafety Officer as soon as possible for follow-up and assistance with actions to reduce future occurrences.
12. Prion-risk materials may be subject to permit requirements for shipment and receipt. USDA permits apply to interstate and international shipment of animal-related materials capable of transmitting infection. CDC permits apply to import of materials that are potentially infectious to humans. Additionally, shipment of these materials requires specific training for the shipper. Contact the EHS Biosafety Staff for further information.

Notes on chemical disinfection

Sodium hydroxide (NaOH, or soda lye)

Be familiar with and observe safety guidelines for working with NaOH. 1N NaOH is a solution of 40 g NaOH in 1 liter of water. 1 N NaOH readily reacts with CO2 in air to form carbonates that neutralize NaOH and diminish its disinfectant properties. 10 N NaOH solutions do not absorb CO2, therefore, 1N NaOH working solutions should be prepared fresh for each use either from solid NaOH pellets, or by dilution of 10 N NaOH stock solutions.

Sodium hypochlorite (NaOCl solution, or bleach)

Be familiar with and observe safety guidelines for working with sodium hypochlorite. Household or industrial strength bleach is sold at different concentrations so a standard dilution cannot be specified. Efficacy depends upon the concentration of available chlorine and should be 20,000 ppm available chlorine.

These solutions are corrosive and appropriate personal protective equipment must be worn when preparing and using them.

APPENDIX J – REQUIREMENTS FOR HANDLING EXCLUDED STRAINS OF SELECT AGENTS

Introduction

The United States Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA) have established regulations for the possession, use and transfer of Select Agents and toxins (see 42 CFR Part 73, 7 CFR Part 331 and 9 CFR Part 121). These regulations have also established a procedure by which an attenuated strain of a Select Agent that does not pose a severe threat to public health and safety, animal health, or animal products may be excluded from the requirements of the regulations when used for specific purposes. Please note that if an excluded attenuated strain is manipulated in such a way that virulence is restored or enhanced, or if factors associated with virulence are reintroduced, it will then be subject to the regulations. Because of the nature of these excluded strains and the potential for them to be manipulated for use as a biological weapon, the Office of Environmental Health and Safety (EHS) has implemented the following containment and security requirements for handling excluded strains of Select Agents. This list was last updated on selectagents.gov on November 19, 2020 and is still current as of August 2022, see the website for more up to date information.

Applicability

The containment and security requirements apply to the following excluded strains of Select Agents (underlined titles are links):

Nontoxic HHS toxins, Section 73.3 (d)(2)

- Botulinum neurotoxins
- Conotoxins
- Staphylococcal Enterotoxins (SE)

Excluded Toxins Modified to be Less Potent or Toxic, Section 73.3 (e)

- Tetrodotoxin

Excluded Attenuated Strains of HHS Select Agents, Section 73.3 (e)

- Botulinum neurotoxin producing species of *Clostridium*
- *Coxiella burnetii*
- Eastern Equine Encephalitis virus
- Ebola virus
- *Francisella tularensis*
- Junin virus
- Lassa fever virus
- Monkeypox virus
- SARS-Coronavirus
- *Yersinia pestis*

Excluded Attenuated Strains of Overlap Select Agents, Section 73.4 (e) and 121.4 (e)

- *Bacillus anthracis*
- *Brucella abortus*

- *Brucella melitensis*
- *Burkholderia mallei*
- *Burkholderia pseudomallei*
- Rift Valley Fever Virus
- Venezuelan Equine Encephalitis virus

Attenuated Strains of USDA-only Select Agents Excluded, Section 121.3 (e)

- African swine fever viruses
- Avian influenza virus (low pathogenic)
- Avian influenza virus (highly pathogenic)
- Foot-and-mouth disease virus

Requirements

After conducting a risk assessment, the EHS/EHS has determined that biosafety level 2 precautions in addition to specific security measures are not only appropriate, but prudent practice for handling exempt strains of Select Agents. Therefore, the following requirements have been implemented:

- All biosafety level 2 practices, safety equipment and facility requirements must be followed. For specific information on those requirements please contact Dr. Jamie Sue Willard-Smith (353-1877):

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets, freezers, or refrigerators designated for this purpose only.
4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
9. An insect and rodent control program is in effect.

B. Special Practices

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility

for assessing each circumstance and determining who may enter or work in the laboratory or animal room.

2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.
3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate signage will be provided by the EHS.
4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).
5. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
6. Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
7. The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
8. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
    a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plasticware should be substituted for glassware whenever possible.
    b. Only needle-locking syringes or disposable syringe needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
    c. Syringes which re-sheathe the needle, needleless systems, and other safety devices are used when appropriate.
    d. Broken glassware must not be handled directly by hand but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or Federal regulations.
9. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
10. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or Federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.
11. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
12. Animals not involved in the work being performed are not permitted in the lab.

C. Safety Equipment (Primary Barriers)

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:
    a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.
    b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.
2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.
3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces, or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching “clean” surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

D. Laboratory Facilities (Secondary Barriers)

1. Provide lockable doors for facilities that house restricted agents (as defined in 42 CFR 72.6).
2. Consider locating new laboratories away from public areas.
3. Each laboratory contains a sink for handwashing.
4. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
5. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
6. Laboratory furniture can support anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
7. Install biological safety cabinets in such a manner that fluctuations of the room supply, and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment to maintain the biological safety cabinets’ air flow parameters for containment.
8. An eyewash station is readily available.
9. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
10. There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

- The following security measures must be adhered to:

- An accurate and up-to-date inventory must be maintained. The following information must be included in the inventory:
    - Date of use
    - Name of person using the materials
    - Beginning amount of material
    - Amount of material used for procedure
    - End amount of material
    - Procedure the material was used for
- All exempt strains of Select Agents (i.e., stock solutions, working solutions, etc.) must be stored in a lockable storage unit.
- Storage units that house exempt strains of Select Agents must be kept locked when not actively being used; and
- Only those people approved by the principal investigator and the EHS may have access to the strains.

- Please notify the Biosafety Office at 353-1877 or 432-5262 if you possess or plan to possess any of the exempted Select Agent strains. A lab inspection must be conducted prior to working with these agents.

- If inconsistencies exist with the inventory, contact the EHS at 355-0153.

Contacts

Any questions regarding these requirements should be directed to Dr. Jamie Sue Willard-Smith (353-1877)

APPENDIX K – SITE SPECIFIC TRAINING CHECKLIST

Location and Review of Safety Protocols	Radiation	Chemical	Biological/Toxin	Bloodborne	Completed
Emergency Contacts	X	X	X	X	
Emergency Response Procedure	X	X	X	X	
Standard Operating Procedures (task specific)	X	X	X	X	
MSU's food in lab policy	X	X	X	X	
Chemical Hygiene Plan		X	X	X	
MSDS		X	X	X	
Radiation Safety Manual	X				
Hazardous Waste Guide	X	X	X	X	
Biohazardous Waste Plan			X	X	
Biological Safety Manual			X	X	
Exposure Control Plan				X	
Source Protocol				X	

Inventory, Storage, Labeling, and Proper Use	Radiation	Chemical	Biological/Toxin	Bloodborne	Completed
Chemical Storage		X	X	X	
Hazardous Chemicals		X	X	X	
Biohazardous Materials (and toxins)			X	X	
Radioactive materials	X				

Location, Proper Use , and Maintenance:	Radiation	Chemical	Biological/Toxin	Bloodborne	Completed
Fume Hood		X	X		
Personal Protective Equipment		X	X	X	
Emergency Eyewash/Shower		X	X	X	
Compressed Gases		X	X	X	
Chemical Spill Kit		X	X	X	
Biological Spill Kit			X	X	
Biosafety Cabinet/Laminar Flow Hood			X	X	
Autoclaves			X	X	
Disinfectants			X	X	
Safer Sharps				X	
Survey Instruments	X				
Radiation license	X				

Waste Segregation, Storage, Transport, Treatment	Radiation	Chemical	Biological/Toxin	Bloodborne	Completed
Sharps Waste		X	X	X	
Glass Waste		X	X	X	
Solid Waste		X	X	X	
Liquid Waste		X	X	X	
Waste Tags		X	X	X	
90 Day Disposal		X	X	X	
Transport		X	X	X	
Treatment/Decontamination	X		X	X	
Radioactive waste	X				

Security	Radiation	Chemical	Biological/Toxin	Bloodborne	Completed
Laboratory Security	X	X	X	X	
Inventory	X	X	X	X	

Worker Consent:

I certify that I have been provided with and understand the information indicated above. I understand that this is a certification of PI/Manager/Precept/Trainer site specific training and informed consent, and does not constitute a waiver of my rights. I understand that I am responsible for adhering to all safety practices, laws, rules and guidelines.

Print Employee's/Student Name (Trainee)	Employee's/Student Signature (Trainee)	Date
Print PI/Manager/Precept/Trainer Name	PI/Manager/Precept/Trainer Signature	Date

APPENDIX L – LARGE SCALE BIOSAFETY GUIDELINES

BMBL 6th Edition, Appendix M—Large-Scale Biosafety

Introduction

When working with biological agents in large – scale quantities, there are unique consideration that must be addressed in order to ensure worker and environmental protection. Large-scale biological production facilities should use the laboratory scale principles of risk assessment set forth in BMBL Section II, and by ISO 35001, Biorisk Management for Laboratories and Other Related Organizations.

In addition to laboratory scale risk assessment requirements, the utilization of larger equipment and volumes of chemicals or raw materials requires risk management strategies beyond biological safety alone. The following sections apply risk management steps to give readers the most pertinent information for managing risk in large-scale production. The recommendations assume that those performing risk assessments for large-scale work will involve industrial hygienists and other process safety specialists when implementing risk assessment and control measures for large-scale operations.

Appendix K of *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* prescribes safety practices and containment procedures for large-scale (i.e., >10 liters per container) facilities. These guidelines can be applied to all large-scale work with biological materials (e.g., genetically modified organisms [GMO] and non-GMO, human, and animal/zoonotic pathogens). Please ensure familiarity with local regulations as these may differ from recommendations in this text.

Risk Assessment

Integrate the steps and processes utilized in laboratory biological risk assessment for any large-scale project. Risk assessment should be done during planning, when elements of the process change, and during periodic reviews of existing biological production processes, particularly after incidents or process failures. Risk control measures must be installed to mitigate unacceptable risk. Systems must be evaluated to determine their contribution to risk. The Good Practice quality guidelines and regulations (GxP) include three commonly used GxPs: Good Clinical Practices (GCP), Good Laboratory Practices (GLP),1 and Good Manufacturing Practices (GMP);2 GxP product Impact Assessment (IA) analysis can be extended to evaluate biosafety and laboratory biosecurity-related systems that govern exposure control, process room and environmental protection, decontamination, access control and accountability. Risk assessments should focus on the biological, chemical, physical, product, and equipment biosafety and laboratory biosecurity risk points. Production technologies and equipment with the potential for misuse (laboratory biosecurity/dual-use/export control) may also be included in the risk assessment. Subject matter experts in engineering; Heating, Ventilation, and Air Conditioning (HVAC); quality control; occupational health; security; and health, safety, and environment (HSE) should always be consulted when making risk-based determinations.

Hazard Identification

The first step of risk assessment is hazard identification. Review additional factors that are unique to large-scale biological processes. Additional factors include but are not limited to:

1. Unique strains utilized primarily for research or manufacturing processes (e.g., producing high titers of a toxin);
2. High volumes (>10 liters) and high concentrations of product;
3. Specialized equipment and processes with unique risk points require a Hazard Analysis of Critical Control Points and/or Hazard and Operability studies;

4. Pressurized vessels and lines for biological and chemical reactions pose a risk for aerosol generation (e.g., bioreactors, fermenters, thermal inactivation tanks); and
5. Atypical routes of transmission (e.g., inhalation of biological agents or toxins not normally transmitted via the aerosol route).

Non-biological hazards to consider when performing a risk assessment may include, but are not limited to:

1. Hazardous chemicals: formaldehyde or similar for inactivation, large quantities of detergents, disinfectants and caustics, adjuvants, preservatives, solvents for down-stream processing, allergens or toxins, and asphyxiants;
2. Physical hazards: noise, steam, heat, cold, and radiation including UV and lasers;
3. Life-safety hazards: confined space, working at heights, line breaking, and pressurized systems;
4. Ergonomics;
5. Process safety-relevant controls (e.g., fire/explosions; pressurized systems);
6. Preventative maintenance (PM): solid and process effluent waste streams and control measures employed, including PM of relevant equipment;
7. Processes to control release of material (i.e., human and environmental risks), including corresponding emergency procedures; and
8. Risk points associated with equipment.

Hazard Evaluation

As with laboratory risk assessment, the hazards associated with the biological agent/material and process equipment must be evaluated. In addition, the operational integrity of containment equipment and facility safeguards and the capability of area staff to effectively control potential hazards must be considered. Staff capability will depend on the training, technical proficiency, and good habits of all team members.

Large-scale research and production pose additional risks that require evaluation. Increased growth, vessel size, and enhanced aeration magnify the aerosol generation risk. By design, the biological agent concentration is greatly increased. Therefore, protection from aerosol transmission must be considered for agents normally transmitted by insect bite or injection.

Chemical risks are also increased due to handling of dry powders for media preparation, pumping of acid or base for pH control, and preparation/addition of inactivation chemicals for vaccine preparation. Closed system transfer technology may be foreign to those with experience limited to the laboratory.

Risks due to hazardous energy (i.e., electrical, steam, pressurized gases) are also magnified. Hazardous energy control procedures such as removing the power cord or closing a supply valve become complex and may be poorly understood by those with experience limited to the laboratory.

Risk Control

Risk mitigation strategies identified in large-scale research and production follow the same principles (i.e., hierarchy of controls) established to control HSE risks.3 Those performing risk assessments for large-scale work may be able to eliminate a hazard or substitute to reduce risk. When this is not possible, engineering, administrative and/or work practice controls, and PPE are utilized.

Engineering Controls

Selecting the proper engineering solution is an iterative process.4,5 The design provisions for a large-scale biological production facility will differ greatly depending on whether the work is dealing with an exotic, indigenous, eradicated, novel, or emerging disease-causing agent; a highly allergenic compound; a GMO, carcinogenic or highly toxic product; or a well-characterized and attenuated childhood vaccine.

Many controls must be considered in the process, including HSE-risk, biosafety, and laboratory biosecurity. In addition, large-scale GxP facilities must evaluate quality design controls for product as well as personnel and environmental protection. Consider state and local regulations when implementing the design of a large-scale biological production facility. A large-scale facility balancing GxP and biosafety requirements will need to evaluate the following basic facility principles:

Clean to Dirty The process design must include controls to prevent contamination spread within the facility and to the environment. If applicable, an assessment of conflicts between GxP and biosafety requirements must also occur to achieve two different definitions of clean. If there are two competing requirements, implement controls that address the highest consequence events and identify alternate methods to meet the intent of the competing requirement. For example, if an operation requires positive-pressure environment to achieve product protection, you can create an air pressure sink in an anteroom to ensure containment of the biological agent.

Change Rooms and Barriers Establish donning and doffing needs by creating an operational flow diagram. This will help clarify how many actions an operator must take for a given procedure or process step when passing through a personnel barrier or door. The review should cover normal operations, planned and unplanned maintenance, and emergencies. This process should identify the potential demand in PPE for the facility, the number and locations of room(s), and room size(s) necessary for storing PPE and changing. Facilities covered by GxP requirements must consider PPE and workflow requirements to achieve product protection in addition to personnel and environmental protection.

Airlocks and high/low-risk rooms (i.e., biologicals vs. cleanrooms) The design must address biosafety concerns as well as applicable GxP requirements to achieve personnel, environment, and product protection, if required.

Surfaces Floor, wall and ceiling, door and window, and other exposed component surfaces must be impervious and easy to clean. The materials must be resistant to a host of chemicals including liquid and gaseous disinfectants, if needed, for decontamination or prevention of cross-contamination. Construction attributes of floor strength, ceiling height, segregation need, piping (i.e., materials, product, and waste) and energy lines must support and promote large-scale processes.

HVAC system, room pressure, and airflow The design of the airflow must provide personnel and environmental protection. In the event a process area must be positive-pressure, consider designing the room airlock or changing area as a pressure sink. Exhaust air filtering systems may be required, as in the case of vaccine plants producing live attenuated vaccines, to prevent ductwork contamination. GxP requirements may also require product protection design considerations.

Gaseous Decontamination The HVAC system, walls, and wall penetrations must be made such that the room can be decontaminated without a negative impact to adjacent spaces. The decontaminant employed must be appropriate for the process and biological agents handled. Use the same principles for gaseous decontamination of a laboratory, but the quantities used and the clearing times will differ substantially.

Spill Containment When designing for spill containment, consider the biological, chemical, and physical processes in an area. Always review spill scenarios while designing a facility. Identify what and how much can be released, where spilled materials will flow (e.g., are there drains leading to an effluent decontamination system (EDS) or will materials released be captured within a containment dike), if manual inactivation will be required, and what emergency response activities will encompass.

Kill Tanks/EDS Systems Ensure EDS systems can inactivate effluent from production waste and spills. It is particularly beneficial to have a facility designed with secondary failsafe systems when large amounts of material are processed. The exact method used will depend on local regulations and the materials in question. Numerous options exist, including chemical inactivation using acids or caustics, and heat inactivation (batch or continuous). Ensure holding tanks have stirrers when volumes are large. Most

facilities employ hard piping, and a process to clean and decontaminate these lines between production areas and the EDS must be integrated into the plan.

Those performing risk assessments for large-scale work will also determine the type of equipment to be used by considering production needs and risk assessment results.6 Historically, the standard has been fixed equipment (i.e., stainless steel bioreactors) with a combination of hard and flexible hose piping for upstream (i.e., biological agent propagation) and downstream (i.e., biological agent purification, concentration, and potentially inactivation) processes. Increasingly, single-use (SU) equipment is replacing fixed equipment for upstream processes. The “ballroom” concept, where both upstream and downstream processes are in one large production facility, is now accepted for select biological processes.7 The ballroom concept relies on maintaining closed systems at all times.

1. Ballroom Layout Advantages
    a. More flexibility to accommodate different process trains;
    b. Improved operational efficiency and oversight (e.g., avoids having to move equipment between rooms); and
    c. Reduction of footprints and cost.
2. Ballroom Layout Disadvantages
    a. Increased risk of contamination spread in upset conditions to downstream processes;
    b. Need for typically open operations (e.g., cell expansion, column packing or powder addition) to be handled in closed systems;
    c. Need for enhanced environmental monitoring to be conducted to detect a breach in any closed system and need to ensure contamination or cross-contamination has not occurred; and
    d. Challenging area and equipment decontamination when production areas are shared.

A non-comprehensive list of containment requirements and associated risk points is provided below to assist in the assessment of risks associated with SU equipment.

Containment Requirements and Example Risk Points7–10

1. Viable organisms should be handled in a closed system or other primary containment.
    a. Ensure the bioreactor bag is compatible with maximum output temperature of heating control circuit;
    b. Ensure the tubing is compatible with process media, including pH control solutions and stability testing has been performed; and
    c. Implement procedures to ensure that probes are not removed during operation.
2. Culture fluids are not removed from a system until organisms are inactivated.
    a. Implement procedures for removing bioreactor bag(s) containing infectious agent(s).
3. Inactivation of waste solutions and materials with respect to their biohazard potential.
    a. Implement procedures for processing used bioreactor bags containing infectious agents;
    b. Ensure presence of biosafety cabinet for removing reusable components before destruction;
    c. Ensure the waste disposal procedure compatible with bioreactor bags;
    d. Implement a procedure for safely autoclaving used bag;
    e. Implement a procedure for safe packing and transport to incinerator if the used bag will be directly incinerated; and
    f. Ensure the incinerator facility can burn large quantities of silicone tubing and bag film.
4. Control of aerosols by engineering or procedural controls to prevent or minimize release of organisms.
    a. Implement controls to prevent bioreactor bag overfilling during additions;
    b. Ensure proper procedure for tubing welding;
    c. Ensure proper procedure for tube weld integrity test;
    d. Ensure regular PM of tubing welders to prevent misalignment; and
    e. Ensure that plastic quick connectors (non-steamable) release viable organism(s) when released.

5. Treatment of exhaust gases from a closed system to minimize or prevent release of viable organisms.
    a. Consider exhaust gas filtration;
    b. Consider controls of exhaust filter clogging with foam and humidity; and
    c. Ensure there is an exhaust filter holder positioned to encourage condensate drainage.
6. Closed system that has contained viable organisms not opened until sterilized by a validated procedure.
    a. Ensure the bioreactor bag is compatible with inactivation chemical.
7. Closed system to be maintained at as low a pressure as possible to maintain integrity of containment features.
    a. Implement a process safety management study of gas overlay and sparging system to determine susceptibility to overpressure, including post-power failure;
    b. Ensure bag installation procedures to prevent damage;
    c. Ensure pressure control to limit aeration and overlay pressure;
    d. Ensure the pressure alarms are interlocked to the gas supply;
    e. Ensure pressure relief devices are installed on gas supplies and properly sized;
    f. Consider installing in-line pressure relief before the bioreactor to protect against gas regulator failure; and
    g. Ensure the gas supply valves fail closed upon power interruption.
8. Rotating seals and other penetrations into closed system designed to prevent or minimize leakage.
    a. Consider magnetic couplings to eliminate rotary seals;
    b. Implement procedures to ensure stirrer operates during pre-use integrity test;
    c. Ensure rotary seals engineered to prevent infectious agent release; and
    d. Consider that over-speed may result in decoupling and in-bag rupture.
9. Closed system shall incorporate monitoring or sensing devices to monitor the integrity of containment.
    a. Consider bioreactor bag pressure logging;
    b. Ensure that loss of pressure (low-pressure alarm) results in sparge/overlay shutdown; and
    c. Ensure that the sensors respond quickly enough to pressure changes.
10. Validated integrity testing of the closed containment system.
    a. Consider integrity test procedures pre-inoculation.
11. Emergency plans required for handling large losses of cultures.
    a. Implement a leak detection system for bottom- or side-mounted probes;
    b. Consider bottom- or side-mounted sensors guarded to prevent impact damage;
    c. Consider respiratory PPE as part of operating PPE or ensure respiratory PPE availability for emergency cleanup;
    d. Ensure a contaminated worker emergency procedure available;
    e. Ensure a large spill clean-up procedure available, including a spill kit;
    f. Ensure personnel trained in large-scale clean-up of infectious organisms; and
    g. Consider gas decontamination of production suite post-incident.
12. Requirements for controlled access area.
    a. Ensure aerosol-containment within skid (i.e., process module);
    b. Consider a spill containment pan to contain or divert entire bioreactor contents for inactivation;
    c. Ensure the pan will divert a worst-case leak scenario to biowaste without spill to the floor;
    d. Consider spill containment within the suite (dike, bund, raised door threshold) to contain entire bioreactor contents for inactivation;
    e. Ensure the suite exhaust HEPA filtration for fluid transfers outside bioreactor containment; and
    f. Ensure the suite is designed to prevent the release of infectious aerosols using differential pressure and sealing of room penetrations.

Those performing risk assessments for large-scale work will also need to review equipment types and assist in the evaluation of the choice that will best balance the needs of GxP and biosafety. These equipment types include:

Pumps and Pipes The type of piping used will depend on how the process is laid out. Hard piping will need clean-in-place (CIP) and sterilization-in-place (SIP) for both GxP and biosafety reasons. Soft hoses allow for quick changes and cleaning. The type of pump will have to meet the volume demands of production. Peristaltic pumps are often used in combination with soft hoses. The risk assessment must show what type of piping and pump to use to meet GxP (if applicable), biosafety, and general HSE demands. Make sure that points where pipes penetrate walls are correctly sealed to promote safe gaseous decontamination. Additionally, pump operation should be evaluated for hearing protection implementation.

Compressed Air and Gases Compressed air is one means of transferring fluids between vessels. The safety review will identify elevated pressure points, type of relief valve protection required, and rupture disc failure scenarios. Some processes require asphyxiants, such as CO2 or N2, and safety measures are to be established to mitigate associated risk.

Electrical Power Power should be installed in a manner that prevents water ingress in all production and failure modes. Planning and construction must follow local electrical codes and the Occupational Safety and Health Administration electrical standards. Large fixed equipment fermenters and equipment often require high voltage power, which creates the need for additional safety measures including emergency stop buttons to shut down equipment and installation of water and dustproof electrical enclosures.11,12 Special care must be taken when solvents are used in production; follow applicable national codes, such as NFPA, UL, and OSHA. UPS needs must be evaluated based on the equipment and facility needs. An emergency generator may be essential to maintain biocontainment.

Production equipment including bioreactors, fermentors, filtration units and centrifuges In all upstream and some downstream processes, equipment is used while the product is still infectious. These units must be set up to eliminate the risk of aerosol release. Prior to charging process equipment with live biological material, the integrity of the closed system should be verified. Before opening a closed system for maintenance or cleaning, in situ decontamination of the vessel is required. To prevent an aerosol release occurring as a result of an upset condition, small equipment can be placed inside a containment device such as a biological safety cabinet. Larger equipment containing infectious agents should reside in rooms under negative pressure. If negative pressure can’t be achieved, room entry and exit airlocks may be used as negative air pressure “sinks” to prevent the escape of aerosols into adjacent areas.

Work Practice and Administrative Controls

Good microbiological practices are vital and apply in the same way as they do in biological research laboratories. Chemical hygiene, hearing protection evaluations in equipment areas, ergonomic, and safety principles apply to large-scale biological production areas as they do in other research laboratories and production areas. Access should be restricted to trained personnel only. Other administrative controls include:

Occupational Health Employers should offer workers appropriate medical surveillance programs to identify immune suppression and other underlying medical conditions, which could be risk factors that necessitate adaptations or accommodations. Occupational physicians should advise on, from a medical point of view, protection measures and procedures (e.g., fitness for duty to wear respirators or perform specific tasks). Where appropriate, the physician will offer vaccination, or provide vaccines, with follow up on titers. In addition to surveillance, clinical treatment procedures for accidental exposure should be developed. For biological agents susceptible to antibiotics, antimicrobial susceptibility testing results should be obtained before large-scale operations begin.

Emergency Response Plans for different emergency situations should be established, including spill protocols. Where appropriate, post-exposure prophylaxis and policies for isolation of potentially infected people should be established. One differentiating factor between small and large spill clean-up is that, unless there is an immediately dangerous for life and health (IDLH) situation, the operator in a large-scale facility must remain in the room long enough to stop and contain the release to minimize HSE consequences. Further information on emergency preparedness and response can be found in Biological Safety: Principles and Practices.13

Laboratory Biosecurity The risk management strategy for a large-scale risk assessment should define both a biosafety containment strategy (refer to BMBL Section II, NIH Guidelines' Appendix K, and the area-specific risk assessment) and a laboratory biosecurity strategy. The biosafety containment strategy defines controls that mitigate risk from an unintentional release, and the laboratory biosecurity strategy defines controls that prevent theft of biological agents that are associated with human health and/or agricultural industry impact. Likewise, materials, equipment, technology, and knowledge of dual-use potential needs to be addressed and a strategy developed to address misuse.14–18

Training Biosafety, laboratory biosecurity, and GxP training (if applicable) are essential in large-scale biological production. For large-scale processes, training should review the epidemiology, signs/symptoms of infection, mode of transmission, risk-mitigating controls including donning and doffing of PPE, and emergency response procedures, area-specific SOPs, including spill response protocols, required for the biological agent/material handled. Workers should understand when PPE is required for product protection vs. personnel protection. An understanding of the handling requirements for inactivated vs. unconfirmed inactivated materials is critical. Training should include a knowledge check.

Ergonomics The ergonomic issues associated with large-scale operations differ from those encountered in the laboratory. Material handling in large-scale operations will present a larger risk of ergonomic injury. To address the ergonomic issues associated with material handling, include the nature of the load in the risk assessment (i.e., the weight distribution and shape of the load), the capabilities of the individual performing the task, the duration and frequency of the task, and the environment in which the material handling task is performed (e.g., space limited or extreme temperature environments). Mitigate ergonomic risks by mechanical means (e.g., lifts, hand trucks, pushcarts), redesign of the work area (e.g., ramps to replace stairs, automated transfer of materials to replace manual transfer), redesign of the work task (e.g., pushing rather than pulling), and training of personnel (e.g., proper lifting technique).

Waste Handling The processes of waste handling are the same as for research laboratories but larger amounts require different logistics. For guidance on validation of decontamination agents and procedures, refer to Appendix B. Key considerations include inactivation of organisms in situ vs. external to process vessel or container. Consider inactivation methodologies for solid infectious waste streams as well as wastewater from production effluent (i.e., determine if there will be an impact to the site wastewater treatment permit due to the presence of organics including preservatives such as thimerosal or adjuvants).

Review and Checking of Risk Control Measures Risk control measures need to be evaluated for efficacy in order to protect people and the environment. The organization should maintain a risk control register, which should be periodically reviewed. The strategy should address the major risk streams (e.g., chemical, physical, biological, and ergonomic).

Preventative Maintenance Preventative maintenance is vital to avoiding process contamination and to ensuring biocontainment. Safety and security-related equipment and infrastructure should be incorporated into a preventive maintenance program that incorporates a change control process. For example, rotary seals in fermenters must be monitored for increased loss of seal water or steam pressure and should be replaced before failure; high-pressure piston seals of homogenizers must be replaced regularly to prevent aerosol release; autoclave temperature and pressure sensors require regular calibration, and steam traps must be maintained. Depending on design, autoclave bioseal or air differential seals should be tested (e.g., smoke, pressure hold, soap bubble, and helium leak testing) to

determine whether they have deteriorated. When required, HEPA filters (i.e., HVAC and equipment) should be integrity tested annually and critical barrier HEPAs should be monitored for pressure differential. Thermal or chemical inactivation systems should undergo regular inspection for corrosion and preventative maintenance of gaskets, seals, and sensors, as well as addition pumps, to ensure proper operation. Validation of inactivation parameters is also required by using spore-based indicators or the actual production organisms. Continuous flow thermal inactivation systems should undergo regular chemical clean-in-place cycles to remove coagulated protein residues, which can reduce system efficiency.

PPE/Gowning

PPE and gowning are used for both personnel and product protection. When PPE is utilized for product protection, it is designed to prevent shedding of foreign material into the production process and final product and to contain skin and respiratory shedding from the worker. Standard cotton or synthetic materials are not acceptable because they are prone to shedding. When PPE is utilized for worker protection, it should be assessed against physical, chemical, and biological hazards. Cotton laboratory coats or jumpsuits are easily saturated with chemical and biological liquids during a large release or spill and do not provide adequate protection. Man-made, water-resistant polymers are a better choice; they are less apt to become saturated. Refer to the material permeation rate or breakthrough detection time. The most protective options for personnel protection are gowns made of microporous laminated materials or jumpsuits with covered zippers.

Depending on the chemicals and/or biological materials handled, large volumes at high concentration plus the inherent increased risk of aerosol generation may require respiratory protection. Common disposable, half-face respirators (e.g., N95) may be sufficient for biological material protection, but they are not designed for chemical protection and may not be sufficient to protect against large volumes of a concentrated high-risk pathogen. Therefore, a risk assessment should be performed to identify the appropriate respirator required for the operation (i.e., filtering facepiece, tight-fitting facepiece, PAPR or SCBA).

Conclusion

Large-scale growth of biological agents is necessary in a variety of settings and requires an evaluation of both the GxP and biosafety requirements. With careful planning and a robust risk assessment of the unique requirements of a large-scale facility, it is possible to design and operate a facility that protects the product, workers, and the environment.

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Additional Resources:

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APPENDIX M – BIOSAFETY PRACTICES FOR VIRAL VECTORS

Introduction

Viral vectors used in recombinant techniques are regulated by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines). Understanding the origins and implications of their use is necessary prior to working with them. There is a potential to infect not only experimental specimens but also lab personnel using these viral vectors. Rendering an infectious virus replication incompetent or otherwise attenuated, lowers the risk, including the later generation viral vector systems. Recombination events or contamination from wild-type virus can still result in the presence of replication competent viruses in a population of replication deficient viral vectors. Principal Investigators are responsible for updating their exposure plans and task procedure documents to address these issues.

Note: The Biosafety Officer must be contacted for a consultation and risk assessment when:

- this appendix does not cover an investigators vector system or
- they contain genes that make products such as:
    - oncogenes
    - growth factors
    - other growth regulating products
    - products released into the circulation
    - have a general effect on the host-immune system
    - that may be shed from animals (toxins)

Containment

Suggested biosafety containment levels are provided for each replication incompetent vector system, higher level containment or PPE may be required in some cases, these are recommendations, risk assessment will determine final requirements. Care should be given to the design and handling of the viral vectors containing genes that: make oncogenes, growth factors, and/or other growth regulating products, products released into the circulation, products that have a general effect on the host-immune system, or products that may be shed from animals (toxins).

Adenovirus

BSL-2

Infectious human viruses, causing a mild respiratory illness, pink eye, or gastroenteritis. Rare cases of severe infection can occur, therefore, requiring adequate containment practices especially vectors containing genes that make products like those of the deleted adenovirus genes.

Disinfectants: 1% sodium hypochlorite, 2% glutaraldehyde, or 0.25% sodium dodecyl sulfate

Biosafety Cabinet required unless IBC approves standard operating procedures outlining mitigation of hazard.

PPE: Safety goggles, face shields, face mask (surgical or N-95), lab coat, and gloves

ABSL-2 then may drop to ABSL-1 depending on pre-dosing discussions.

Adeno – Associated Virus

BSL-2 unless approved by IBC for lower containment level

The potential generation of replication competent virus and for oncogenesis through insertional mutagenesis remain the top concerns.

New research demonstrates that Adeno-associated Virus (AAV) can integrate into known cancer driver genes in cells, leading to formation of tumors (i.e., insertional mutagenesis). The research, published in Nature Genetics [Oct. 2015, Vol. 47(10): 1187-1195], has found AAV genes in patients' human hepatocellular carcinomas. Insertional mutagenesis and cancer are a concern as AAV vectors lose site specific integration into chromosome 19.

There is evidence of AAV infection in human embryos, association with male infertility, along with a significant correlation between AAV DNA in amnion fluids and premature amniorrhexis (rupture).

AAV may be transmitted through direct contact with infected individual or contaminated environment, via respiratory, gastrointestinal and body fluid transmission. May also be transmitted from mother to fetus. Most adults are seropositive for AAV, with only 30% having neutralizing antibodies.

In the lab, transmission can occur through ingestion, inhalation of aerosolized droplets, mucous membranes, and accidental injection. Can also infect a wide range of mammalian cell types.

This indicates a pathogenic role for virus, which is commonly used in research. In the past, AAV has been classified as a Risk Group 1 virus because it was considered a defective virus requiring coinfection with adenovirus to become infectious.

Institutions such as Duke University and University of Iowa, are now requiring BSL 2 as the default for work with the vectors, however ABSL 1 is sufficient for working with animals forty-eight hours post injection.

Disinfectants: 1% sodium hypochlorite, 2% glutaraldehyde, or 0.25% sodium dodecyl sulfate

Biosafety Cabinet required unless IBC approves standard operating procedures outlining mitigation of hazard.

PPE: Safety goggles, face shields, face mask (surgical or N-95), lab coat, and gloves

ABSL-1 housing sufficient unless in the presence of helper virus then ABSL-2.

Retrovirus

BSL-2 unless approved by IBC for lower containment level

Infectious viruses which can integrate into transduced cells with high frequency, which may have oncogenic potential. Containment for vectors with the ability to infect human cells are recommended to have BSL-2/2+, ecotropic vectors with no ability to infect human cells with IBC approval may be handled at BSL-1.

MMLV

The host range is dependent on the specificity of the viral envelope. Clinical events reported involve leukemogenesis and oncogenesis. Many are amphotropic and require BSL-2 handling or higher depending upon the construct.

Disinfectants: 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol, and others

PPE: Safety goggles, face shields, face mask (surgical or N-95), lab coat, and gloves

ABSL-2 for amphotropic or pseudotyped vectors; for ectotrophic IBC may approve ABSL-1

Lentivirus

Ability to integrated into host chromosomes to infect non-dividing cells. May cause severe immunologic and neurologic disease in their natural hosts. Latest generation vectors have been designed to significantly reduce the possibility of recombination resulting in a wild type – potentially infectious virus. Clinical adverse events include leukemogenesis and oncogenesis through insertional mutagenesis. Ones that are incapable of causing productive infections in humans are to be handled at BSL-2, with others such as simian or human lentiviruses conducted at higher containment BSL-2+.

IBC recommends third generation or better.

Disinfectants: 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol

PPE: Safety goggles, face shields, face mask (surgical or N-95), lab coat, and gloves

ABSL-2 then may drop to ABSL-1 depending on pre-dosing discussions.

Rabies virus

Common zoonotic infection from bats and other wild mammals, results in encephalitis or paralysis and is often fatal. It's neuronal tropism, these vectors can be used to study neuronal trafficking or to express genes efficiently in neurons. BSL-2 is required for most constructs, but the use of BSL-3 will depend on a risk assessment.

Vaccination may be recommended per the University Physician and Occupational Health Clinic on campus.

Disinfectants: 70% ethanol, phenol, formalin, ether, trypsin, beta-propiolactone and others

PPE: Safety goggles, face shields, face mask (surgical or N-95), lab coat, and gloves

ABSL-2 housing.

Baculovirus

Non-mammalian viral vector that infects insects and are very stable, remaining dormant for years before infecting the insects. Most handled at BSL-1 conditions.

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