

Biological Safety Manual



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Environmental
Health and Safety
MICHIGAN STATE UNIVERSITY

Table of Contents

Emergency Contact	7
Foreword.....	8
Biohazard Definition.....	9
Rules, Regulations & Guidelines	9
Risk Assessment	11
Agent Hazards:.....	11
Laboratory Procedure Hazards	12
Biological Safety and Biosafety Levels	12
General Laboratory Practices	13
Routes of Infection	13
Access.....	14
Personal Protective Equipment (PPE)	14
Eye and Face Protection	14
Laboratory Clothing	14
Gloves.....	15
Respirators	15
Laundry	15
Food and Drink Policy	15
Housekeeping	16
Inventory Log.....	16
Training	16
Health and Medical Surveillance	17
The Biosafety Level 1 Laboratory	18
Laboratory Design and Facilities	18
Standard Microbiological Practices	18
Special Practices.....	19
Safety Equipment	19
The Biosafety Level 2 Laboratory	20
Laboratory Design and Facilities	20
Standard Microbiological Practices	21
Special Practices.....	22

Safety Equipment	23
Biological Safety Level 3 Laboratories	24
Laboratory Design and Facilities	24
Standard Microbiological Practices	25
Special Practices	26
Safety Equipment	27
Laboratory Animal Facilities	28
Animal Facility – Biosafety Level 1 (ABSL-1)	28
Standard Microbiological Practices	28
Safety Equipment (Primary Barriers and Personal Protective Equipment)	30
Laboratory Facilities (Secondary Barriers)	30
Animal Facility – Biosafety Level 2 (ABSL-2)	31
Standard Microbiological Practices	31
Special Practices	33
Safety Equipment (Primary Barriers and Personal Protective Equipment)	34
Laboratory Facilities (Secondary Barriers)	35
Animal Facility – Biosafety Level 3 (ABSL-3)	36
Standard Microbiological Practices	36
Special Practices	38
Safety Equipment (Primary Barriers and Personal Protective Equipment)	39
Laboratory Facilities (Secondary Barriers)	39
Invertebrates	41
Plant Biological Safety Levels	41
Plant Biosafety Level 1 (PBSL-1)	42
Standard Practices	42
Facilities	42
Plant Biosafety Level 2 (PBSL-2)	42
Standard Practices	42
Facilities	43
Laboratory Biosecurity	44
Safety Equipment	45
Biological Safety Cabinets (BSCs)	46

Pipetting aids.....	51
Homogenizers, shakers, blenders, and sonicators	51
Disposable transfer loops, needles and cell spreaders	51
Recommended Work Practices	51
Autoclaves.....	51
Flow Cytometers	52
Pipettes and Pipetting Aids	52
Sharps	53
Cryostats	53
Centrifuge Equipment.....	54
Safety Blenders	54
Lyophilizers and Ampoules	54
Loop Sterilizers and Bunsen Burners.....	55
Guidelines for Working with Tissue Culture/Cell Lines	55
Guidelines for Preventing the Transmission of Tuberculosis.....	55
Guidelines for Clinical Laboratories	56
Guidelines for Prion Use	56
Guidelines Regarding Select Agents	56
Considerations for Colleges & Departments	57
Guidelines for Handling Exempt Strains of Select Agents.....	57
Guidelines for the Use of Exempt Levels of Select Agent Toxins.....	58
Decontamination	58
Cleaning Laboratory Materials	59
Ways to Decontaminate	59
Heat	59
Liquid Chemicals Used as Disinfectants.....	60
Vapors and Gases	62
Radiation.....	62
Incineration	62
Biohazardous Waste.....	63
General Labeling, Packaging and Disposal Procedures	63
Waste Specific Procedures for BSL-1 and 2	64

Waste Specific Procedures for Biosafety Level 3 (BSL-3)	65
Pathological Waste.....	65
Animal Waste	65
Department or Facility Specific Waste Procedures	65
Biohazard Spill Clean-Up Procedures	65
Basic Biological Spill Kit Contents:.....	66
Spills Inside the Laboratory	66
Spills with NO broken glass/sharps:.....	66
Spills involving broken glass/sharps:.....	66
Spills Inside the Biological Safety Cabinet	67
Spills Inside a Centrifuge.....	67
Spills During Transport.....	67
Spill kit maintenance:	67
Handwashing and Hand Decontamination.....	68
Introduction to the Transport of Biological Substances.....	68
Transporting Biological Materials on Campus.....	68
Shipping of Biological Materials to an Off Campus Destination	69
Use of Animals in Research.....	70
Use of Human Subjects and Materials in Research	70
Biosafety and Recombinant DNA technology	71
Guidelines for Working with Genetically Modified Animals	71
NIH recombinant DNA review categories	71
Review Process Overview	72
Responsibilities of the Principal Investigator (PI) for Recombinant DNA Research	72
APPENDICES.....	74
Appendix A – Biosafety Resources	75
Appendix B – Select Agent List DHHS/USDA.....	76
Appendix C – Risk Assessment Form.....	78
Appendix D – Examples of RG-2, RG-3, and RG-4 Agents	81
Appendix E – Equipment Release Form	86
Appendix F- Exposure Response Procedures	87
Appendix G – Lab and Animal Biosafety Level Summaries	88

Appendix H – Example Inventory Log91

Appendix I – Biosafety Practices for Handling Prions92

Prions & General Biosafety Recommendations92

Appendix J – Requirements for Handling Exempt Strains of Select Agents96

Appendix K – Site Specific Training Checklist..... 101

Appendix L – Large Scale Biosafety Guidelines 103

Emergency Contact

In case of emergency: Dial 911

Non – Emergency Medical Attention

Faculty, staff and paid student employees

Lansing Urgent Care

<http://lansingurgentcare.com/>

Non-employee students

Olin Student Health Center

(517) 353-5557

Or nearest emergency room or after hours urgent care facility

Outside of the Lansing area, go to the nearest emergency room.

For assistance involving occupational, environmental, radiation, chemical and/or biological safety

Environmental Health & Safety (EHS)

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 the Environmental Health and Safety (EHS) Department at Michigan State University. The manual is part of MSU's biosafety program, which was established to accomplish the following goals:

- Protect personnel from exposure to 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 biosafety manual provides university-wide safety guidelines, policies and procedures for the use and manipulation of biohazards. Although the implementation of these procedures is the responsibility of the Principal Investigator (PI), its success depends largely on the combined efforts of laboratory supervisors and employees. Planning for and implementation of biological safety must be part of every laboratory activity in which biohazardous materials are used.

In general, the handling and manipulation of biological agents and toxins, as well as recombinant or synthetic nucleic acid molecules, requires the use of various precautionary measures depending on the material(s) involved. This manual will provide assistance in the evaluation, containment and control of biohazards. However, it is imperative that all parties involved or working with these materials seek additional advice and training when necessary. The EHS is available at MSU to assist in this endeavor.

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, rickettsiae, chlamydiae, 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

The following is a brief 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 for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. These guidelines address the safe conduct of research that involves 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. It has been amended and revised many times since then. 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. For more information, please 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 <http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines>.

Centers for Disease Control and Prevention (CDC) and the National Institute of Health (NIH) Guidelines on: Biosafety in Microbiological and Biomedical Laboratories (BMBL). In 1984, the CDC/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 a variety of infectious agents in various 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. MSU is using the BMBL as the basis for this biosafety manual. <http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>

Michigan Occupational Safety and Health Administration: Bloodborne Infectious Disease Standard. In 1992, the Occupational Safety and Health Administration (OSHA) promulgated 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: https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051 and MIOSHA Standard: http://www.michigan.gov/documents/CIS_WSH_part554_35632_7.pdf

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 in order 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. A copy of the most current list of restricted agents and toxins covered under this rule is included in Appendix B.

United States Department of Agriculture (USDA): Agricultural Bioterrorism Protection Act of 2002; Possession, Use, and Transfer of Biological Agents and Toxins. The USDA has also established a set of rules that require facilities and institutions to be registered and approved in order to transfer or receive certain biological agents and toxins. A copy of the most current list of restricted agents and toxins covered under this rule is included in Appendix B. <http://www.selectagents.gov/>

Michigan Department of Public Health: Michigan Medical Waste Regulatory Act (MMWRA). In 1990, the MMWRA was promulgated 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 with this Act is outlined in the MSU Biohazardous Waste Management Plan, which is used in conjunction with the MSU Hazardous Waste Disposal Guide. <http://www.deq.state.mi.us/documents/deq-whm-hwrp-mwRegAct-Rules.pdf>

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 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 a process used to examine the various factors associated with a procedure involving biological materials in order 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. 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. Please refer to Appendix C for a form to assist with risk assessments. 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*
- *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*
The predominant routes of transmission in the laboratory include mucous membrane exposure, parenteral inoculation, ingestion and inhalation of infectious aerosols.
- *Infective dose*
- *Stability in the environment*
- *Host range*
- *Its endemic nature*
- *Reports of laboratory acquired infections*
- *Origin of the agent*

Classification of Infectious Agents on the Basis of Hazard (Risk Groups)

Risk groups (RG) are a method used by the World Health Organization (WHO) and by the National Institutes of Health (NIH) to classify human etiological agents based on hazard to both the individual and to the community. There are four risk groups. These correlate to but are not equivalent to biosafety levels. Determining the risk group of a biological agent can be part of the biosafety risk assessment and helps in assigning the correct biosafety level for containment. 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.

Table 1: Basis for the Classification of Biohazardous Agents by Risk Group

Risk Group	Risk to the individual and the community
Risk Group 1 (RG-1)	Agents that are not associated with disease in healthy adult humans (no or low individual and community risk).
Risk Group 2 (RG-2)	Agents that are associated with human disease which are rarely serious and for which preventive or therapeutic interventions are often available (moderate individual risk but low community risk).
Risk Group 3 (RG-3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).
Risk Group 4 (RG-4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk)

Examples of RG-1 agents include microorganisms like *Escherichia coli*-K12 or *Saccharomyces cerevisiae*. A list of Risk Group 2, 3 and 4 agents can be found in Appendix D. It is important to note however, that no list is all inclusive. Also, those agents not listed in RG-2, RG-3 or RG-4 are not automatically classified in RG-1. Those unlisted agents need to be subjected to a risk assessment based on the known and potential properties of the agents.

Hazards of Genetically-Modified Agents

When conducting a risk assessment of genetically modified agents, consideration of the same factors used in risk assessment of the wild-type organism should be done. However, it is important to address 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. Sometimes, important information may not be available for a newly engineered agent and the risk assessment may be difficult or incomplete. In these cases, due diligence should be practiced and the biosafety level assignment should be made conservatively. Once more information is available another risk assessment should be completed.

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 please 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 a needle, other contaminated sharp 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

Aerosols, or respirable sized particles, are extremely hazardous because they are generated in many lab procedures and are usually undetected. The creation of infectious aerosols places the person carrying out the procedure and others in the laboratory at risk. Any procedure that breaks the surface tension of a liquid will produce aerosols. Pipetting, blenders, non-self-contained centrifuges, sonicators and vortex mixers all produce aerosols. Procedures and equipment that create aerosols also create larger droplets that rapidly settle out of the air. These droplets can settle on surfaces and therefore contaminate gloved hands, work spaces 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, persons outside of the laboratory, and the environment to potentially infectious agents. It can be accomplished through the following means:

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 can be made to achieve different levels of physical containment. Currently four biosafety levels (1- 4) define the levels of containment necessary to protect personnel and the environment. A 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. Since most of the 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. For more information on biosafety level 4 requirements 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 must be aware of the potential risks. In addition, they must be 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

An infection occurs when disease-causing microorganisms enter the human body in sufficient numbers and by a particular route and overcome the body's defense system. The following routes of infection have been reported for laboratory-acquired infections:

1. *Through the mouth*

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

2. *Through the skin*

- Accidental inoculation with a hypodermic needle, other sharp instrument or glass
- Cuts, scratches

3. *Through the eye*

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

4. *Through the lungs*

- Inhalation of airborne microorganisms

Most of the laboratory-acquired infections reported in the literature point to accidents during work with some type of infectious agent. These include spills, splashes and accidents involving needles or other sharp objects. The general laboratory procedures outlined in this manual address those issues and provide for guidance in handling infectious or potentially infectious materials.

Access

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

Biohazard Warning Sign

A biohazard label is required for all areas or equipment in which RG-2 or higher agents are handled or stored or where BSL-2 or higher procedures are required. Labels should be 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 to be obtained from the EHS (355-0153).

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

- Biosafety level
- Supervisor's or other responsible person's name
- Telephone number
- Procedures required for entering and exiting the lab

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 devices and equipment, must be provided to all employees under the appropriate circumstances. The employees have the responsibility of properly using the equipment.

Eye and Face Protection

Safety glasses must be worn in the lab whenever procedures are underway involving a low probability of splash, work with low hazard chemicals, or an impact hazard.

Whenever possible, lab operations should be performed in containment devices such as a biological safety cabinet or fume hood, or behind a bench-top shield in order 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, then appropriate 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 must be worn.
- Full face protection (i.e., face shield) must be 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 (www.EHS.msu.edu) 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, and gowns. Long-sleeved garments should be used to minimize the contamination of skin or street clothes. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration. If the garment is not disposable, it must be capable of withstanding sterilization, in the event it becomes contaminated. The garment should be flame resistant when working with or around open flames or high temperature heat sources.

A laboratory coat is recommended for all work at BSL-1 and it or other suitable protective clothing is required when handling potentially infectious materials at BSL-2 or higher. Additional criteria for selecting

clothing are: comfort, appearance, closure types and location, antistatic properties and durability. Protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas. Disposables should be available for visitors, maintenance and service workers in the event it is required. All protective clothing should be either discarded in the laboratory or laundered (Department facilities or MSU's laundry – Spartan Linen Services). Personnel must not take laboratory clothing home. Long hair and loose clothing (like neck ties, dangling jewelry, scarves, or long sleeves) should be secured to avoid contamination and material getting caught in equipment.

Gloves

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

When latex gloves have been chosen, alternatives should be made available. Gloves should be changed as soon as possible after they have become contaminated; when their integrity has been compromised or when necessary. Hands should be properly washed with soap and water after removing gloves. Disposable gloves must not be washed or reused and should be disposed of as soon as removed.

Gloves should be removed and hands washed when work with potentially infectious materials is complete or when leaving the laboratory. If you are 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 door knobs 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 HIV 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 must contact the EHS for assistance in selection of proper equipment and training in its usage. All personnel wearing respirators need to be 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.

Laundry

All personal protective clothing must be cleaned, laundered and disposed of by the employer at no cost to employees. Apparel contaminated with human blood or other potentially infectious materials should be handled as little as possible and needs to be 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 must be worn by employees who handle contaminated laundry.

Food and Drink Policy

The following statement is the accepted practice for food and drink items in campus laboratories and should be abided by at all times:

“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 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 must be relied upon to provide work areas free of significant sources of contamination. Housekeeping procedures should be based on the highest degree of risk to which personnel and experimental integrity may be subjected.

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

Inventory Log

A written or computerized inventory log must be kept. The inventory should be complete enough so that the PI would know if materials are missing, what those materials are, the quantity of materials, and the potential hazards of the materials. The log should be 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. In addition, all personnel working with RG-2 or 3 agents or at BSL-2 or 3 should receive adequate laboratory specific training from the Principal Investigator (PI) or laboratory supervisor. See Appendix K for a site-specific training checklist that can facilitate and document this training. Training should include at a minimum:

- 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.

In addition, it is important that all personnel working in a laboratory handling biological materials take the appropriate biological safety-related trainings offered by the EHS:

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:** If your job or coursework involves 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:** This training course has been developed specifically to address the needs of MSU personnel whose job duties or coursework involves contact with and care of animals or animal diagnostic products, or other animal materials (e.g., animal-derived cell lines).

- **Other:** If your research does not fall under the previous descriptions and your job or coursework involves: 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 , microbes, viruses

Biosafety Principles Refresher Training: This is an online course that is required each year after taking the Biosafety Principles Training initially.

Bloodborne Pathogens Initial: This is a MIOSHA required in-person class that is needed by anyone who will be handling human-derived materials, including blood and cell lines. Certain groups, such as healthcare workers can take this class online.

Bloodborne Pathogens Refresher: This is an online course that is required by MIOSHA each year after taking the Bloodborne Pathogens Initial course.

Medical Waste Training: This is for supervisors and employees who must comply with the Michigan Medical Waste Regulatory Act (MMWRA) training requirements. The MMWRA training requirements apply to every employee who generates, handles, treats and/or disposes of biohazardous waste (including sharps) at MSU. It includes general policies that apply to MSU biohazardous waste generators. This training is included in Biosafety Principles Training, Bloodborne Pathogens Initial Training. It is also 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: This training is required for anyone who works in or who has access to a laboratory. It is available on the EHS website and is also included in Biosafety Principles Training.

Others: The Biosafety Office also offers specialized courses as requested. These include, but are not limited to, Non-Primate Biohazard training, Infectious Substance and Biological Materials Shipping, and Biosafety Cabinet training. If you have a need for a specialized class, please contact our office.

Health and Medical Surveillance

Medical surveillance of personnel in general 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. In the case of exposures to potentially infectious materials, medical surveillance will include health monitoring as prescribed by MSU Occupational Health in order to facilitate recovery (Refer to Appendix F for exposure response procedures).

The Principal Investigator is responsible for assuring that all lab and support personnel and visitors are fully informed of:

- 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 any 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 should 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 you believe that you may be immunocompromised. Please remember that events such as pregnancy, recent illnesses caused by an infectious agent (i.e., the flu), chemotherapy, etc. can result in an immunocompromised state of health.

Vaccinations

Specific projects may use infectious materials and techniques that warrant consideration of vaccines. In these instances, the Principal Investigator should notify the MSU Biosafety Officer and MSU Occupational Health to further assess this need.

In the event that restricted access entry or vaccination requirements are implemented for a study underway, this information will be clearly posted on the lab door to communicate elevated risk.

The Biosafety Level 1 Laboratory

Laboratory Design and Facilities

The facilities required in a biosafety level one laboratory include the following:

Doors

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

Sink

A sink must be available and supplied for handwashing (i.e., stocked with soap and paper towels).

Easily cleaned

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

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

Windows

If the lab has windows that can be opened to the outdoors, they must be fitted with screens.

Standard Microbiological Practices

The following standard microbiological practices must be used in a BSL-1 lab:

Controlled access

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

Handwashing

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

Eating, drinking, handling contact lenses and applying cosmetics

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

Pipetting

Mechanical pipetting devices must be available and used. Mouth pipetting is prohibited.

Safe sharps practices

All policies regarding the safe use of sharps must be 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 should be 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 must be decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant should be used. If bench paper or plastic backed absorbents are used, they should be discarded and the space beneath decontaminated.

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.

Proper decontamination and transport of waste

All cultures, stocks and other biohazardous materials must be properly 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 must ensure that the waste is placed in a leak-proof container and is secured. Please 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.

Door signage

All laboratory doors must have an "Admittance to Authorized Personnel Only" label. This label contains appropriate contact information for general and emergency entrance to the lab.

Pest management program

A pest management program is managed through EHS. They should be contacted at the first sign of a problem.

Training

In addition to the completion of EHS required training courses, the principal investigator must ensure that all lab personnel receive site-specific training. This training should include information specific to their job duties, precautions to prevent exposures, and exposure response procedures. In addition, lab personnel should be given information about immune competence and conditions that could predispose them to infection, as appropriate. See Appendix K for a checklist to assist with and document this training.

Special Practices

There are no special practices required in a BSL-1 lab.

Safety Equipment

The following safety equipment must be used in a BSL-1 lab:

Personal protective equipment (PPE)

The use of laboratory coats, gowns or uniforms is recommended. Flame retardant material must be used when working with open flames or high heat sources. 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 at all times while in the laboratory. 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.

The Biosafety Level 2 Laboratory

Laboratory Design and Facilities

The facilities required in a biosafety level two laboratory include the following:

Doors

Self-closing doors are required for access control. They must be closed when work is in progress inside the lab and they should be kept locked when no one is present in the laboratory.

Sink

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

Easily cleaned

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

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 conjunction with lab work must be covered with a non-porous material that can be easily cleaned and disinfected.

Windows

If the lab has windows that can be opened to the outdoors, they must be fitted with screens.

Biological safety cabinets

Biological safety cabinets (BSC) must be installed in a manner so that changes in room air do not interfere with the operation of the cabinet. They should be 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 must be tested and certified at least annually and whenever they are relocated or serviced. BSCs should be operated in accordance with the manufacturer's recommendations. See the following section of this manual for additional information: *Safety Equipment- Biological Safety Cabinets*.

Vacuum lines

Vacuum lines must be protected by High Efficiency Particulate Air (HEPA) filters. A dual flask system should be utilized. Flasks should be stabilized within a vessel that can contain a potential spill. Flask should contain appropriate disinfectant to decontaminate waste and be emptied at the end of work that day.

Eyewash stations

An eyewash station must be readily available.

Airflow

Ventilation systems must allow for inward flow of air without recirculation to spaces outside of the laboratory.

Waste decontamination

A method for decontaminating lab wastes (i.e., autoclave, incineration, etc.) must be available. It is the responsibility of the generating department to decontaminate all solid non-sharps biohazardous waste

and all liquid biohazardous waste. The EHS is responsible for the removal and proper treatment of sharps waste. See the MSU Biohazardous Waste Management Plan for additional information.

Standard Microbiological Practices

The following standard microbiological practices must be used in the BSL-2 lab:

Controlled access

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

Handwashing

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

Eating, drinking, handling contact lenses and applying cosmetics

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

Pipetting

Mechanical pipetting devices must be available and used. Mouth pipetting is prohibited.

Safe sharps practices

All policies regarding the safe use of sharps must be 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 should be 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 must be decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant should be used. If bench paper or plastic backed absorbents are used, they should be discarded and the space beneath decontaminated.

Proper decontamination and transport of waste

All cultures, stocks, and other biohazardous materials must be 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 must ensure that the waste is placed in a leak-proof container and is secured. Please 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.

Door signage

All laboratory doors 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 Biosafety Level 2 door sign. Both of these labels should be obtained from EHS.

Pest management program

A pest management program is managed through EHS. They should be contacted at the first sign of a problem.

Training

In addition to the completion of EHS required training courses, the principal investigator must ensure that all lab personnel receive site-specific training. This training should include information specific to their job duties, precautions to prevent exposures, and exposure response procedures. In addition, lab personnel should be given information about immune competence and conditions that could predispose them to infection, as appropriate. See Appendix K for a checklist to assist with and document this training.

Special Practices

The following special practices must be utilized in a BSL-2 lab:

Laboratory entrance

Before entering the laboratory, all people must be made aware of the potential hazards. They must also meet all entry and exit requirements (e.g., donning and doffing of personal protective equipment, immunization requirements, handwashing, etc.).

Medical surveillance

All laboratories 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. Occupational Health as well as the EHS should be contacted for assistance.

Laboratory specific biosafety manual

Each laboratory must supplement this biosafety manual with information that is specific for the individual laboratory. 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. Written standard operating procedures are required for tasks that are performed with BSL-2 materials.

Training

Lab personnel must demonstrate proficiency in microbiological practices before handling BSL-2 agents. It is the responsibility of the PI to ensure that proficiency has been demonstrated.

Containers for potentially infectious materials

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

Decontamination of laboratory equipment

Lab equipment must be decontaminated routinely. It must also be decontaminated after spills, splashes or when potentially contaminated. All spills must be cleaned by personnel who are properly trained and have the proper equipment to handle infectious materials. All BSL-2 labs should 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*. All equipment must be decontaminated before being repaired, maintained, or removed from the laboratory. When any of these is to occur, lab personnel must complete an Equipment Release Form and attach it to the piece of equipment. See Appendix E for an example of the form.

Exposure incidents

Exposure response procedures should be posted in an easily accessible location in the laboratory. All lab personnel should be 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.

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.

Personal Electronic Device Use in the BSL-2 Laboratory

Personal Electronic Devices (PED) can include, but are not limited to head phones, 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.

Music Listening – Ear buds and headphones are prohibited. MP3 players must be placed in a sealed plastic bag (i.e. Ziplock) and disinfected prior to leaving the lab. Utilize a benchtop Bluetooth speaker that remains in the facility and disinfected regularly instead of any listening devices that are worn on the person.

Laptops and Computers – Personal computers are discouraged from being brought into the laboratory from home unless:

- the device will remain within the facility as a laboratory only computer and is disinfected regularly
- the device is kept in an area far from experiments or hazardous materials, such as a laboratory desk designated for documentation

Smart Phones and Tablet Computers – It is suggested that if they must be used in the laboratory they must be inside of a sealed bag (i.e. Ziplock) The bag must be disinfected before leaving the laboratory. The item can then be removed from the bag.

Aerosol generating procedures

All procedures that may result in the generation of potentially infectious aerosols (pipetting, mixing, vortexing, etc.) must be conducted within a biological safety cabinet or other approved containment device.

Safety Equipment

The following safety equipment must be used in a BSL-2 lab:

Biological safety cabinets (BSC)

A biological safety cabinet, 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. 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*.

Personal protective equipment (PPE)

The use of laboratory coats, gowns or uniforms is required when handling hazardous materials. It is recommended that long hair and loose clothing be secured. 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 should wear safety glasses or other eye protection at all times while in the laboratory. Gloves must be worn as protection from hazardous materials. Two pairs should be worn as appropriate. 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 must not be washed or reused. Hands must be washed after removing gloves, and before leaving the laboratory. All protective equipment must be removed before leaving the laboratory. Used disposable PPE should be disposed of with other contaminated waste. Reusable PPE (i.e., goggles) should be appropriately decontaminated before reuse. Reusable laboratory clothing should be laundered through MSU Laundry. It must not be taken home. If visibly contaminated, laundry should be placed in a biohazard bag before being placed with other items to go to laundry.

Animal rooms

Eye, face and respiratory protection should be used as appropriate in rooms containing infected animals.

Biological Safety Level 3 Laboratories

The containment laboratory- 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.

Laboratory Design and Facilities

The facilities required in a biosafety level three laboratory include the following:

Location

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

Doors

A series of two self-closing doors are required for access control. They must be closed when work is in progress inside the lab and they should be kept locked when no one is present in the laboratory.

Sink

A hands-free or automatic sink must be available and supplied for handwashing (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 must have a sink available and supplied for handwashing.

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 allowed. Seams, floors, walls and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed for whole room decontamination. Floors must be slip resistant, impervious to liquids and resistant to chemicals. Walls and ceilings should have a smooth, sealed finish to allow for decontamination. Whole lab decontamination should be considered when gross contamination has occurred, when there is a change in lab usage, for renovations, and for maintenance shutdowns.

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 conjunction with lab work must be covered with a non-porous material that can be easily cleaned and disinfected.

Windows

If the lab has windows they must be sealed.

Biological safety cabinets

Biological safety cabinets (BSC) must be installed in a manner so that changes in room air do not interfere with the operation of the cabinet. They should be 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 must be tested and certified at least annually and whenever relocated or serviced. BSCs should be operated in accordance with the manufacturer's recommendations. Refer to the following section of this manual for additional information: *Safety Equipment- Biological Safety Cabinets*.

Vacuum lines

Vacuum lines must be protected by High Efficiency Particulate Air (HEPA) filters. Filters must be replaced as needed. Liquid disinfectant trap may be required.

Eyewash stations

An eyewash station must be readily available.

Airflow

A ducted ventilation system that provides directional airflow from “clean” areas to “potentially contaminated” ones is required. The lab must be designed so that under failure conditions that airflow will not be reversed. A means of visual verification of airflow must be available. Audible alarms should be considered. Exhaust air should be 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.

Biological safety cabinet exhaust air

The HEPA filtered exhaust air from a Class II BSC can be re-circulated within the laboratory as long as 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 must be directly connected to the building exhaust. Air supply must be provided in a way that does not allow for positive pressurization of the cabinet.

Waste decontamination

A method for decontaminating lab wastes (i.e., autoclave, incineration, etc.) must be available. It is the responsibility of the generating department to decontaminate all solid non-sharps biohazardous waste and all liquid biohazardous waste. The EHS is responsible for the removal and proper treatment of sharps waste. See the MSU Biohazardous Waste Management Plan for additional information.

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.

Equipment decontamination

The facility must be designed so that large pieces of equipment can be decontaminated before being removed from the laboratory.

Facility verification

The facility design, operational parameters and procedures must be verified and documented before initial operation. Annual facility re-verification is required.

Standard Microbiological Practices

The following standard microbiological practices must be used in the BSL-3 lab:

Controlled access

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

Handwashing

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

Eating, drinking, handling contact lenses and applying cosmetics

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

Pipetting

Mechanical pipetting devices must be available and used. Mouth pipetting is prohibited.

Safe sharps practices

All policies regarding the safe use of sharps must be 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 should be 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 pipetting devices, conducting work inside of a biological safety cabinet, etc.

Decontaminate work surfaces

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

Proper decontamination and transport of waste

All cultures, stocks, and other biohazardous materials must be 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 must ensure that the waste is placed in a leak-proof container and is secured. Please 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.

Door signage

All laboratory doors 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 Biosafety Level 3 door sign. Both of these labels should be obtained from the EHS.

Pest management program

A pest management program is managed through EHS. They should be contacted at the first sign of a problem.

Training

In addition to the completion of EHS required training courses, the principal investigator must ensure that all lab personnel receive site-specific training. This training should include information specific to their job duties, precautions to prevent exposures, and exposure response procedures. In addition, lab personnel should be given information about immune competence and conditions that could predispose them to infection, as appropriate.

Special Practices

The following special practices must be utilized in a BSL-3 lab:

Laboratory entrance

Before entering the laboratory, all people must be made aware of the potential hazards. They must also meet all entry and exit requirements (e.g., donning and doffing of personal protective equipment, immunization requirements, handwashing, etc.).

Medical surveillance

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.

Laboratory specific biosafety manual

Each laboratory must supplement this biosafety manual with information that is specific for the individual laboratory. 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.

Training

Lab personnel must demonstrate proficiency in standard and special microbiological practices before handling BSL-3 agents. It is the responsibility of the PI to ensure that proficiency has been demonstrated.

Containers for potentially infectious materials

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

Decontamination of laboratory equipment

Lab equipment must be decontaminated routinely. It must also be decontaminated after spills, splashes or when potentially contaminated. All spills must be cleaned by personnel who are properly trained and have the proper equipment to handle infectious materials. All BSL-3 labs should 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 must be decontaminated before being repaired, maintained, or removed from the laboratory. When any of these is to occur lab personnel must complete an Equipment Release Form and attach it to the piece of equipment. See Appendix E for an example of the form.

Exposure incidents

Exposure response procedures should be posted in an easily accessible location in the laboratory. All lab personnel should be 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.

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.

Manipulating infectious materials

All procedures that involve the manipulation of infectious materials must be 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 can be used if approved by the Biological Safety Officer.

Safety Equipment

The following safety equipment must be used in a BSL-3 lab:

Biological safety cabinets (BSC)

A biological safety cabinet must be used whenever working with infectious materials. Other physical containment devices may be used with the approval of the Biological Safety Officer.

Personal protective equipment (PPE)

The use of laboratory coats, gowns or uniforms with a solid front is required when in the laboratory. Work with certain agents may require that street clothes be removed and dedicated lab clothing be worn. It is recommended that long hair be secured or placed within a hair net to avoid contamination. Protective clothing cannot be worn outside of the lab. 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 should wear safety glasses or other eye protection at all times while in the laboratory. Gloves must be worn as protection from hazardous materials. Two pairs should be worn as appropriate.

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 must not be washed or reused. Hands must be washed after removing gloves, and before leaving the laboratory. All protective equipment must be removed before leaving the laboratory. Used disposable PPE should be disposed of with other contaminated waste. Reusable PPE (i.e., goggles) should be appropriately decontaminated before reuse. Reusable laboratory clothing must be decontaminated before being laundered and must be laundered at the University. It must not be taken home. If visibly contaminated, laundry should be placed in a biohazard bag before being placed with other items to go to laundry.

Animal rooms

Eye, face and respiratory protection should be used in rooms containing infected animals.

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.

Animal Facility – Biosafety Level 1 (ABSL-1)

This is suitable for the maintenance of most stock animals after quarantine, and for animals that are deliberately inoculated with agents in Risk Group 1.

Standard Microbiological Practices

The following practices must be used in an ABSL-1 lab:

Prior to initiation of work:

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

Facility specific biosafety manual

Each animal facility must supplement 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 must complete required EHS training courses. See the following section of this manual for a description of courses: *General Laboratory Practices- Training*. The facility director must ensure 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 must receive annual updates or additional training when procedures or policies change. Records are maintained.

Medical surveillance

All personnel involved in animal research must 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 must 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 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: 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 must ensure that access to the animal areas is controlled. Only those people necessary should be allowed into the facility. When procedures are in progress the lab door should be shut and when no one is present in the lab the doors should be locked. Anyone requesting access to the facility should be questioned as to their purpose and identification should be provided. All people requesting access must be 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 must be worn as protection from hazardous materials and when handling animals. Two pairs should be worn as appropriate. 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 must not be washed or reused. All PPE should be doffed so that the transfer of infectious materials to areas beyond where they or animals are being handled is minimized. Hands must be washed after removing gloves, and before leaving the animal room. Used disposable PPE should be disposed of with other contaminated waste. Reusable PPE (i.e., goggles) should be appropriately decontaminated before reuse. Reusable protective clothing should be laundered through MSU Laundry. It must not be taken home. If visibly contaminated, laundry should be placed in a biohazard bag before be placed with other items to go to laundry.

Eating, drinking, handling contact lenses and applying cosmetics

Eating, drinking, contact lens handling and cosmetic application must be done outside of animal and procedure rooms. Food and beverages for human consumption must be 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 should be 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 must be washed with soap and water after handling potentially infectious materials. Hands should be washed before leaving the laboratory and before touching common use surfaces (i.e, computers, telephones, etc.).

Pipetting

Mechanical pipetting devices must be available and used. Mouth pipetting is prohibited.

Safe sharps practices

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

Decontaminate work surfaces

Work surfaces must be decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant should be 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 EHS. They should be 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 must be 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 must ensure that the waste is placed in a leak-proof, covered container and is secured. Please refer to the following 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)

The following safety equipment must be used in an ABSL-1 lab:

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)

The facilities required in an ABSL-1 laboratory include the following:

Location

The animal facility is located 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 must be self-closing. They must be closed when animals are present inside the room and they should be kept locked when no one is present in the room.

Sink

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

Easily cleaned

The lab must be designed in a way that allows it to be cleaned easily. Spaces between benches, cabinets and equipment must be accessible for cleaning. Interior surfaces must be water resistant. Floors must be 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 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 conjunction with lab work must be covered with a non-porous material that can be easily cleaned and disinfected.

Windows

If the facility has windows they must be break resistant. If they can be opened to the outdoors, they must be 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*.

Appurtenances

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

Floor drains

Traps must be filled with water or disinfectant as appropriate.

Cage washers

Cages are preferentially washed with an automatic cage washer. The cage washer must have a final rise temperature of 180°F.

Lighting

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

Eyewash stations and showers

An eyewash station must be readily available.

Animal Facility – Biosafety Level 2 (ABSL-2)

This is suitable for work involving animals that are infected with agents assigned to Risk Group 2.

Standard Microbiological Practices

The following practices must be used in an ABSL-2 lab:

Prior to initiation of work

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

Facility specific biosafety manual

Each animal facility must supplement 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 must complete required EHS training courses. See the following section of this manual for a description of courses: *General Laboratory Practices- Training*. The facility director must ensure 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 must receive annual updates or additional training when procedures or policies change. Records are maintained.

Medical surveillance

All personnel involved in animal research must 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 must 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 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 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.

Controlled access

The facility supervisor must ensure that access to the animal areas is controlled. Only those people necessary should be allowed into the facility. When procedures are in progress the lab door should be shut and when no one is present in the lab the doors should be locked. Anyone requesting access to the facility should be questioned as to their purpose and identification should be provided. All people requesting access must be 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 must be used when there is the potential for splashes of microorganisms or other hazardous materials. Respirators must be worn as appropriate. It is recommended that long hair be secured or placed within a hair net to avoid contamination. Gloves must be worn as protection from hazardous materials and when handling animals. Two pairs should be worn as appropriate. 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 must not be washed or reused. All PPE should be doffed so that the transfer of infectious materials to areas beyond where they or animals are being handled is minimized. Hands must be washed after

removing gloves, and before leaving the animal room. Used disposable PPE should be disposed of with other contaminated waste. Reusable PPE (i.e., goggles) should be appropriately decontaminated before reuse. Reusable protective clothing should be laundered through MSU Laundry. It must not be taken home. If visibly contaminated, laundry should be placed in a biohazard bag before be placed with other items to go to laundry.

Eating, drinking, handling contact lenses and applying cosmetics

Eating, drinking, contact lens handling and cosmetic application must be done outside of animal and procedure rooms. Food and beverages for human consumption must be 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 should be 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 must be washed with soap and water after handling potentially infectious materials. Hands should be washed before leaving the laboratory and before touching common use surfaces (i.e, computers, telephones, etc.).

Pipetting

Mechanical pipetting devices must be available and used. Mouth pipetting is prohibited.

Safe sharps practices

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

Decontaminate work surfaces

Work surfaces must be decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant should be 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 EHS. They should be 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 must be 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 must ensure that the waste is placed in a leak-proof, covered container and is secured. Please 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

The following special practices must be utilized in an ABSL-2 lab:

Medical surveillance

A medical surveillance program will be 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 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.

Aerosol generating procedures

A biological safety cabinet, 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. 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 and practices that reduce risk of exposure while handling animals should be considered as appropriate.

Proper decontamination and transport of waste

All cultures, stocks, wastes from animal rooms, and other biohazardous materials must be 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 you must ensure that the waste is placed in a leak-proof, covered container and is secured. The container should be surface disinfected before transport and should bear a biohazard label. Please 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 should be decontaminated routinely. All equipment must be decontaminated before being repaired, maintained, or removed from the laboratory. When any of these is to occur lab personnel must complete an Equipment Release Form and attach it to the piece of equipment. See Appendix E for an example of the form. It must also be decontaminated after spills, splashes or when potentially contaminated. All spills must be cleaned by personnel who are properly trained and have the proper equipment to handle infectious materials. All ABSL-2 labs should 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 should be posted in an easily accessible location in the laboratory. All lab personnel should be 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)

The following safety equipment must be used in an ABSL-2 lab:

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 should be determined by the risk assessment. The use of laboratory coats, gowns or uniforms and other required PPE must be worn while in areas where infectious materials or animals are manipulated or housed. 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 secured or placed within a hair net 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. All reusable protective clothing must be laundered by MSU laundry or at the animal facility. It cannot be taken home.

Laboratory Facilities (Secondary Barriers)

The facilities required in an animal biosafety level two laboratory include the following:

Location

The animal facility is located 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 must open inward and be self-closing. They must be closed when animals are present inside the room and they should be kept locked when no one is present in the room.

Sink

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

Easily cleaned

The lab must be designed in a way that allows it to be cleaned easily. Spaces between benches, cabinets and equipment must be accessible for cleaning. Interior surfaces must be water resistant. Floors must be 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 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 conjunction with lab work must be covered with a non-porous material that can be easily cleaned and disinfected.

Windows

If the facility has windows they must be break resistant and sealed.

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*.

Appurtenances

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

Floor drains

Traps must be filled with water or disinfectant as appropriate.

Cages

Cages should be decontaminated before being washed. The cage washer must have a final rinse temperature of 180°F.

Lighting

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

Biological safety cabinets (BSCs)

Biological safety cabinets (BSC) must be installed in a manner so that changes in room air do not interfere with the operation of the cabinet. They should be 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 must be 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 must be 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

The following practices must be used in an ABSL-3 lab:

Prior to initiation of work

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

Facility specific biosafety manual

Each animal facility must supplement 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 must complete required EHS training courses. See the following section of this manual for a description of courses: *General Laboratory Practices- Training*. The facility director must ensure 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 must receive annual updates or additional training when procedures or policies change. Records are maintained.

Medical surveillance

All personnel involved in animal research must 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 must 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 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 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 must ensure that access to the animal areas is controlled. The fewest number of individuals possible should be allowed access. Only those people necessary should be allowed into the facility. When procedures are in progress the lab door should be shut and when no one is present in the lab the doors should be locked. Anyone requesting access to the facility should be questioned as to their purpose and identification should be provided. All people requesting access must be 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 must be used when there is the potential for splashes of microorganisms or other hazardous materials. Respirators must be worn as appropriate. It is recommended that long hair be secured or placed within a hair net to avoid contamination. Gloves must be worn as protection from hazardous materials and when handling animals. Two pairs should be worn as appropriate. 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 must not be washed or reused. All PPE should be doffed so that the transfer of infectious materials to areas beyond where they or animals are being handled is minimized. Hands must be washed after removing gloves, and before leaving the animal room. Used disposable PPE should be disposed of with other contaminated waste. Reusable PPE (i.e., goggles) should be appropriately decontaminated before reuse. Reusable protective clothing should be laundered through MSU Laundry. It must not be taken home. If visibly contaminated, laundry should be placed in a biohazard bag before be placed with other items to go to laundry.

Eating, drinking, handling contact lenses and applying cosmetics

Eating, drinking, contact lens handling and cosmetic application must be done outside of animal and procedure rooms. Food and beverages for human consumption must be 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 should be 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 must be washed with soap and water after handling potentially infectious materials. Hands should be washed before leaving the laboratory and before touching common use surfaces (i.e, computers, telephones, etc.).

Pipetting

Mechanical pipetting devices must be available and used. Mouth pipetting is prohibited.

Safe sharps practices

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

Decontaminate work surfaces

Work surfaces must be decontaminated after work is finished and after a spill of potentially hazardous materials. Appropriate disinfectant should be 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 EHS. They should be 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 must be 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 must ensure that the waste is placed in a leak-proof, covered container and is secured. Please 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

The following special practices must be utilized in an ABSL-3 lab:

Medical surveillance

A medical surveillance program will be 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 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. 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) must be 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 handling animals should be considered as appropriate.

Containment caging systems

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

Ventilated caging systems

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

Proper decontamination and transport of waste

All cultures, stocks, wastes from animal rooms, and other biohazardous materials must be decontaminated before disposal. This includes potentially infectious animal tissues, carcasses, bedding, feed, sharps, etc. An approved method of decontamination must be 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 you must ensure that the waste is placed in a leak-proof, covered container and is secured. The container should be surface disinfected before transport and should bear a biohazard label. Please 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 should be decontaminated routinely. All equipment must be decontaminated before being repaired, maintained, or removed from the laboratory. When any of these is to occur lab personnel must complete an Equipment Release Form and attach it to the piece of equipment. See Appendix E for an example of the form. It must also be decontaminated after spills, splashes or when potentially contaminated. All spills must be cleaned by personnel who are properly trained and have the proper equipment to handle infectious materials. All ABSL-3 labs should 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 should be posted in an easily accessible location in the laboratory. All lab personnel should be 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)

The following safety equipment must be used in an ABSL-3 lab:

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 for all procedures involving infectious materials and animals (when possible). 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. 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 must be 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 must be worn while in areas where infectious materials or animals are manipulated or housed. Front button lab coats are not appropriate. Disposable 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 secured or placed within a hair net 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. Appropriate respiratory protection and foot protection must be worn when entering areas where infectious materials or animals are housed. All reusable protective clothing must be decontaminated before being laundered by MSU laundry or at the animal facility. It cannot be taken home.

Laboratory Facilities (Secondary Barriers)

The facilities required in an animal biosafety level three laboratory include the following:

Location

The animal facility is located 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 must open inward and be self-closing. They must be closed when animals are present inside the room and they should be kept locked when no one is present in the room. Entry into the area is through a double-door entry.

Showers

Showers should be considered based on the risk assessment.

Sink

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

Easily cleaned

The lab must be designed in a way that allows it to be cleaned and decontaminated easily. Spaces between benches, cabinets and equipment must be accessible for cleaning. Interior surfaces must be water resistant. Floors must be 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 must be appropriate for the anticipated use. Cabinets and bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis and other chemicals. Chairs used in animal areas must be covered with a non-porous material that can be easily cleaned and disinfected.

Windows

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

Airflow

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

Appurtenances

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

Floor drains

Traps must be filled with water or disinfectant as appropriate.

Cages

Cages should be decontaminated before being removed from the ABSL-3 space. They must be washed using an automatic cage washer. The cage washer must have a final rise temperature of 180°F.

Lighting

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

Biological safety cabinets (BSCs)

Biological safety cabinets (BSC) must be installed in a manner so that changes in room air do not interfere with the operation of the cabinet. They should be 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 must be tested and certified at least annually and operated in accordance with the manufacturer's recommendations.

Autoclave

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

Eyewash stations and showers

An eyewash station and safety shower must be readily available.

Design and operational procedures

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

Additional environmental protection

Additional protective measures should be 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 will be 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 should be taken into consideration before handling arthropods. For additional information on arthropod containment guidelines, please 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 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 should be 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 should be 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 shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress. Prior to entering the greenhouse, personnel shall be required to read and follow instructions on PBSL-1 greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.

Records

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

Decontamination and inactivation

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

Control of undesired species and motile macroorganisms

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

Concurrent experiments conducted in the greenhouse

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

Records

A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility. A record shall be kept of experiments currently in progress in the greenhouse facility. The Principal Investigator shall report 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 shall be prepared and maintained.

Decontamination and inactivation

Experimental organisms shall be 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 should be made periodically, to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

Control of undesired species and motile macroorganisms

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

Concurrent experiments conducted in the greenhouse

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

Greenhouse practices manual

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

Facilities

Definitions

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 shall be available for the treatment of contaminated greenhouse materials.

Supply and exhaust air ventilation systems

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

Other

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.

Laboratory Biosecurity

Recent 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, exempt levels of toxins on the select agents list or exempt 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, exempt levels of select agent toxins, exempt strains of select agents, other agents of public health or agricultural concern, or agents of high commercial value please contact the Biosafety Team for 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 should be limited to authorized personnel based on the necessity of entering sensitive areas. At a minimum, laboratory doors must be locked when no one is present in the lab, all storage units housed in shared space (i.e., hallway, storage room, etc.) must be locked, and all persons entering the laboratory should be asked for identification and questioned as to their purpose for being there.
- 2. Inventory and accountability:** It is the responsibility of each laboratory to establish material accountability procedures. These should be 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.
- 3. Transport of biological agents:** Material transport policies are in place that outline requirements for transporting locally on campus and outside of campus. See the following

section of this manual for additional information: *Introduction to the Transport of Biological Materials*.

4. **Reporting and communication:** In addition to following departmental reporting requirements should a security breach occur, the laboratory must also notify DPS and the Biological Safety Officer. Investigation into the breach will occur as appropriate.
5. **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.

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
--Class II	Aerosol and spatter	Minimum inward airflow (face velocity) at work access opening. Adequate filtration of exhaust air. Provides product protection
--Class III	Aerosol and spatter	Maximum containment. Provides product protection if laminar flow air is included.
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	Ease of use Controls contamination of suction end of pipette, protecting pipetting aid, user, and vacuum line

	end of pipette	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 microorganisms	Robust
		Watertight primary and secondary containers to contain spills
		Absorbent materials to contain spills
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, 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 also protect the environment.

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 unrecirculated 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 located 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 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 III Biological Safety Cabinet

This type provides the highest level of personnel protection and is 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 or 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.

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

TYPE OF PROTECTION	BSC SELECTION
Personnel protection, microorganisms in Risk Groups 1-3	Class I, Class II, Class III
Personnel protection, microorganisms in Risk Group 4, glovebox 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
Volatile radionuclide/chemical protection	Class I, Class IIB2, Class III

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 Risk Group 1-4 microorganisms; 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 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 and IIB2 BSCs, which have hard-duct installations, must have airflow through them at all times 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 absolutely 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 come into contact with 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 work day, 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 where 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 in order 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 formaldehyde or chlorine dioxide gas. BSC decontamination should be performed by a qualified professional.

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. 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 amount 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 located 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 at all 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 actually 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 through the use of 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 $\frac{3}{4}$ full or within 90 days 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. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material. Gloves should be worn during preparation of frozen sections. When working with biohazardous material in a cryostat, the following is recommended:

- Consider the contents of the cryostat to be contaminated and decontaminate it frequently with a disinfectant suitable for the agent(s) in use.
- Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.
- 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.
- Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.

- Consider solutions for staining potentially infected frozen sections to be contaminated.

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:

- Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Fill and open centrifuge tubes, rotors and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
- Add disinfectant to the space between the tube and the bucket to disinfect material in the event of breakage during centrifugation.
- Always balance buckets, tubes and rotors properly before centrifugation.
- Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters.
- Work in a BSC when resuspending sedimented material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.
- Small low - speed centrifuges may be placed in a BSC during use to reduce the aerosol escape.

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 BL-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. A number of 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) regulates 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 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's 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.

Registration of Select Agent & Toxin Possession is MANDATORY

Under previous select agent regulations, an individual was permitted to use select agent materials without registering with the federal authorities. Registration was only required if an individual planned to send or receive materials on the select agent list. Under the revised regulations which take effect on February 7, 2003, 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 Director 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 in order 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 in order to address this potential. In the meantime, the following actions can be taken to prevent this from happening:

- Screen all research materials received in order 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 Exempt 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 exempt strains and the potential for them to be manipulated for use as a biological weapon, the Office of Environmental Health and Safety/Office of Radiation, Chemical and Biological Safety (EHS/EHS) has implemented the containment and security requirements outlined in Appendix J for handling exempt strains of select agents.

The containment and security requirements apply to the following exempt strains of select agents:

Attenuated strains of HHS Select Agents and Toxins excluded

- *Botulinum neurotoxins*
- Conotoxins
- *Coxiella burnetii*
- Eastern Equine Encephalitis virus
- Ebola virus
- *Francisella tularensis*
- Junin virus
- Lassa fever virus
- Monkeypox virus
- SARS-Coronavirus
- Staphylococcal Enterotoxins (SE)
- Tetrodotoxin
- *Yersinia pestis*

Attenuated strains of Overlap Select Agents excluded

- *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

- Avian influenza virus (highly pathogenic)

Guidelines for the Use of Exempt Levels of Select Agent Toxins

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. Generally speaking, 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 must be carried out with care to avoid exposure to infectious 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 incubation time.

Heat

In order 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. In order 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 rough 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.

Table 4: Increasing Resistance to Chemical Disinfectants

LEAST RESISTANT	LIPID OR MEDIUM-SIZE VIRUSES	Examples Herpes simplex Virus Cytomegalovirus Hepatitis B virus HIV
	VEGETATIVE BACTERIA	<i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> <i>Salmonella choleraesuis</i>
	FUNGI	Trichophyton sp. Cryptococcus sp. Candida sp.
	NONLIPID OR SMALL VIRUSES	Poliovirus Coxsackievirus Rhinovirus
	MYCOBACTERIA	<i>Mycobacterium tuberculosis</i> ; <i>M. bovis</i>
	MOST RESISTANT	BACTERIAL SPORES

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 glutaraldehydes are 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 cold sterilants since they inactivate bacterial spores. 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 commonly 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

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:** 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:

- a. 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.
 - b. 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.
 - c. 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.
 - d. Sharps: Defined as needles, syringes, scalpels, and intravenous tubing with needles attached regardless of whether they are contaminated or not.
 - e. Contaminated wastes from animals that have been exposed to agents infectious to humans, these being primarily research animals.
2. **Regulated waste** as defined by the *Michigan Occupational Safety and Health Act on Bloodborne Infectious Diseases* (MIOSHA) including:
 - a. Liquid or semi-liquid blood or other potentially infectious materials;
 - b. Contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state if compressed;
 - c. Items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling;
 - d. Contaminated sharps which includes any contaminated object that can penetrate the skin;
 - e. Pathological and microbiological wastes containing blood or other potentially infectious materials.
 3. **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.

General Labeling, Packaging and Disposal Procedures

Currently, biohazardous waste is to be decontaminated before leaving MSU. Most of the waste can be autoclaved prior to disposal, while some waste will be incinerated. The responsibility for decontamination and proper disposal of biohazardous waste lies with the producing facility (e.g., laboratory and department). The EHS assists only in the disposal of sharps and pathological waste including animal carcasses.

All biohazardous waste needs to be packaged, contained and located in a way that protects and prevents the waste from release at any time at the producing facility prior to ultimate disposal. If storage is necessary, putrefaction and the release of infectious agents into the air must be prevented.

No biohazardous waste can be accumulated for more than 90 days at MSU.

If not stated otherwise (see below), most biohazardous waste will be disposed of in biohazard bags. MSU requires the use of biohazard bags that include the biohazard symbol and a built-in heat indicator with the word ("AUTOCLAVED"). Bags that meet these requirements are available in various sizes at General Stores and Biochemistry Stores. All waste disposed of in these bags is to be autoclaved in an approved autoclave until the waste is decontaminated. The built-in heat indicator will turn dark. All autoclaves used for the decontamination of biohazardous waste will be tested by the EHS at least on an annual basis. After successful autoclaving (decontamination), all biohazard bags need to be bagged in opaque (black) plastic non-biohazard bags that are leakproof. These opaque bags can be put in the lodal or picked up by custodial services. Biohazardous waste that has been successfully decontaminated by autoclaving is no longer considered hazardous.

Since autoclaves are an integral part of MSU's biohazardous waste treatment procedure, proper operation and maintenance is very important. All users of autoclaves need to be trained in the proper operating procedures either through the laboratory supervisor or Principal Investigator or whoever was put in charge by the department. Maintenance and repair of autoclaves used for the decontamination of biohazardous waste are the responsibility of the individual departments. If the department chooses to not use autoclaves for their biohazardous waste treatment, alternative procedures (e.g., outside biomedical waste hauler) need to be established.

Waste Specific Procedures for BSL-1 and 2

Cultures, Stocks and Related Materials

Cultures and stocks of infectious agents and associated biologicals (as defined above), shall be placed in biohazard bags and decontaminated by autoclaving. Double or triple bagging may be required to avoid rupture or puncture of the bags.

Bulk Liquid Waste, Blood and Blood Products

All liquid waste from humans or animals such as blood, blood products and certain body fluids, not known to contain infectious agents, can be disposed of directly by flushing down a sanitary sewer. However, due to coagulation, flushing of large quantities of blood is impractical. Contact the EHS for additional information on disposal of large volumes of blood. All other liquid biohazardous waste needs to be autoclaved prior to disposal or treated with a disinfectant.

Sharps

All sharps must be placed in a rigid, puncture resistant, closable and leakproof container, which is labeled with the word "**Sharps**" and the biohazard symbol. MSU/EHS approved sharps containers are available through General Stores. Food containers (e.g., empty coffee cans) are **not permissible** as sharps containers. When a sharps container is first put into use it must be labeled with a completed sharps label. All sharps must be handled with extreme caution. The clipping, breaking, and recapping of needles is not recommended. Sharps containers should not be filled more than 3/4. After use, the container needs to be closed and labeled with a MSU Hazardous Materials Pick Up Tag. To comply with the 90 day accumulation limit, contact the EHS for pick-up as soon as possible. **Never place any type of sharps in the lodal.**

Contaminated Solid Waste

Contaminated solid waste includes cloth, plastic and paper items that have been exposed to agents

infectious or hazardous to humans, animals, or plants. These contaminated items shall be placed in biohazard bags and decontaminated by autoclaving. Double or triple bagging may be required to avoid rupture or puncture of the bags. **Contaminated Pasteur pipettes are considered sharps** and need to be disposed of in a sharps container.

Waste Specific Procedures for Biosafety Level 3 (BSL-3)

All biohazardous waste including RG-2 and 3 agents that are handled at BSL-3 is to be autoclaved at the point of origin (laboratory, or facility). Transportation of non-autoclaved BSL-3 waste outside of the building is generally not permitted. Exceptions might include animal carcasses that need to be incinerated.

Pathological Waste

The Environmental Compliance office provides removal, transportation and disposal services for University units that generate pathological waste. According to the MMWRA, pathological waste consists of 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. At MSU, animal carcasses are also considered pathological waste. Although not all pathological waste is infectious, it is prudent to handle such waste as if it were because of the possibility of unknown infection in the source. All human pathological waste is also covered by "Universal Precautions" according to the MIOSHA Bloodborne Pathogen Standard. For more information on this subject, refer to MSU's *Exposure Control Plan*. Typically, carcasses or tissues are collected in plastic bags, labeled, stored in area freezers, cold rooms or refrigerators and removed for incineration by EHS. Many units have routine weekly EHS pickups. For non-scheduled pickups, contact the EHS Environmental Compliance Office.

Animal Waste

Follow practices outlined by Campus Animal Resources <https://animalcare.msu.edu/car>

Campus Animal Resources

4660 S. Hagadorn, Suite 210
East Lansing, MI 48823
T: 517-353-5064

Department or Facility Specific Waste Procedures

If required, departments or facilities may establish biohazardous waste procedures that are more stringent than the above listed procedures. A written copy of these procedures should be made available to the EHS prior to initiation.

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 should have a basic biological spill kit ready to use at all times. For most instances the basic kit can be assembled with materials already used in the laboratory. All labs operating at BSL-2 or higher must 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, as long as 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. It should be enhanced 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)
- 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 situation, 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.
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.
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 labcoat, 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:

- Don't 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 should be restocked after each use. It should also be checked for completeness on an annual basis. The following maintenance activities should be done:

- Check expiration on disinfectant and replace as needed (e.g., bleach should be replaced every 6 months once opened and after 24 hours when diluted);
- Replace gloves;
- Replace antimicrobial towelettes; and

- Check straps on splash goggles for deterioration.

Handwashing 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-washing 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-washing is 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 Substances

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 in the event that 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. In light of recent current events, there is 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.

The EHS offers training and consultation for campus personnel who plan to ship biological materials including: diagnostic specimens, infectious substances, genetically modified organisms, and biological materials on dry ice.

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).

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 www.technologies.msu.edu

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) or view the MSU Export Controls Web Site: www.exportcontrols.msu.edu

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 are 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 Logistics regarding the MSU contracted Custom Broker.

When in doubt, PLEASE 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.

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.

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. This can be found on the Biosafety in Research website (www.biosafety.msu.edu).

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 has to 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 has to be 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, in order 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.

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.

APPENDICIES

Appendix A – Biosafety Resources

MSU Safety Manuals:

Biohazardous Waste Management Plan
Chemical Hygiene Plan
Exposure Control Plan for Bloodborne Pathogens (updated annually)
Radiation Safety Manual
Waste Disposal Guide

Websites:

The Office of Radiation, Chemical and Biological Safety
www.EHS.msu.edu

Biosafety in Research
www.biosafety.msu.edu

Biosafety-related Products:

There are many biosafety related products available through University Stores and through Biochemistry Stores. For product details and pricing information please contact:

- University Stores (355-1700)
www.universitystores.msu.edu
- Biochemistry Research Store (353-0813)
www.bch.msu.edu/~bmbstore/

Available products include, but are not limited to:

- Sharps containers
- Biohazardous waste containers
- Biohazardous waste bags
- Splash goggles
- Gloves (latex, nitrile, vinyl, etc.)
- Disposable inoculating loops
- Disposable spreaders
- Safer sharps devices
- Lab coats

Laundry Services:

Laboratory coats cannot be taken home to be washed. They must be laundered by a facility that is certified to handle biological contaminated items. Spartan Linen Services offers laundering services. Contact them for details (355-8520; www.spartanlinen.com).

Appendix B – Select Agent List DHHS/USDA

HHS and USDA Select Agents and Toxins 7CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

HHS SELECT AGENTS AND TOXINS

Abrin
Botulinum neurotoxins*
Botulinum neurotoxin producing species of *Clostridium**
Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence X₁CCX₂PACGX₃X₄X₅X₆CX₇)¹
Coxiella burnetii
Crimean-Congo haemorrhagic fever virus
Diacetoxyscirpenol
Eastern Equine Encephalitis virus³
Ebola virus*
*Francisella tularensis**
Lassa fever virus
Lujo virus
Marburg virus*
Monkeypox virus³
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)
Ricin
Rickettsia prowazekii
SARS-associated coronavirus (SARS-CoV)
Saxitoxin
South American Haemorrhagic Fever viruses:
Chapare
Guanarito
Junin
Machupo
Sabia
Staphylococcal enterotoxins A,B,C,D,E subtypes
T-2 toxin
Tetrodotoxin
Tick-borne encephalitis complex (flavi) viruses:
Far Eastern subtype
Siberian subtype
Kyasanur Forest disease virus
Omsk hemorrhagic fever virus
Variola major virus (Smallpox virus)*
Variola minor virus (Alastrim)*
*Yersinia pestis**

OVERLAP SELECT AGENTS AND TOXINS

*Bacillus anthracis**
Bacillus anthracis Pasteur strain
Brucella abortus
Brucella melitensis
Brucella suis
*Burkholderia mallei**
*Burkholderia pseudomallei**
Hendra virus
Nipah virus
Rift Valley fever virus
Venezuelan equine encephalitis virus³

USDA SELECT AGENTS AND TOXINS

African horse sickness virus
African swine fever virus
Avian influenza virus³
Classical swine fever virus
Foot-and-mouth disease virus*
Goat pox virus
Lumpy skin disease virus
*Mycoplasma capricolum*³
*Mycoplasma mycoides*³
Newcastle disease virus^{2,3}
Peste des petits ruminants virus
Rinderpest virus*
Sheep pox virus
Swine vesicular disease virus

USDA PLANT PROTECTION AND QUARANTINE (PPQ)

SELECT AGENTS AND TOXINS

Peronosclerospora philippinensis
(*Peronosclerospora sacchari*)
Phoma glycinicola (formerly *Pyrenochaeta glycines*)
Ralstonia solanacearum
Rathayibacter toxicus
Sclerophthora rayssiae
Synchytrium endobioticum
Xanthomonas oryzae

*Denotes Tier 1 Agent

¹ 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 α -MI and α -GI (shown above) as well as α -GIA, Ac1.1a, α -CnIA, α -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.

² 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.

³ 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. 9/10/13

Appendix C – Risk Assessment Form
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		
<i>Pathogenicity of the organism & Routes of transmission</i>	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 – Examples of RG-2, RG-3, and RG-4 Agents

NIH Guidelines

Appendix B-I. Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in healthy adult humans. 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.

Appendix B-II. 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.

Appendix B-II-A. 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)

Appendix B-II-B. 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*
- Microsporium*
- Paracoccidioides braziliensis*
- Penicillium marneffeii*
- Sporothrix schenckii*
- Trichophyton*

Appendix B-II-C. 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. granulosus*, *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

Appendix B-II-D. 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

Appendix B-III. Risk Group 3 (RG3) Agents

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

Appendix B-III-A. 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](#))

Appendix B-III-B. Risk Group 3 (RG3) - Fungal Agents

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

Appendix B-III-C. Risk Group 3 (RG3) - Parasitic Agents

None

Appendix B-III-D. 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-associated coronavirus (SARS-CoV)
--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](#))

Appendix B-IV. 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.

Appendix B-IV-A. Risk Group 4 (RG4) - Bacterial Agents

None

Appendix B-IV-B. Risk Group 4 (RG4) - Fungal Agents

None

Appendix B-IV-C. Risk Group 4 (RG4) - Parasitic Agents

None

Appendix B-IV-D. 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

Equipment Release Form

Date: _____ Location of Origin: _____

Principle Investigator: _____

Destination/Service Department: _____

Service To Be Performed: _____

Type of Equipment: _____

Contaminated (Yes/No): _____

Contaminants Identified/Suspected: _____

Method of Decontamination: _____

Name of Person Decontaminating: _____

I certify that the above listed equipment is free of contamination or hazardous agents, and that it is safe to release to unrestricted areas and/pr perform the work described above on this equipment.

Signature of Responsible Person

Appendix F- Exposure Response Procedures

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** (Frandon 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

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	None required	Open bench and sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: <ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • 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 <u>in combination with</u> full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: <ul style="list-style-type: none"> • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decon systems • Other requirements outlined in the text

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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 <ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • Controlled access • Decontamination of clothing before laundering • Cages decontaminated before bedding removed • Disinfectant foot bath as needed 	ABSL-2 equipment plus: <ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive- 	ABSL-3 facility plus: <ul style="list-style-type: none"> • Separate building or isolated zone • Dedicated supply and exhaust, vacuum and decontamination systems • Other requirements outlines in the text

			pressure personnel suit) used for all procedures and activities	
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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, 5th ed., 2007*)

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	TME prion	MkPrP ^{Sc}	2
Chronic wasting disease (CWD)	mule deer, elk and white tail deer	CWD prion	MdePrP ^{Sc}	2
Bovine spongiform encephalopathy (BSE)	cattle	BSE prion	BoPrP ^{Sc}	2/3
Feline spongiform encephalopathy (FSE)	cats	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
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 2007). 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 2007, 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).

Although there are no documented laboratory-acquired prion infections, the primary hazard is from accidental parenteral inoculation or ingestion. 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.

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 2007, 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 1 hour (porous load) (BMBL 2007, 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 disinfective 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 Exempt 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 exempt strains and the potential for them to be manipulated for use as a biological weapon, the Office of Environmental Health and Safety/Office of Radiation, Chemical and Biological Safety (EHS/EHS) has implemented the following containment and security requirements for handling exempt strains of select agents.

Applicability:

The containment and security requirements apply to the following exempt strains of select agents:

Attenuated strains of HHS Select Agents and Toxins excluded

- [Botulinum neurotoxins](#)
- [Conotoxins](#)
- [Coxiella burnetii](#)
- [Eastern Equine Encephalitis virus](#)
- [Ebola virus](#)
- [Francisella tularensis](#)
- [Junin virus](#)
- [Lassa fever virus](#)
- [Monkeypox virus](#)
- [SARS-Coronavirus](#)
- [Staphylococcal Enterotoxins \(SE\)](#)
- [Tetrodotxin](#)
- [Yersinia pestis](#)

Attenuated strains of Overlap Select Agents excluded

- [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

- [Avian influenza virus \(highly pathogenic\)](#)

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 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-sheath 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:
 - e. 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.
 - f. 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 is capable of supporting 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 so as 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 please 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

Required on-site training	Chemical	Biological/Toxin	Bloodborne	Complete if checked
Location and Review of Safety Protocol Guides				
Emergency Contacts	X	X	X	
Chemical Hygiene Plan	X	X	X	
MSDS	X	X	X	
Hazardous Waste Guide	X	X	X	
Standard Operating Procedures (task specific)	X	X	X	
Emergency Response Procedures	X	X	X	
Biological Safety Manual		X	X	
Biohazardous Waste Plan		X	X	
Exposure Incident Response Procedure		X	X	
Exposure Control Plan			X	
Source Protocol			X	
Inventory, Storage, Labeling, and Proper Use of:				
Chemical Storage	X	X	X	
Hazardous Chemicals	X	X	X	
Biohazardous Materials (including toxins)		X	X	
Location, Proper Use, and Maintenance of:				
Personal Protective Equipment	X	X	X	
Emergency Eyewash/Shower	X	X	X	
Fume Hood	X	X	X	
Compressed Gasses	X	X	X	
Chemical Spill Kit	X	X	X	
Biological Spill Kit		X	X	
Biosafety Cabinet		X	X	
Laminar Flow Hood		X	X	
Autoclaves		X	X	
Disinfectants		X	X	
Safer Sharps			X	
Waste Segregation, Storage, Transport, Treatment				
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	
Security				
Laboratory Security	X	X	X	
Inventory	X	X	X	

Emergency Contacts - Same as posted on door signs
Chemical Hygiene Plan - Online or hard copy in lab and present upon inspection
MSDS - know location and present upon inspection
Hazardous Waste Guide - Online or hard copy in lab and present upon inspection
Standard Operating Procedures - Online or hard copy in lab and present upon inspection
Emergency Response Procedures - Post in prominent place in lab or near phone
Biological Safety Manual - Hard copy in lab and present upon inspection
Biohazardous Waste Plan - Hard copy in lab and present upon inspection
Exposure Incident Response Procedure - Post in prominent place
Exposure Control Plan - Hard copy in lab and present upon inspection
Source Protocol - Hard copy in lab and present upon inspection
Chemical Storage - Know what types are stored where and how to label
Hazardous Chemicals - Know what types are stored where and how to label
Biohazardous Materials - Know what types are stored where and how to label
Personnel Protective Equipment - know what types, when to use, and how to maintain them
Emergency Eyewash/Shower - Know location and maintenance
Fume Hood - Know when and how to use
Compressed Gasses - Know how and when to use
Chemical Spill Kit - Location and maintenance
Biological Spill Kit - Location and maintenance
Biosafety Cabinet/Laminar Flow Hood - Location, use and maintenance including certification
Autoclaves - Location, use and maintenance including certification
Disinfectants - Location, use, concentration, MSDS, expiration and disposal
Safer Sharps - Use, annual review, and evaluation
Sharps/Glass/Solid/Liquid Waste - Location, labeling, use and disposal of container
Waste Tags - Use
90 day Disposal - which wastes fall under this law
Transport - secondary container use
Treatment - how to treat each type of waste
Laboratory Security - Aware of security plan for MSU, department policies, and lab policy
Inventory - Online or hard copy of hazardous/biohazardous material, present upon inspection

(Print Employee's/Student Name)

(Manager/Precept/Trainer signature - Date)

(Faculty/Student/Employee Signature - Date)

I certify that the site-specific training items were reviewed and understood as required by the MSU EHS.

(This must be completed and signed at each facility the student or employee is working in)

Appendix L – Large Scale Biosafety Guidelines



https://www.asm.org/index.php?option=com_content&view=article&id=2703

Large Scale Biosafety Guidelines

The final version of the Large Scale BioSafety Guidelines was incorporated into Biological Safety: Principles and Practices, 3rd edition, by Diane O. Fleming and Debra L. Hunt, available from [ASM Science](#). The guidelines can be found in Section V. Special Considerations for Biosafety.

Draft Document considered by the Laboratory Practices Committee April 13, 1999 until September 1, 1999:

Biosafety guidelines for work with small volumes of infectious agents, i.e., those amounts typically used for diagnosis, characterization, or basic research, have been established by CDC-NIH and WHO. Additionally, guidelines for working with recombinant DNA molecules in large volumes exist in the NIH Guidelines for Research with Recombinant DNA molecules. However, no specific biosafety guidelines have been established for large scale work with organisms that do not contain recombinant molecules or organism. This document has been developed by including additional equipment and practices for safe large scale work to the existing guidelines from NIH and CDC.

It is understood that the organism, quantity, frequency, and process have a significant impact on the choice of an appropriate biosafety level for the work to be conducted. There is no specific volume which constitutes "large scale" for microbial agents. Certain CDC-NIH guidance documents have referred to large scale as those volumes typically in excess of those used for identification, typing, assay performance or testing. The risk analysis must include an assessment of the infectivity of the agent, the routes of exposure, the severity of infection, availability of prophylaxis, the level of containment afforded by the process and equipment used, etc., not just the volume of material being handled. Similarly, there is little scientific evidence to support the premise that only volumes greater than 10 liters merit large scale requirements. Certainly that is not true for BSL-2 and BSL-3 organisms. The CDC-NIH Guideline recommends raising the biosafety level for culturing and purification of many BSL-2 organisms, however that was only done in an effort to provide considerations for the Biosafety Officer and scientists in the establishment of the appropriate level of protection.

This document serves as an effort to collect best practices for maximizing the safety for large scale work, and can be utilized by an Institutional Biosafety Committee and/or a Biological Safety Officer to develop biosafety procedures for the work to be done.

The guidelines will cover 4 different levels for large scale work: Good Large Scale Practices (GLSP), Biosafety Level 1 - Large Scale (BSL1-LS), Biosafety Level 2 - Large Scale (BSL2-LS), and Biosafety Level 3 - Large Scale (BSL3-LS). The containment conditions for Biosafety Level 4 - Large Scale are not defined here, but should be determined on a case by case basis.

Only the biological hazard of the organism or cell line is addressed here. Other hazards, such as the toxicity or biological activity of the products produced, should be considered separately. These guidelines do not specifically address animal or plant pathogens, however, the containment principles and practices may be useful for some of those agents.

It is recommended that all institutions that engage in large scale research or production with microorganisms appoint a Biological Safety Officer (BSO) to oversee the procedures, facilities and equipment used.

I. Good Large Scale Practices (GLSP)

The GLSP level is recommended for organisms that are not known to cause disease in healthy adults. (Risk Group I), are non-toxigenic, are well characterized and/or have an extended history of safe large scale work. These organisms should not be able to transfer antibiotic resistance to other organisms. Examples of these organisms include *Saccharomyces cerevisiae*, *E. coli* K12 etc. These organisms should have limited survival and/or no adverse consequences if released into the environment.

A. Standard Microbiological Practices

1. Individuals wash their hands after handling viable material.
2. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not allowed in the work area.
3. Mouth pipetting is prohibited.
4. Work surfaces are capable of being cleaned and disinfected.
5. An insect and rodent control program is in effect.

B. Special Practices

1. Institutions that engage in large scale work should have a health and safety program for their employees.
2. Written instructions and training are provided for personnel who work at GLSP conditions.
3. Processing, sampling, transfer, handling, etc. of viable organisms are done in a manner that minimizes employee exposure and the generation of aerosols.

4. Discharges of viable organisms are disposed of in accordance with applicable local, state, and federal requirements.
5. The facility should have an emergency response plan which includes the handling of spills.

C. Safety Equipment

1. Protective clothing, e.g. uniforms, laboratory coats, etc., is provided to minimize the soiling of personal clothing.
2. Safety glasses are worn in the facility. Face shields and/or goggles and face masks are provided for procedures that may involve splashing or spraying of viable organisms.

D. Facilities

1. Sinks, eyewash stations, and safety showers are provided in the work area.

II. Biosafety Level 1 - Large Scale (BSL1-LS)

BSL1-LS is recommended for the large scale growth of organisms that are not known to cause disease in healthy adult humans and pose minimal hazard to personnel and the environment..These organisms would be handled at BL-1 laboratory scale.

A. Standard Microbiological Practices

1. Access to the work area may be restricted at the discretion of the project manager when work is ongoing.
2. Persons wash / clean their hands after they handle viable organisms, after removing gloves, and on leaving the work area.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work area.
4. Food is stored outside of the work area in cabinets or refrigerators designated and used for this purpose only.
5. Mouth pipetting is prohibited. Only mechanical pipetting devices are used.
6. Work surfaces are decontaminated on a routine basis and after any spill of viable organisms.
7. Procedures are performed carefully in a manner which minimizes aerosol generation.
8. All discharges of the viable organisms are disposed of in accordance with applicable local, state and federal regulations..
9. An insect and rodent control program is in effect.

B. Special Practices

1. Institutions that engage in large scale work have a health and safety program for their employees.
2. Written procedures and training in basic microbiological practices are provided and documented.
3. Medical evaluation, surveillance and treatment are provided where indicated; e.g. determine functional status or competency of employees' immune system when working with opportunistic pathogens, etc.
4. Spills and accidents which result in overt exposure to viable organisms are reported to the facility supervisor/manager. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
5. Emergency plans shall include methods and procedures for handling spills and employee exposures.
6. Cultures of viable organisms are handled in a closed system or other primary containment equipment, e.g. biological safety cabinet, which is designed to reduce the potential for the escape of viable organisms.
7. Sample collection and material addition to a closed system, and transfer of culture materials from one closed system to another are conducted in a manner which minimizes employee exposure, the release of viable material and the generation of aerosols.
8. Culture fluids may be removed from a closed system or other primary containment system in a manner which minimizes employee exposure, the release of viable material and the generation of aerosols.
9. Exhaust gases removed from a closed system or other primary containment system minimize the release of viable organisms to the environment by the use of appropriate filters or procedures.
10. A closed system or other primary containment equipment that has contained viable organisms shall not be opened for maintenance or other purposes until it has been decontaminated.

C. Safety Equipment

1. Protective clothing; e.g. uniforms, laboratory coats, etc., is provided to prevent the contamination or soiling of personal clothing.

2. Safety glasses must be worn. Protective face protection consisting of a face shield, or goggles and face mask is worn for procedures that may involve splashing or spraying of viable organisms.
3. Gloves are worn if the skin on the hands is broken, irritated, or otherwise not intact.

D. Facilities

1. Each work area contains a sink for handwashing, an eyewash station, and an emergency shower. The sink is located near the exit of the work area.
2. The work area has a door which can be closed when large scale work is ongoing.
3. The work area is designed to be easily cleaned.
4. Floors are able to be cleaned and disinfected in case of spills of viable organisms. Rugs are not allowed.
5. Work surfaces are impervious to water and resistant to acids, alkali, organic solvents, and moderate heat.
6. Furniture in the work area is sturdy and placed so that all areas are accessible for cleaning.
7. If the work area has windows that open, they are fitted with fly screens.
8. Facilities are designed to prevent the release of large volumes of viable organisms directly to sewer; e.g. floor drains are capped or raised, fitted with liquid tight gaskets to prevent release of untreated organisms to sewer.

III. Large Scale Biosafety Level 2 (BSL2-LS)

BSL2-LS is recommended for the propagation and cultivation of infectious organisms that would be handled at BSL-2 in laboratory scale. The following guidelines have been developed for facilities that routinely handle large volumes of these materials.

A. Standard Microbiological Practices

1. Access to the work area is restricted to personnel who meet the entry requirements.
2. Persons wash their hands after they handle viable organisms, after removing gloves and before leaving the work area.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the facility.
4. Food is stored outside of the facility in cabinets or refrigerators designated and used for this purpose only.

5. Mouth pipetting is prohibited. Only mechanical pipetting devices are used.
6. Work surfaces are decontaminated on a routine basis and after any spill of viable organisms.
7. Procedures are performed carefully in a manner which minimizes aerosol generation.
8. All contaminated wastes are decontaminated by an approved method prior to disposal in accordance with local, state, and federal regulations. Wastes that need to be transported to a different area or facility are closed and placed in a durable, leakproof container for transfer. Material to be transferred off site for decontamination is packaged and labeled in accordance with the applicable regulations.
9. All discharges of viable organisms are inactivated by a validated process, i.e. one that has been demonstrated to be effective using the organism in question, or with an indicator organism which is known to be more resistant to the physical or chemical methods used; e.g. *Bacillus stearothermophilus* for steam heat.
10. An insect and rodent control program is in effect.

B. Special Practices

1. Institutions that engage in large scale work have a health and safety program for their employees.
2. Doors to the work area are kept closed when work is ongoing.
3. Access to the work area is restricted to personnel whose presence is required and who meet entry requirements, i.e., immunization, if any. Individuals who can not take / do not respond to the vaccine, who cannot take the recommended prophylaxis in the event of an exposure incident, who are at increased risk of infection, or for whom infection may prove unusually hazardous, are not allowed in the work area until their situation has been reviewed by appropriate medical personnel. The individuals are informed of the potential risks and sign an acknowledgement / consent form, or similar vehicle, which indicates that they understand and accept the potential risk.
4. Written procedures and policies for handling infectious organisms are provided.
5. Personnel are able to demonstrate proficiency in standard microbiological practices and procedures, handling of human pathogens at a Biosafety Level 2. This can consist of previous experience and /or training. Training in the hazards associated with the organisms involved, and the practices and operations specific to the large scale work area are provided and documented.
6. Appropriate immunizations, medical evaluation surveillance, and treatment are provided where indicated; e.g., immunization, survey of immune status, etc.

7. A hazard warning sign, incorporating the universal biohazard symbol, identifying the infectious agents, listing the name and telephone numbers of the persons knowledgeable about and responsible for the work area, along with any special entry requirements for entering the work area, is posted at the entry to the work area.
8. When appropriate, baseline serum samples or other surveillance samples are collected and stored for all personnel working in or supporting the work area.
9. A biosafety manual is available which details required safety practices and procedures, spill clean-up, handling of accidents, and other appropriate safety information.
10. The use of sharps is avoided. If required, additional safety devices or personal protective equipment are used to prevent accidental exposure. Plasticware is substituted for glassware whenever possible. If glassware is used, it is coated or shielded to minimize the potential for breakage.
11. Viable organisms are placed in a container that prevents leakage during collection, handling, processing, and transport.
12. Viable organisms are handled in a closed system or other primary containment equipment which prevents their release into the environment.
13. Sample collection and material addition to a closed system, and transfer of culture materials from one closed system to another are conducted in a manner which prevents employee exposure and the release of material from the closed system.
14. Culture fluids shall not be removed from a closed system (except as allowed in #13) unless the viable organisms have been inactivated by a validated procedure, or the organism itself is the desired product.
15. Exhaust gases removed from a closed system or other primary containment system are filtered or otherwise treated to prevent the release of viable organisms to the environment.
16. A closed system that has contained viable organisms will not be opened for maintenance or other purposes unless it has been decontaminated.
17. Rotating seals and other mechanical devices directly associated with a closed system used for the propagation of viable organisms are designed to prevent leakage or are fully enclosed in ventilated housings that are exhausted through filters or otherwise treated to prevent the release of viable organisms to the environment.
18. Closed systems used for the propagation of viable organisms and other primary containment equipment are tested for the integrity of the containment features prior to use, and following any changes/modifications to the system that could affect the containment characteristics of the equipment. These systems are equipped with a sensing device which monitors the integrity of the containment while in use. Containment

equipment for which the integrity cannot be verified or monitored during use, are enclosed in ventilated housings that are exhausted through filters or otherwise treated to prevent the release of viable organisms.

19. Closed systems that are used for propagation of viable organisms or other primary containment equipment are permanently identified. This identifier is used on all records regarding validation, testing, operation, and maintenance.
20. Contaminated equipment and work surfaces are decontaminated with a suitable disinfectant on a routine basis, after spill cleanup, etc. Contaminated equipment is decontaminated prior to servicing or transport. Absorbent toweling / coverings can be used on work surfaces to collect droplets and minimize aerosols and are discarded after use.
21. Individuals seek medical attention immediately after an exposure incident. Spills and accidents that result in overt exposure to infectious materials are immediately reported to the facility supervisor / manager and the BSO. Appropriate medical treatment, medical evaluation, and surveillance are provided, and written records maintained.
22. Emergency procedures includes provisions for decontamination and clean-up of all spills/releases of viable material, including proper use of personnel protective equipment..
23. Animals not involved in the work being performed, are not permitted in the work area.

C. Safety Equipment

1. Protective clothing, e.g.. lab coats, protective coveralls, etc., is worn to prevent contamination of personal clothing. If the organism can be transmitted through the skin, the protective clothing should be waterproof with a solid-front, e.g.. wrap-around , or back- or side-tie coats. Protective clothing is removed when leaving the work area.
2. Protective eyewear is worn at all times in the work area. Protective face protection, i.e., face shield or goggles and face mask / respirator are worn for any procedures that may involve splashing or spraying. Respirators are worn if the agents involved are respiratory transmissible.
3. Impervious gloves are worn at all times in the work area when work is ongoing. Double gloving and/or the use of latex gloves is considered if personnel are working over extended periods of time, or with processes that may require direct contact with the infectious material. Gloves are discarded upon leaving the work area.
4. The selection of a respirator / face mask is made based on the transmissibility of the agent. If the agent is transmitted through the respiratory route, a respirator with a filtration efficiency capable of protecting the individual from the organism is used, e.g., HEPA for viruses, N95s for Mycobacteria tuberculosis, etc. If the agent is transmitted

through mucous membrane contact, a face mask which prevents droplet penetration, e.g., plastic molded, is preferred. Personnel are trained in the use of respirators / face masks for procedures that may involve aerosol generation, and for emergency situations that involve the release of viable organisms in the work area.

5. Biological safety cabinets or other ventilated containment devices are used to contain processes of viable organisms if removed from a closed system.
6. Only centrifuge units with sealed rotor heads or safety cups that can be opened in a biological safety cabinet are used; or the centrifuge is placed in a containment device.

D. Facilities

1. Each facility contains a sink for handwashing, an eyewash station, and an emergency shower. The sink is foot, elbow, automatic, etc., or otherwise not hand operated, and located near the door of each room in the work area.
2. The work area has a door which can be closed when large scale work is ongoing.
3. The work area is designed to be easily cleaned and disinfected. Furniture and stationary equipment are sealed to the floor or raised to allow for cleaning and disinfection of the facility.
4. Floors, walls, and ceilings are made of materials that allow for cleaning and disinfection of all surfaces. Light fixtures are covered with a cleanable surface.
5. Work surfaces are impervious to water and resistant to acids, alkali, organic solvents, and moderate heat.
6. Windows to the facility are kept closed and sealed while work is ongoing.
7. General laboratory-type work areas are designed to have a minimum of 6 air changes per hour. For large scale facilities, the number of air changes per hour will depend on the size of the area, the chemicals and agents handled, the procedures and equipment utilized, and the microbial / particulate requirements for the area.
8. The ventilation in the work area is designed to maximize the air exchange in the area,, i.e., the supply and exhaust are placed at opposite ends of the room, ceiling supply with low level exhaust, etc.
9. The work areas in the facility where the infectious organisms are handled is at negative pressure to the surrounding areas.
10. Provisions are made to contain large spills of viable organisms within the facility until appropriately decontaminated. This can be accomplished by placing the equipment in a diked area, or sloping or lowering the floors in those areas to allow for sufficient capacity to contain the viable material and disinfectant.

11. Drainage from the facility is designed to prevent the release of large volumes of viable material directly to sewer, e.g., floor drains is capped, raised, or fitted with liquid tight gaskets to prevent release of untreated organisms to sewer.

IV. Large Scale Biosafety Level 3 (BSL3-LS)

BSL3-LS is recommended for the propagation and cultivation of infectious organisms classified as Risk Group 3.

There is no specific volume which constitutes "large scale" for infectious agents, since the risks are more dependent on the agent, the procedures being used, and the frequency of activity, as opposed to the volume of the organism being handled. That is why it is recommended that work involving the cultivation or handling of Risk Group 3 organisms, beyond the amounts usually used for the identification or characterization, be done under the direction/auspices of a Biological Safety Officer who can take into account the infectivity of the agent, the routes of exposure, the level of containment afforded by the process and equipment used, the severity of infection, availability of prophylaxis, etc.

The following guidelines have been developed for facilities that routinely handle large volumes of these materials.

A. Standard Microbiological Practices

1. Access to the facility is restricted to personnel who meet the entry requirements. Individuals who have not been trained in the operating and emergency procedures of the facility, are accompanied by trained personnel at all times while in the facility.
2. Persons wash their hands after they handle viable materials, after removing gloves and before leaving the work area.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work area.
4. Food is stored outside of the work area in cabinets or refrigerators designated and used for this purpose only.
5. Mouth pipetting is prohibited. Only mechanical pipetting devices are used.
6. Work surfaces are decontaminated on a routine basis and after any spill of viable material.
7. Procedures are performed carefully in a manner which minimizes aerosol generation.
8. All contaminated wastes are decontaminated by an approved method prior to disposal in accordance with local, state, and federal regulations. Wastes that need to be transported to a different area or facility, are closed and placed in a durable, leakproof container for

transfer. Material to be transferred off site for decontamination is packaged and labeled in accordance with the applicable regulations.

9. All discharges of the viable materials are inactivated by a validated process, i.e. one that has been demonstrated to be effective using the organism in question, or with an indicator organism which is known to be more resistant to the physical or chemical methods used; e.g. *Bacillus stearothermophilus* for steam heat.
10. An insect and rodent control program is in effect.

B. Special Practices

1. Institutions that engage in large scale work must have a health and safety program for their employees.
2. Doors to the facility is kept closed except for entry and egress
3. Access to the facility is restricted to personnel whose presence is required and who meet entry requirements, i.e., immunization, if any, and comply with all entry and exit procedures. Individuals who can not take / do not respond to the vaccine, who cannot take the recommended prophylaxis in the event of an exposure incident, who are at increased risk of infection, or for whom infection may prove unusually hazardous, are not allowed in the work area until their situation has been reviewed by appropriate medical personnel. The individuals are informed of the potential risks and sign an acknowledgement / consent form, or similar vehicle, which indicates that they understand and accept the potential risk.
4. Written procedures and policies for handling infectious materials are provided.
5. All personnel working at a BSL3-LS must demonstrate proficiency in standard microbiological practices and techniques, and in handling human pathogens at a Biosafety Level 3. This can consist of previous experience and/or training program. Training in the hazards associated with the materials involved, and the specific practices and operations specific to the facility are provided and documented.
6. Appropriate immunizations, medical evaluation surveillance, and treatment are provided where indicated; e.g., immunization, survey of immune status, etc.
7. A hazard warning sign, incorporating the universal biohazard symbol, identifying the infectious agents, listing the name and telephone numbers of the persons knowledgeable about and responsible for the facility, along with any special entry requirements for entering the work area, is posted at the entry to the facility.
8. Baseline serum samples and / or other appropriate specimens are collected and stored for all personnel working in or supporting the facility. Additional specimens may be collected periodically depending on the agents handled

9. A biosafety manual is available which details required safety practices and procedures, spill clean-up, handling of accidents, and other appropriate safety information.
10. The use of sharps is avoided. If required, additional safety devices or personal protective equipment is used to prevent accidental exposure. Plasticware is substituted for glassware whenever possible. If glassware is used, it is coated or shielded to minimize the potential for breakage.
11. Viable organisms are placed in a container that prevents leakage during collection, handling, processing , and transport.
12. Viable organisms are handled in a closed system or other primary containment equipment which prevents their release into the environment.
13. Sample collection and material addition to a closed system, and transfer of culture materials from one closed system to another are conducted in a manner which prevents employee exposure and the release of material from the closed system.
14. Culture fluids are not be removed from a closed system (except as allowed in #14) unless the viable organisms have been inactivated by a validated procedure.
15. Exhaust gases removed from a closed system or other primary containment system are filtered or otherwise treated to prevent the release of viable organisms to the environment.
16. A closed system that has contained viable organisms will not be opened for maintenance or other purposes unless it has been decontaminated.
17. Rotating seals and other mechanical devices directly associated with a closed system used for the propagation of viable organisms are designed to prevent leakage or are fully enclosed in ventilated housings that are exhausted through filters or otherwise treated to prevent the release of viable organisms..
18. Closed systems used for the propagation of viable organisms and other primary containment equipment are tested for the integrity of the containment features prior to use, and following any changes/modifications to the system that could affect the containment characteristics of the equipment. These systems are equipped with a sensing device which monitors the integrity of the containment while in use. Containment equipment for which the integrity cannot be verified or monitored during use, are enclosed in ventilated housings that are exhausted through filters or otherwise treated to prevent the release of viable organisms.
19. Closed systems that are used for propagation of viable organisms or other primary containment equipment is permanently identified. This identifier is used on all records regarding validation, testing, operation, and maintenance.

20. Contaminated equipment and work surfaces are decontaminated with a suitable disinfectant on a routine basis, after spill cleanup, etc. Contaminated equipment is decontaminated prior to servicing or transport. Absorbent toweling / coverings can be used on work surfaces to collect droplets and minimize aerosols, however, they should be discarded after use.
21. Individuals seek medical attention immediately after an exposure incident. Spills and accidents that result in overt exposure to infectious materials are immediately reported to the facility supervisor / manager and the BSO. Appropriate medical treatment, medical evaluation, and surveillance are provided, and written records maintained.
22. Emergency procedures include provisions for decontamination and clean-up of all spills/releases of viable material, including proper use of personnel protective equipment.
23. Animals not involved in the work being performed, are not permitted in the work area.

C. Safety Equipment

1. Persons entering the facility will exchange or completely cover their clothing with garments such as solid-front or wrap-around gowns, coveralls, etc. If the organism can be transmitted through the skin, the protective clothing must be waterproof. Head and shoe covers, or captive shoes are provided. Protective clothing is to be removed when leaving the facility.
2. Protective eyewear is worn at all times in the work area. Protective face protection, i.e., face shield or goggles and face mask / respirator are worn for any procedures that may involve splashing or spraying. Respirators are worn if the agents involved are respiratory transmissible.
3. Impervious gloves are worn at all times in the work area when work is ongoing. Double gloving and/or the use of latex gloves is considered if personnel are working over extended periods of time, or with processes that may require direct contact with the infectious material. Gloves are discarded upon leaving the work area.
4. The selection of a respirator / face mask is made based on the transmissibility of the agent. If the agent is transmitted through the respiratory route, a respirator with a filtration efficiency capable of protecting the individual from the organism is used, e.g., HEPA for viruses, N95s for Mycobacteria tuberculosis, etc. If the agent is transmitted through mucous membrane contact, a face mask which prevents droplet penetration, e.g., plastic molded, is preferred. Personnel are trained in the use of respirators / face masks for procedures that may involve aerosol generation, and for emergency situations that involve the release of viable organisms in the work area.
5. Biological safety cabinets Class II or Class III, or other ventilated containment devices are used to contain processes of viable materials if removed from a closed system.

6. Only centrifuge units with sealed rotor heads or safety cups that can be opened in a biological safety cabinet are used; or the centrifuge are placed in a containment device.
7. Continuous flow centrifuges or other aerosol generating equipment are contained in devices that are exhausted through filters or otherwise treated to prevent the release of viable organisms.
8. Vacuum lines are protected with liquid disinfection traps and HEPA filters or equivalent, which are routinely maintained and replaced as needed.

D. Facilities

1. The facility is separated from areas which are open to unrestricted traffic flow within the building. The entry area to the facility consists of a double doored entry area, such as an airlock or pass-through.
2. Each major work area contains a sink for handwashing, which is not hand operated, e.g. automatic, foot, or elbow operated.
3. An eyewash station and emergency shower is available in the facility.
4. The facility is designed to be easily cleaned and disinfected. Furniture and stationary equipment is sealed to the floor, raised, or placed on wheels to allow for cleaning and disinfecting of the facility.
5. Work surfaces are impervious to water and resistant to acids, alkali, organic solvents, and moderate heat.
6. Floors, walls, and ceilings are made of materials that allow for cleaning and disinfection of all surfaces. Light fixtures are sealed, or recessed and covered with a cleanable surface..
7. Penetrations into the containment facility are kept to a minimum and sealed to maintain the integrity of the facility.
8. Windows to the facility are kept closed and sealed.
9. Liquid and gas services to the facility are protected from backflow unless they are dedicated to the facility. Fire protection sprinkler systems do not require backflow preventors.
10. The ventilation system for the facility is designed to control air movement;
 - The position of the supply and exhaust vents is designed to maximize the air exchange in the area, i.e., the supply and exhaust is placed at opposite ends of the room, ceiling supply with low level exhaust, etc.

- General laboratory-type work areas are designed to have a minimum of 6 air changes per hour. For large scale facilities, the number of air changes per hour will depend on the size of the area, the chemicals and agents handled, the procedures and equipment utilized, and the microbial / particulate requirements for the area.
 - The facility is at negative air pressure to the surrounding areas or corridors. The system shall create directional airflow that draws air from the "clean" areas of the facility into the "contaminated" areas. If there are multiple contaminated areas, the area of highest potential contamination is the most negative.
 - The exhaust air from the facility is not recirculated to any other area in the facility, and is discharged to the outside through HEPA filters or other treatments which prevent the release of viable microorganisms.
 - The facility has a dedicated air supply system for the facility. If the supply system is not dedicated to the facility, it contains HEPA filters or appropriate dampers, which can protect the system from potential backflow in the event of a system failure..
 - The supply and exhaust systems for the facility are interlocked to prevent the room pressure from going positive in the event of power or equipment failure. The system is alarmed to indicate system failures or changes in desired air flow.
11. A method for decontaminating all wastes is available in the facility i.e., autoclave, chemical disinfection, incineration, or other approved method.
12. Provisions are made to contain large spills of viable organisms within the facility until appropriately decontaminated. This can be accomplished by placing the equipment in a diked area, or sloping or lowering the floors in those areas to allow for sufficient capacity to contain the viable organisms and disinfectant.
13. Drainage from the facility is designed to prevent the release of large volumes of viable organisms directly to sewer, e.g., floor drains are capped, raised, or fitted with liquid tight gaskets to prevent release of untreated organisms to sewer.