Chapter 4 – WORKING SAFELY WITH BIOHAZARDOUS MATERIALS

This chapter contains guidance on working safely with biohazardous materials. Emphasis is placed on integrating biosafety practices and procedures with standard laboratory operations. Classification of biosafety levels is included in this chapter.

4.1 Basic Biosafety Practices

Persons working with biohazardous materials must be aware of potential hazards and must be trained and proficient in specific safety practices and techniques. The following biosafety practices are fundamental in using biohazardous materials safely:

- **Identify and evaluate biohazardous material exposure risk.**
  Research laboratory procedures or techniques must be designed to safely handle biohazardous material and anticipate the worst-case exposure potential. Knowing how infectious organisms are transmitted (aerosol, ingestion, dermal, injection), organism infectious dose, and types of laboratory activities (centrifuging, pipetting, shaking, vortexing, sonicating, opening containers of infectious materials, necropsy, etc.) can help to evaluate potential risks and avoid infection. Information about the organism(s) and activities must be gathered prior to commencing work. Refer to Sections 2.4 and 4.3.3. If human blood, tissue or cell lines are part of your research activities, follow the template in Appendix F to develop your specific laboratory exposure control plan (refer to 4.3.5).

- **Attend required EHS biosafety training as well as complete lab specific training with PI.**
  Proficiency in laboratory practices (training provided by PI). Refer to Section 2.2, Appendix O.

- **Know where information resources for biohazardous materials can be found.**
  Collect and communicate all the facts and information resources for biohazardous materials to appropriate personnel to minimize exposure risk. Refer to Chapter 2 and the Appendix O of this manual**.**

- **Make sure all biohazard signs and labels are present.**
  Post appropriate biohazard signs and labels to assure only authorized personnel, informed of potential risks, enter areas where biohazardous material are used. Any equipment where biohazardous materials are used or stored must be labeled. Refer to Section 4.3.8 of this manual.

- **Utilize appropriate safety equipment and facility design for the Biosafety level.**
  Primary containment safety equipment, such as biological safety cabinets, is designed to reduce or eliminate exposure to biohazardous materials. Secondary containment facility design is intended to contain biohazardous materials in the laboratory so that they cannot cause harm to the general public or the environment. Refer to Sections 4.2.7 and 4.3.7 of this manual.

- **Maintain good housekeeping and personal hygiene.**
  Good housekeeping is the most important step to improve safety. Good housekeeping also leaves a good impression upon visitors. Floors, laboratory benches, equipment, and other surfaces should be disinfected routinely. All biohazardous material waste should be autoclaved, sterilized, or placed in a biohazard Unwanted Materials container for disposal. Refer to Appendix G of this manual. Personal hygiene, such as frequent hand and laboratory clothes washing, should be observed at all times. Refer to Section 4.3.1 and Appendices E and F of this manual.

4.2 Biosafety Principles and Concepts

The purpose of this section is to provide definitions of biosafety concepts.

4.2.1 Pathogenicity or Virulence

Pathogenicity or virulence is the ability of a biohazardous material to produce or develop a rapid, severe, or deadly disease. Some materials are highly pathogenic, even in healthy adults, whereas others are opportunistic pathogens able to infect only hosts with lowered immunity or sites other than their normal habitat. Some biohazardous materials are attenuated, or weakened, and do not produce significant disease. The more severe the health consequences of a potentially acquired disease, the higher the risks.

4.2.2 Routes of Entry

An infection occurs when pathogenic microorganisms enter the human body in sufficient numbers and by a particular route which overcomes the body's defense system. By understanding the mode of transmission (pathway from source to you) and route of entry (entry route into body), procedures or controls to prevent exposure and infection can be developed.

Inhalation hazards: Inhalation of aerosolized biohazardous materials is the most common route of entry into the body. Inhalation of aerosols involves microscopic solid or liquid particles small enough to remain dispersed and suspended in air for long periods of time. Sources of aerosols include:

- Aerosolized solid material (spores, dust, particulate, etc.).
- Liquid material (mists and sprays, coughing, spittle, sputum, etc.).
- Technical process (blending, grinding, sonicating, lyophilizing, sawing, centrifuging, etc).

Ingestion hazards: Ingestion of biohazardous materials occurs frequently as the result of poor personal hygiene and poor laboratory practice. Proper hand washing minimizes the opportunity for mouth and eye exposures. Examples of how ingestion occurs include:

- Eating, drinking, and smoking in laboratory
- Mouth pipetting and suction techniques
- Transfer of microbes to mouth by contaminated fingers or articles

Direct (Skin/Eye) Contact hazards: Direct contact to biohazardous materials occurs through cross-contamination and mucous membrane exposure including the skin, eyes, inside of the mouth, nose, and the genitals. The main avenues by which biohazardous materials enter the body through the skin are hair follicles, sebaceous glands, sweat glands, and cuts or abrasions. Examples of how direct contact occurs include:

- Splash or spray of biohazardous material onto skin, eye, mouth, or nose
- Handling contaminated equipment with unprotected non-intact skin
- Transfer or rubbing by contaminated fingers or gloved hand
- Applying cosmetics or contact lens in laboratory

Injection or inoculation hazards: Inoculation or injection occurs when biohazardous material is accidentally introduced into the body with contaminated objects through the intact skin barrier. Inadequate control of sharp instruments and infected animals or arthropod vectors usually results in accidental inoculation or injection. Examples of injection and inoculation hazards include:

- Inoculation with a hypodermic needle, broken glassware, scalpels, or other sharp instruments
- Sharps injuries (needle sticks, glass pipettes, syringes, etc.)
- Animal bites, scratches, kicks, abrasions, punctures

4.2.3 Agent Stability or Viability

Stability and viability refer to the ability of a biohazardous material to retain its biohazardous characteristics such as aerosol infectivity and survival time in environment. Factors such as temperature, humidity, pH, oxygen, sunlight or ultraviolet light, chemical disinfectants, growth factors (food reservoir or media), and competition with endemic organisms must be considered.

4.2.4 Infectious Dose

The infectious dose is the number of microorganisms required to initiate an infection. This dose can range from one to hundreds of thousands of units depending on agent, exposure route, virulence, and host immune status or susceptibility for the disease.

4.2.5 Concentration (Amount of Agent)

Concentration is the number of infectious organisms per unit volume. As the viable agent concentration and volume increases, the risk potential gets higher. The media/reservoir, laboratory activity, volume (especially >10 liters) need to be considered in risk determination.

4.2.6 Immune Status

Immune status is the current condition of a living organism to resist and overcome infection or disease. The primary function of the immune system is to protect the body from foreign substances by an acquired ability to distinguish self from non-self. Host susceptibility or immune status helps determine the level of risk of acquiring a disease upon exposure. CDC and NIH guidelines presume a population of immuno-competent individuals.

4.2.7 Laboratory Biosafety Level Criteria

There are four recommended laboratory biosafety levels. The biosafety levels consist of laboratory practices, safety equipment, and facilities combinations which are specifically appropriate for the operations performed, suspected routes of biohazardous material transmission, and laboratory function or activity.

- Biosafety Level 1 (BSL-1)
  - Suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment.
  - Special containment equipment or facility design is neither required nor generally used.
  - Laboratory personnel have specific training in the procedures conducted in the laboratory.
  - Supervision by a scientist with general training in microbiology or a related science.

- Biosafety Level 2 (BSL-2)
  - Suitable for work involving agents of moderate potential hazard to laboratory personnel and the environment.
  - Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists.
  - Access to the laboratory is limited when work is being conducted.
  - Extreme precautions are taken with contaminated sharp items.

- Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

- Biosafety Level 3 (BSL-3)
  - Clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route.
  - All procedures involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment.
  - The laboratory has specific engineering and design features.
  - Laboratory personnel have specific training in handling pathogenic and potentially lethal agents.
  - Supervision by a competent scientist who is experienced in working with these agents.

- Biosafety Level 4 (BSL-4)
  - Work with dangerous and exotic agents that pose a high individual risk of aerosol transmitted laboratory infections and life threatening disease.

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 top sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: • Limited access • Biohazard warning signs • "Sharps" precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers = Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPE: laboratory coats, gloves, and face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: • Controlled access • Decontamination of all waste • Decontamination of lab clothing before laundering • Baseline serum	Primary barriers = Class I or II BSCs or other physical containment devices used for all open manipulations of agents; PPE: protective lab clothing, gloves, and respiratory protection as needed	BSL-2 plus: • Physical separation from access corridors • Self-closing, double-door access • Exhausted air not recirculated • Negative airflow into laboratory
4 **(Not At MU)**	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 practice plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers = All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit	BSL-3 plus: • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decon systems • Other requirements outlined in the BMBL text

4.2.8 Vertebrate Animal Biosafety Level Criteria

There are four recommended vertebrate animal biosafety levels. The recommendations below describe practices, safety equipment and facilities for experiments with animals infected with agents that cause, or may cause, human infection. In general, the biosafety level recommended for working with biohazardous material *in vivo* and *in vitro* are comparable.

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

- Animal Biosafety Level 1 (ABSL-1)
  - Suitable for work involving well-characterized agents <u>not</u> known to consistently cause disease in healthy adult humans, and of <u>minimal potential hazard</u> to personnel handling the animals and the environment.

- Animal Biosafety Level 2 (ABSL-2)

- This level involves practices for work with those agents associated with human disease. It addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure. ABSL-2 builds upon the practices, procedures, containment equipment, and facility requirements of ABSL-1.
- ABLS-2 is suitable for work involving agents of moderate potential hazard to laboratory personnel, animals, and the environment.
- Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists.
- Access to the animal facility is limited to the fewest number of individuals possible. Personnel who must enter the room for program or service purposes when work is in progress are advised of the potential hazard.
- Certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

- Animal Biosafety Level 3 ABSL-3

  - ABSL-3 level involves practices suitable for work with animals infected with indigenous or exotic agents that present the potential of aerosol transmission and of causing serious or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2.
  - All procedures involving the manipulation of infected animal and infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment.
  - The animal facility has specific engineering and design features.
  - Laboratory personnel have specific training in handling infected animals with pathogenic and potentially lethal agents.

- Animal Biosafety Level 4 (ABSL-4)
  - This level involves practices suitable for addressing dangerous or exotic agents that pose high risk of life threatening disease, aerosol transmission, or related agents with unknown risk of transmission.

4.2.9 Risk Group Levels

The following are NIH requirements for Campus Research with recombinant or synthetic nucleic acid molecules in humans, animals, and plants. The investigator must make an initial risk assessment based on the Risk Group (RG) of an agent. Agents are classified into four Risk Groups (RGs) according to their relative pathogenicity for healthy adult humans by the following criteria:

- Risk Group 1 (RG1): agents are not associated with disease in healthy adults.

- Risk Group 2 (RG2): agents are associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.

- Risk Group 3 (RG3): agents are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available.

- Risk Group 4 (RG4): agents are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

4.2.10 Recombinant or Synthetic Nucleic Acid Molecules Biosafety Level Criteria

NIH Guidelines also address physical and biological containment for recombinant or synthetic nucleic acid molecule research involving humans, animals, plants and large scale use, including standard microbiological practices, special practices, containment equipment and laboratory facilities.

- Biosafety Level 1-4 (BL1 to BL4): standard research laboratory experiments.
- Biosafety Level 1-4 Large Scale (BL1-Large Scale to BL4-Large Scale): large scale (over 10 liters) research and production with Good Large Scale Practices (GLSP).
- Biosafety Level 1-4 Plants (BL1-P to BL4-P): standard plant greenhouse facility experiments.
- Biosafety Level 1-4 Animals (BL1-N to BL4-N): standard whole animal facility experiments.

4.2.11 Dual Use

Researchers must consider not only the ways in which their research findings may add to scientific knowledge but also potential misuse of their findings. In general "dual use" research refers to technologies that could benefit civilian use but also be of concern to national security. In 2011 research teams in The Netherlands and the United States worked together to engineer a highly pathogenic form of H5N1 bird flu virus which caused the scientific community as well as the U.S. National Science Advisory Board for Biosecurity (NSABB) to ask that certain portions of the collaborators research be redacted from scientific publications. The NSABB developed a short video to provide education on this topic "Dual Use Research: A Dialogue" http://oba.od.nih.gov/biosecurity/biosecurity.html. If you have additional questions concerning this topic, please contact the MU Biosafety Office.

In the life sciences, dual-use research "encompasses biological research with legitimate scientific purpose, the results of which may be misused to pose a biologic threat to public health and/or national security." Generally, the term tends to refer to technologies that have both a civilian and a military use. The dual-use research dilemma in the life sciences refers to the conundrum of producing and publishing research within the life sciences that is directed toward or intended to improve public health, animal health, or agricultural productivity, but that in the hands of a rogue state, terrorist group, or individual, could be used to impair public health. As early as the 18th century, greater understanding of the smallpox virus led to the first viral vaccine, as well as use of the virus as a bioweapon. In the wake of the anthrax attacks of 2001 and heightened concerns regarding terrorism, the more tangible possibility of bioterrorism has increased fears and concerns regarding the performance and publication of dual-use research.

Within the last 50 years alone, the scientific community has solved the structure of DNA and sequenced the entire genomes of 10 mammals (including the human, chimpanzee, mouse, rat, dog, and cat), 2 other vertebrates, 6 invertebrates, 3 protozoa, 9 plants, and 14 fungi, not to mention numerous viruses and bacteria. Only 30 years ago, one could spend years on a doctoral thesis to sequence one gene. In contrast, with current technology, the sequencing of one gene can occur in a matter of hours. In 2002, a group of researchers published its work describing the synthetic reconstruction of poliovirus, a project that took three years. The next year, the reconstruction of an equivalently sized virus took only two weeks. The exponential increase in sequencing and synthetic biology technologies reflects the increased productivity and advancement throughout the life sciences in general. The rise of biotechnology, informatics, and automation has decreased the labor required and the time to knowledge acquisition, while increasing productivity and the number and types of questions that biologists can address. Such a convergence of biology and technology increases the pace of biological findings and the creation of new fields within biology in unpredictable ways. The discoveries and

innovations that are happening today will precipitate advances over the next 30 years, and most likely even over the next 5 to 10 years—discoveries and innovations that have not even been envisioned at this time. In addition, life sciences research occurs in an increasingly interdisciplinary and international environment. As George Church, Director of the Center for Computational Genetics at the Harvard Medical Center, pointed out at the May 2006 regional meeting, "Biology has a thousand journals and the Internet allows rapid information dispersion." Just as computing and other technological innovations have created new industries and sectors toward the end of the 20th century and during the early part of the 21st, technology also has pushed the boundaries of the life sciences. Now, when research in the life sciences is considered, computational biology, systems biology, nanotechnology, and synthetic biology are at the forefront of such discussions. These fields blend biology—from whole organism biology to microbiology—with computer science, the physical sciences, engineering, and mathematics.

Although the risk that pathogens will be used for harm has been around for centuries, the emerging global, fast-paced, and collaborative nature of the life sciences now makes protecting information, personnel, and materials from abuse that much more difficult. To effectively identify dual-use research of concern, and perhaps restrict it, techniques must be available to determine what types of biological agents could stand as threats, as well as what types of mathematics, software programs, physical materials, and computational tools could enhance biological threats. The ability to understand the ways that these emerging biology applications could be used for offensive purposes poses a formidable challenge because of the unpredictable nature of science and the ways in which new technologies that come along completely alter what can be accomplished.

Despite these important advances, in contrast with other weapons, the materials and equipment required to create and propagate a biological attack using naturally occurring or genetically manipulated pathogens remain decidedly "low-tech," inexpensive, and widely available. In the case of the physical sciences and nuclear proliferation, the development of nuclear weapons R&D required equipment that was specialized and expensive. As a result, the ability to engage in research promoting nuclear proliferation was restricted to the global superpowers and other well-funded non-state entities. In addition, nuclear weapons R&D could be detected by monitoring the acquisitions of the specialized equipment needed for such programs and by other technical means. By contrast, much of the same equipment that can be used to create a dangerous biological agent is also a key part of benign biological research programs. Moreover, in the case of life sciences research, it is not just that much of the same materials and equipment can be used for illegal and benign research, but also that biological research can produce agents and knowledge that in the hands of some would promote human health and welfare, but that in the hands of others would be used for harm. This is the crux of what is called "dual-use research of concern."

"Dual-Use Research of Concern" (DURC) is defined as life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied to pose a threat to public health and safety, agricultural crops and other plants, animal, the environment, or material, or national security. When research activities with agents or potential outcome are of "dual-use" concern the MU Institutional Biosafety Committee will ask seven additional questions of the research proposing the research activities:

1. Enhance the harmful consequences of a biological agent or toxin.
2. Disrupt immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification.
3. Confer to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilities at their ability to evade detection methodologies.
4. Increase the stability, transmissibility, or the ability to disseminate the agent or toxin.
5. Alter the host range or tropism of the agent or toxin.
6. Enhance the susceptibility of a host population to the agent or toxin.
7. Generate or reconstitute an eradicated or extinct agent or toxin.

If the answer to any of the seven questions is “Yes” then the MU Institutional Biosafety Committee must consider the possibility of “dual use” research (DURC) and consider if the research is ethical. The committee will engage representatives of the National Institutes of Health in any DURC discussions.

4.3 Standard Operating Procedures

The following information represents a minimum set of guidelines for handling biohazardous material on campus. Individual administrative units, laboratories, or research groups are expected to develop more detailed procedures as appropriate. Other resources such as those listed in Section 4.3.5, Appendix B and Appendix F may be useful in developing detailed procedures. EHS is available to consult and assist during individual safe procedure development for situations not covered in this guide.

4.3.1 General Laboratory Practices

Understand and respect the safety and health hazards associated with the biohazardous materials and equipment you use, and practice the following general safety guidelines at all times:

- **Accident/injury response**. If an injury requiring emergency medical assistance occurs, call 911. If an emergency occurs in one of the hospitals, refer to the specific hospital's special number to call. See Chapter 6 for emergency response information.

- **Autoclaves.** Personnel should only operate the autoclave after receiving proper instructions on operational procedures. Loosen caps of any containers prior to autoclaving. Open only when temperature and pressure are back to normal. Any leakage or release of contaminated materials should be reported to the PI or supervisor at once. Refer to Appendix I for specific information.

- **Biohazard releases**. If a biohazardous material makes contact with the skin, wash the area with soap and water immediately. If you suspect that a biohazardous material is aerosolized, hold your breath and leave the area immediately. If emergency assistance is required, call 911. Refer to Chapter 6 for additional information. Be sure to report incident to MU Biosafety Office—will discuss reporting requirements.

- **Biological Safety Cabinets**. Biological Safety Cabinets are primary containment devices that protect the personnel, immediate laboratory, and research and teaching environment from exposure to biohazardous materials. EHS (882-7018) must be contacted before the use of any new or relocated Biological Safety Cabinet to schedule certification. Refer to Sections 2.2, 4.3.7 and Appendix L for specific information.

- **Blending, Grinding, Sonicating, Lyophilizing, Vortexing:** The greatest risk when using any of these techniques is the creation of aerosols. Blenders, grinders, sonicators, lyophilizers, etc. should be operated in a biosafety cabinet whenever possible. Safety blenders should be used. Safety blenders are designed to prevent leakage from the bottom of the blender jar and to withstand sterilization by autoclaving. They also provide a cooling jacket to avoid biological inactivation. Avoiding glass blender jars prevents breakage. If a glass jar must be used, it must be covered with a polypropylene jar to contain the glass in case of breakage. A towel moistened with disinfectant must be placed over the top of the blender while operating. This practice can be adapted to grinders and sonicators as well. Aerosols must be allowed to settle for five minutes before opening the blender jar (or grinder or sonicator container). Lyophilizer vacuum pump exhaust should be filtered through HEPA filters or vented into a biosafety cabinet. Polypropylene tubes should be used in place of glass ampoules for storing biohazardous material in liquid nitrogen. Ampoules can explode, causing eye injuries and exposure to the biohazardous material.

- **Centrifuging:** The greatest risk with centrifuging is the creation of aerosols. Sealed tubes and safety cap buckets that seal with O-rings should be used. To avoid spills from broken tubes, the tubes, O-rings and

buckets should be inspected for damage before each use. Leaks can be prevented by not overfilling centrifuge tubes. The outside of the tubes should be wiped with disinfectant after they are filled and sealed. Rotors and centrifuge tubes should be opened inside a biosafety cabinet. If a biosafety cabinet is not available, a minimum of 10 minutes settling time should be allowed before opening.

- **Children and unauthorized persons**. Children and other unauthorized persons should not be in laboratories where biohazardous materials are present.

- **Decontamination**. Work surfaces and equipment must be decontaminated immediately after using biohazardous materials. It is critical that the work area within biological safety cabinets always be cleaned and disinfected thoroughly, using a chemical disinfectant after each use.

- **Dishwashers**. Laboratory staff must remove as much organic material (culture/gels) from glassware prior to placing in the dishwasher. Organic material can clog dishwasher drains and cause the equipment to fail.

- **Disposal of biohazardous materials**. Disposal procedures are described in Chapter 5. Questions about

- **Electrical**. Access to electrical equipment (e.g., plugs, switches, and electrical panels) should be maintained at all times. Obstruction should never prevent immediate access in an emergency. Use polarized and grounded receptacle outlets in general laboratory areas and Ground Fault Circuit Interrupters (GFCIs) in wet or outdoor locations. Cords should not run in aisles or corridors, through doors, walls, partitions, under rugs, or above suspended ceilings.

- **Emergency eyewash and safety showers**. Be certain safety showers and emergency eyewash units are properly located, and maintained (reachable within 10 seconds). There should be no obstructions that might inhibit the use of this equipment.

- **Equipment**. Use proper equipment that is in good condition. Never use chipped or cracked glassware. Shield pressurized or vacuum apparatus. Label contaminated equipment.

- **Fire extinguishers**. Appropriate type fire extinguishers must be available, charged, and hung in a location that is immediately accessible (within 75 feet or as required by EHS). There should be no obstructions that might inhibit the use of this equipment. Contact Campus Facilities (882-8211) for assistance.

- **Food, drink, cosmetics and contact lens**. Eating, drinking, and the application of contact lens or cosmetics are forbidden in areas where biohazardous materials are used. Do not store food in the same refrigerator with biohazardous materials. Food used for research should be labeled, “Not for Human Consumption”.

- **Freezers and Refrigerators.** These should be checked and cleaned out periodically to remove any broken ampoules, tubes, etc. containing toxic or infectious material. Use rubber gloves during this cleaning. Label all biohazardous or toxic material stored in refrigerators or deep freezers (refer to Section 4.3.8). Discard old specimens or samples when no longer needed.
- **Glass tubing**. When inserting glass tubing into stoppers, lubricate tubing and wear leather gloves to protect hands from tubing slips and breaks.

- **Horseplay**. Practical jokes or other behaviors that might confuse, startle, or distract another worker, are forbidden when biohazardous materials are present.

- **Housekeeping**. Exits, aisles, and safety equipment should not be obstructed. A minimum of 36 inches width must be maintained for laboratory aisles. Hallways are not to be used as storage areas.

- **Inoculating Loop Sterilizing**. The greatest risk when sterilizing inoculating loops in an open flame (such as with a Bunsen burner) is the creation of aerosols, which may contain viable microorganisms, and flammable material work. A shielded electric incinerator or hot bead sterilizer should be used to minimize aerosol production. Disposable plastic loops and needles are good alternatives.

- **Open Flames in Biological Safety Cabinets**. Open flames, such as Bunsen burners, **should never** be used in biological safety cabinets (BSC). Open flames inside of a BSC disrupt the airflow, compromising protection of both the worker and the work. Open flames are extremely dangerous around flammable materials, such as ethanol, which is often found in a BSC. Electric incinerators or disposable inoculating loops can be used instead.

- **Personal hygiene**. Hands should be washed frequently, even after wearing gloves, and scrubbed vigorously with soap and water for a full 30 seconds. The physical removal of organisms from the skin is just as important as using a disinfectant. Contaminated hands should be kept away from the mouth, eyes, and non-intact skin.

- **Pipetting**. The greatest risks with pipetting are the creation of aerosols and splashing. Mouth pipetting is prohibited. Mechanical pipetting aids should be used instead. All biohazardous materials should be pipetted in a biosafety cabinet if possible. Cotton-plugged pipettes should be used. Biohazardous materials must never be forcibly discharged from pipettes. “To deliver” pipettes should be used instead of pipettes requiring blowout. To avoid splashing, biohazardous material should be dispensed from a pipette by allowing it to run down the receiving container wall. After using reusable pipettes, they should be placed horizontally in a pan filled with enough liquid disinfectant to completely cover them and the entire pan autoclaved before cleaning the pipettes for reuse. Refer to Appendix J for proper plastic pipette disposal instructions.

- **Security**. Access to biohazardous materials shall be limited to authorized personnel only. The control of biological hazards shall be maintained by securing and locking the laboratory when unattended by authorized personnel and during all off-hours. During transportation between laboratories, biohazardous materials must be properly packaged and must not be left unattended or unsecured. Refer to Appendix M for specific information.

- **Sharps, Needles, and Syringes.** The greatest risks when using sharps are accidental injection and the creation of aerosols. Needles and syringes should only be used when there is no reasonable alternative. Safety needles and syringes should be used in these instances. The sharp should be kept away from the fingers as much as possible. Sharps should never be bent, sheared, recapped, nor have needles removed from syringes after use. If a contaminated needle must be recapped or removed from the syringe, a mechanical device, such as a forceps, must be used. Air bubbles should be minimized when filling a syringe. A pad moistened with disinfectant must be placed over the tip of the needle when expelling air. Work should be performed in a biosafety cabinet whenever possible. An appropriate sharps container must be kept close to the work area to avoid walking around with contaminated sharps. Care should be taken not to overfill sharps containers. They are considered full when they are 2/3 filled. Refer to Section 4.3.5, 5.2 and Appendix J for specific information.

- **Shipping.** See Chapter 7

- **Smoking**. Smoking is not allowed on the MU campus, as of July 1, 2013. http://smokefree.missouri.edu/index.php. A burning cigarette, cigar, or pipe is an ignition source to flammable solvents. The handling of chewing tobacco, cigarettes, cigars or pipes from bench to mouth is a potential route of transmission for microorganisms and toxic material. Wash hands before smoking whenever biohazardous materials have been handled.

- **Spill preparedness**. Before working with biohazardous materials, assess potential spill or release hazards. Become familiar with the general spill response procedures (refer to Chapter 6). Be sure the Laboratory Biosafety Spill Kit/Station and Emergency Notification Signage are current, available and maintained.

- **Transporting Biological Materials (on campus).** See Chapter 7

- **Unattended experiments**. Avoid unattended experiments. If biohazardous material operations are carried out with no one present, it is the responsibility of the worker to design and prevent accidental release in the event of interruption in utility services. Appropriate arrangements should be made for periodic inspection of the operation.

- **Universal Precautions.** All blood or Other Potentially Infectious Materials (OPIM) will be considered infectious regardless of the perceived status of the source individual. These precautions will be observed at the University to prevent contact with potentially infectious materials. Refer to Sections 2.2, 4.3.4, 4.3.5 and Appendix F for specific information.

- **Working alone**. When working with biohazardous materials, it is advisable to have a second person present, or at a minimum, maintain contact via telephone.

4.3.2 Personal Protective Equipment

Wearing appropriate personal protective equipment and practicing good personal hygiene will reduce exposure to biohazardous materials during routine laboratory use, and in the event of an accident.

- **Attire**. Wear a laboratory coat or appropriately similar protective clothing during active use with biohazardous materials. If shorts or skirts are worn, the lab coat must be knee length or longer. Wear closed-toed shoes (no sandals). Confine loose clothing and long hair. Nylons or pantyhose are not recommended because they may melt upon contact with acid or heat source. Hands and arms must be covered when using UV light sources.

- **Eye Protection**. It is a state law and campus policy that personnel including students, staff, and visitors wear approved safety glasses or goggles at all times where eye hazards are a possibility. Goggles are recommended when biohazardous splashes or releases are possible. Proper UV shielded safety glasses must be used with UV light sources.

  Contact lenses may be worn in the laboratory. However, they do not provide any protection for the eyes. Persons who wear contacts must use the same eye protective equipment as those who do not wear contacts.

- **Face Shields**. As a result of a risk assessment a full-face shields may be required to be worn when conducting a procedure which may result in a violent reaction, spray or splash. Full-face shields with bottom caps to protect the neck are preferred as they provide the best protection.

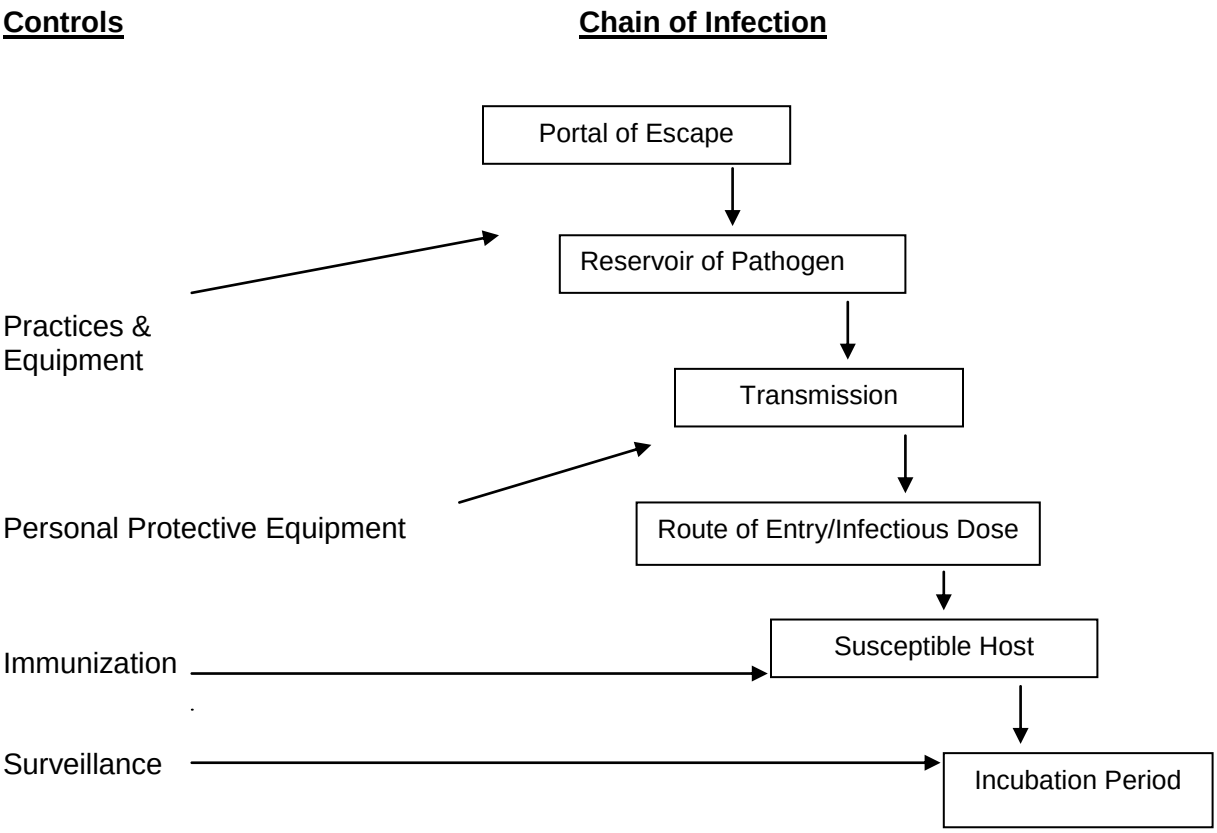
- **Gloves**. Gloves are essential when working with biohazardous materials and observing universal precautions. The proper gloves will prevent skin absorption, contamination, infection, and burns. Disposable gloves (nitrile, latex, etc.) usually provide adequate protection against exposure to biohazardous materials, but rarely provide adequate protection to hazardous materials. Consult a glove manufacturer or contact EHS for assistance in appropriate glove selection.

- **Respiratory Protection**.. If the use of a respirator is required (aerosol generating activities), you must comply with the MU Respiratory Protection Program, which includes an initial medical assessment, annual fit testing, and instructions on proper use. Contact EHS at 882-7018 for assistance.

4.3.3 Risk Assessments

The purpose of the risk assessment is to reduce potential hazards during acquisition, use, and storage of biohazardous materials. The Principal Investigator/Supervisor has responsibility for making the risk assessment and controlling the potential hazards associated with biohazardous materials. This should be done in close collaboration with the Institutional Biosafety Committee and MU Expert Microbiologist Resources available through the EHS Biological Safety Professional.

The following flow chart will help visualize the chain of infection and control options within the research laboratory. Visualize the possible chain of infection and identify proper controls to break the chain (Refer to flow chart below).



4.3.4 Bloodborne Pathogens and Universal Precautions

MU faculty, staff and students may be at risk of exposure to blood borne pathogens such as hepatitis B (HBV), hepatitis C, syphilis, malaria and human immunodeficiency virus (HIV). Universal precautions and the laboratory specific exposure control plan are measures that promote University worker protection.

All research personnel working with unfixed human blood, human tissue, or human cell lines are required to be aware of the Bloodborne Pathogens Program; an;

1. Laboratory specific Exposure Control Plan (Section 4.3.5);
2. Annual bloodborne pathogens training (Section 2.2); and
3. Enrollment of applicable staff in the MU Occupational Health and Safety Program.

The concept of "Universal Precautions" refers to the treatment of all potentially infectious blood or body fluids as if known to be infectious. These precautions are observed at MU for all human blood, blood products, certain body fluids (semen, vaginal, cerebrospinal, synovial, pleural, peritoneal, pericardial, and amniotic), body fluids with visible blood, unfixed human tissue or organ, animal blood and tissue with known zoonotic disease or unknown sources. This laboratory infection control method will help prevent laboratory-associated infections and spread of communicable disease.

The simple "**ABCDE**'s" of laboratory infection control are:

- **A**void contact with potentially infectious materials (Protective gloves, glasses, lab coat & HEPA respirator).
- **B**e prepared with the proper supplies and equipment (Biosafety spill kits, safety equipment, etc.).
- **C**lean and sanitize contaminated surfaces with a proper disinfectant.
- **D**ispose or treat biohazardous waste, sharps, contaminated clothing, etc. properly.
- **E**very time, remember to wash your hands well and no eating, drinking, smoking, etc. in laboratory.

Note: Hepatitis B Vaccine – complete protection against hepatitis B requires administration of 3 intramuscular injections at 0, 1, and 6 months. Contact Occupational Health at 882-9957 for more information.

4.3.5 Laboratory Specific Exposure Control Plan

Each Principal Investigator and Supervisor should review and prepare a specific Exposure Control Plan for their laboratory. The Laboratory Specific Exposure Control Plan is a reference and guide for all personnel who may be exposed to biohazardous materials. A template is presented in Appendix F. Completed Laboratory Specific Exposure Control Plan shall be forwarded to the EHS Office (Biosafety Professional). The Laboratory Specific Exposure Control Plan elements listed below are used to reduce exposure to blood borne pathogens, biohazardous materials and lab-acquired infections.

- Review work assignments to determine employee potential for exposure to lab-acquired infections.
- Identify responsibilities of employees covered by the Exposure Control Plan
- Universal precautions and specific measures on how to minimize the risk of exposure
- Engineering Controls - biosafety cabinet, centrifuge safety cups, sharps containers, etc.
- Work practices - hand washing, personal hygiene, labeling, sharps handling, etc.
- Personal Protective Equipment (PPE) - gloves, lab coat, safety glasses, HEPA mask, etc.
- Housekeeping - cleaning, decontamination and removal of unwanted materials.
- Laboratory Specific Emergency Plan should there be an exposure or release.
- Whether there is a need for Hepatitis B vaccine.
- Exposure Incident Reporting and Recordkeeping.
- Training - Initial and Refreshers.

4.3.6 Handling and Storage of Biohazardous Materials

Hazards associated with biohazardous materials vary widely. Understanding the hazards associated with a biohazard and reducing the quantity used and stored in the lab will decrease the chance of injury.

- **Containers.** Verify the integrity of all containers**.** If deteriorated containers are found, dispose of the biohazard promptly or transfer it to a properly labeled new container. Make sure that the container is appropriate for the biohazard being stored. Example: some biohazards are stored as a liquid and if frozen, the container needs to adequately contain the expansion of the liquid and not leak after thawing.

- **Transferring.** Provide completely sealed secondary containment for all biohazardous material when transferring between work areas. This will prevent release and aerosolizing of the biohazard in the event of an accident.

- **Inventory.** Inventories should be reviewed on a regular basis to identify deteriorating biohazardous materials before problems develop with the material or containment. Avoid excess purchases, growth or stockpiling of biohazardous material. Having up-to-date inventories improves emergency response capability, helps EHS and IBC with activities such as biohazard waste determinations and safety reviews, and allows principal investigator or supervisor to maintain accountability and security of biohazardous material within laboratory.

    - Registered Users are required to submit an annual surveyto EHS of biohazardous material for recombinant or synthetic nucleic acid molecule research activities, Biosafety Level 2 or 3 research activities, Select Agents, High Consequence Livestock Pathogens or Toxins, and others as determined by regulatory requirements.
    - Registered Users are encouraged to maintain an inventory of all other biohazardous materials.

- **Labels**. Make sure all labels are legible. Label all containers of biohazardous materials with the complete name, date, origin (human, animal or plant source) and rDNA information, if applicable. Refer to Section 4.3.8 for specific information.

- **Storage**. Avoid storing biohazardous material containers in hard to reach areas. Containers larger than one gallon should not be stored above shoulder height. Biohazardous materials should be segregated by classification and stored alphabetically. Laboratories with large numbers of biohazard classifications may choose to further segregate these hazards. Biosafety Cabinets are not designed for the storage of chemicals or biohazardous materials.

4.3.7 Biological Safety Cabinets and Other Engineering Controls

Biosafety cabinets and other engineering controls should be inspected annually for proper operation by a qualified person from EHS, or approved outside contractor. A written report of the results must be kept on record by the unit in charge of the laboratory. In most cases the Department or PI is responsible for the cost of maintenance and annual certification for biosafety cabinets. Biosafety cabinets are not designed to control chemical exposures. If you wish to use a hazardous chemical in a biological safety cabinet, contact the EHS Biological Safety Professional (882-7018) to assess potential hazards and assist in developing safe procedures.

Laboratory fume hoods are currently evaluated annually at no charge to the user. Campus Facilities Work Order Desk at 882-8211 should be contacted for the maintenance of laboratory fume hoods and other engineering controls.

Engineering controls, protective equipment, biosafety cabinets and laboratory fume hoods should be checked periodically by the Registered User to ensure that the equipment is functioning properly. EHS will assist upon request. Any questions or requests for assistance in evaluation of biosafety cabinets, laboratory hoods, or other protective equipment maybe directed to EHS (882-7018) or Campus Facilities (882-8211). Refer to Appendix L "Biological Safety Cabinet Summary Chart".

4.3.7.1 What are Biological Safety Cabinets and Laminar Flow "Clean Benches"?

Biological Safety Cabinets have been divided into three classes (Class I, II, and III) based on primary containment capability, design, and cleanliness. Class I and II cabinets are partial containment devices with an air barrier between the operator and biohazard work area. Class III cabinets are "absolute" containment devices with a physical barrier between the operator and biohazard work area.

Laminar Flow "Clean Benches" are not primary or secondary containment devices. They provide the horizontal or vertical positive pressure flow air environment for product protection only. The horizontal flow clean benches are used in clinical, pharmaceutical, and laboratory facilities without toxic, infectious, radioactive, or sensitizing materials. The vertical flow clean benches are useful for certain manipulations of clean materials (e.g. pouring agar plates, etc.) but must not be used for personal protection. All Laminar Flow "Clean Benches" must have a "Product Protection Only" label on the front of the bench. These labels are available by contacting EHS (882-7018) which recommends Laminar Flow "Clean Benches" be certified annually.

Note: All Biosafety Cabinets and Laminar Flow (Clean benches) that use an Ultraviolet (UV) lighting source are required to have a visible warning label that states a UV light source is in the cabinet and should not be on while occupants are in the laboratory. Refer to Section 4.3.8.2 for specific information.

4.3.7.2 Biological Safety Cabinet Certifications

All Biological Safety Cabinets are required to have an initial or annual (re)certification, if the cabinet is used for primary containment. Prior to the annual recertification all materials within the biological safety cabinet must be removed and the interior work surface and side and back walls of the cabinet must be decontaminated with a disinfectant that will effective inactivate agents in use in the laboratory. MU Biosafety staff recommend using a 10% bleach solution (spray and wipedown), then a water solution (to remove salts left by the bleach solution—spray and wipedown), then a 70% ethanol solution (spray and allow to gas off).

Prior to any move or repair of a previously used biological safety cabinet, a member of the MU Biosafety Staff must be contacted. Biosafety Staff will discuss research activities and possible concerns related to the move or repairs planned. A gas decontamination of the biological safety cabinet may be necessary prior to the move or repair. Contact MU EHS Biosafety Staff at 882-7018. It is also recommended that all Laminar Flow "Clean Benches" be certified annually to assure product protection capability.

Annual certifications cover, but are not limited to, the following inspection areas: Down Flow Velocity and Volume; Inflow Velocity (Face Velocity); Airflow Smoke Test; HEPA Filter Leak Test; Electrical Leakage; Ground Circuit Resistance and Polarity; Lighting Intensity; Cabinet Leak Test; Vibration, and Noise Level; and Record of Field Certification. After successful inspection completion, certification labels will be placed on the Biological Safety Cabinet or Laminar Flow "Clean Benches".

EHS administers all Biological Safety Cabinet and Laminar Flow "Clean Benches" inspections and certifications. EHS contracts with an NSF accredited Biological Safety Cabinet Certification Vendor. Each MU Investigator has the option to use the EHS contractors or contract separately with another accredited certification contractor of their choice. The current contractor listing and rates are available from the Biological Safety Professional (882-7018).

4.3.7.3 Safety Procedures for Biological Safety Cabinets

- The cabinet must be on at least 5 minutes before starting biohazard work.
- The researcher mustwear a closed-front lab coat (or surgical gown) and gloves.
- The gloves should overlap the lab coat or surgical gown cuffs.
- All handling materials should be placed in the cabinet before initiating biohazard work, to minimize in-and-out motions.
- Do not cover or obstruct the air intake grill.
- All biohazard work should be at least four inches in front of the cabinet's front grill.
- When a biological safety cabinet is in use, the lab entry door must be kept closed and traffic minimized.
- Do not use electric fans in the room when the biological safety cabinet is operating - this will seriously affect airflow.
- Develop unwanted materials collection and decontamination procedures within the biological safety cabinet to avoid clutter and minimize in-and-out motions
- Decontaminate the cabinet with an appropriate disinfectant at the end of each operation.

4.3.7.4 Other Engineering Controls

- Laboratory Hood (Hazardous and Radioactive Material Use) and Canopy Hood
- Glove Boxes, Autoclaves, Incinerators, Sharps Container, UV Fluorescent Lamps
- Centrifuge w/ O-rings Sealed Lids, Sealed Tubes & Safety Cap Buckets
- Safety Blenders (designed to prevent jar bottom leakage/withstand sterilization by autoclaving)
- Lyophilizer Vacuum Pump Exhaust w/ HEPA filters, Enclosed Incubators and Fermentors
- Open Bench Top Sink and Separate HEPA Filter Ventilation System
- Negative Pressure Rooms, Sealed Floors, Walls, Windows, Ceilings and Doors
- Airlocks or Liquid Disinfectant Barrier, Change/Shower Rooms
- Mechanical Animal Cage Washer, Waste Water Disinfectant System
- 24-Hour Limited Security Access, Pest/Vector-proof Design

4.3.8 Biohazard Warning Signs and Labels

Anyone entering areas where biohazardous materials are used or stored must be aware of the potential hazards. The biohazard symbol must be on all entries to biohazardous material laboratories, containers, biological safety cabinets, infectious waste containers, freezers, refrigerators and other equipment where blood and other potentially infectious materials are used or stored.

Signs - Post permanent area warning signs in a visible, legible, entry location for all laboratory occupants and visitors. Post temporary signs (less than one month) with tape on glass surfaces or on refrigerators, freezers, or entry doors. Remove all temporary signs when the hazard no longer exists. Entry door signs may be requested from EHS. Refer to Appendix B template of the MU Biohazard Warning Signs.

Note: These Biohazard Warning Signs (Appendix B) are in addition to the MU Emergency Notification Signage posting required for all laboratories on the MU Campus.

Labels - Affix labels with appropriate warnings to all biohazardous material in addition to the required area sign designation referred to above. Red or orange biohazard labels should be placed on containers, biological safety cabinets, and storage units (refrigerators, freezers, incubators, waste containers, etc.) that are used for biohazardous materials. Contaminated equipment must be labeled as well. Refer to Section 4.3.8.1 below for specific requirements.

4.3.8.1 Biohazardous Material Container Labels

All biohazardous reagents and materials must be labeled with the following information:

- Content (Name of Biohazardous Material) and Volume
- Origin (human, animal or plant source and rDNA information if applicable)
- Concentration (# organisms/volume, #viable colonies/volume, etc.)
- Dates (received, prepared, placed in service)
- "**Caution Required**" and Biohazard Symbol
- Type of Hazards (i.e. inhalation, skin contact, etc.)
- Precautions and Controls (i.e. avoid skin contact)
- Accident Instructions (i.e. wash immediately, etc.)

4.3.8.2 Ultraviolet (UV) Fluorescent Lamp Labeling Requirements

The use of UV radiation fluorescent lamps (180-400 nm wavelength) may produce acute adverse effects such as corneal injuries (welder's flash), erythema, photokeratitis, and lens cataracts. Appropriate UV lamp warning labeling must state: "CAUTION Ultraviolet Radiation - Protect Eyes and Skin". The UV lamp room warning sign must state, "CAUTION Ultraviolet Radiation - DO NOT ENTER AREA WHILE UV LAMPS OPERATING". Appropriate personal protective equipment (PPE) would include safety glasses w/ proper UV filters, and UV hand and arm covering at a minimum. All UV exposures should not exceed the ACGIH TLVs for occupational exposure to UV radiation incident upon the skin or the eye in an 8-hour period without PPE. Refer below to the UV lamp use applications of biological safety cabinets w/UV lamps, single UV lamp fixture (above lab bench), and UV lamp room:

Biological Safety Cabinets (BSC) w/ UV lamps: use in accordance with manufacturer's guidelines. Assure that "older" BSC have the UV lamp warning labeling and the labeling is legible. The principal investigator is responsible to maintain, certify, label, provide PPE and UV hazard training for all exposed to the BSC w/ UV lamps (laboratory and Campus Facilities staff).

Single UV Lamp Fixture: install and use fixtures and lamps in accordance with manufacturer recommendations and NEC guidelines. Assure that the UV lamp fixture has the UV lamp warning labeling and the labeling is legible. The principal investigator is responsible to maintain, label, provide PPE and UV hazard training for all exposed to the single UV lamp fixtures. CF supervisors are responsible to provide PPE and UV hazard training for all CF staff working on single UV lamp fixtures.

UV Lamp Room: install and use fixtures and lamps in accordance with manufacturer recommendations and NEC guidelines. Assure that the lamp fixture has the lamp warning labeling and the labeling is legible. Post the UV lamp room warning sign on all entrance doors. Verify no room occupancy and lock all entry doors prior to operating the UV lamp room. If appropriate, provide a flashing warning light outside the room, next to the warning sign, when the UV lamps are operating to prevent unauthorized entry. The principal investigator is responsible to maintain, label, provide PPE and UV hazard training for all exposed to the UV lamp room or single lamp fixtures. CF supervisors are responsible to provide PPE and UV hazard training for all CF staff working on UV lamp fixtures or rooms.

4.3.9 Special Provisions for Select Agents and Biological Toxins

In addition to the general safety guidelines mentioned above, special precautions are required when handling select agents and biological toxins, high consequence livestock pathogens or toxins, and plant pathogens with a high degree of acute toxicity. The Registered User should ensure that precautions designed to minimize risk of exposure to these substances are taken. Refer to Appendix N for listing. The following are minimum guidelines:

- Report any newly identified select agents or toxins, high consequence livestock pathogens or toxins and plant pathogens immediately to EHS.
- Quantities of select agents or toxins should be minimized, as should their concentrations such as in cultures, broths, or lyophilizing these select agents and biological toxins.
- Using work practices that block routes of exposure can prevent workplace infection. Good microbiological techniques must always be used in the laboratory.
- Each laboratory using these substances must designate an area for this purpose, sign or mark this area with an appropriate hazard warning such as a Biohazard Warning Sign. Refer to Appendix B for illustration of the specific Biohazard Warning Signs (Human, Animal and Plant).
- All laboratory workers and ancillary workers in a laboratory with an area designated for use with select agents, biological toxins, and acutely toxic chemicals must be trained in the harmful effects of these substances. This should include recognizing signs and symptoms of exposure. Training to safely handle and store these substances is required for those who use or may potentially be exposed to these materials. This training is the responsibility of the Registered User or Principal Investigator and must be done prior to the use of any of these materials.
- Laboratory workers using these select agents and biological toxins must have access to appropriate personal protective equipment (available at no expense to the worker) and must be trained to properly use this equipment.
- Detection equipment may be required if acutely toxic chemicals are used with biohazardous material.
- All unwanted chemical hazardous materials contaminating biohazardous material should be collected and disposed of promptly as outlined in Chapter 5. The designated working area must be thoroughly decontaminated and cleaned at regular intervals that are determined by the Principal Investigator. The interval may be as short as every few minutes to as long as one day depending upon the frequency of usage and the level of hazard.
- Special precautions are required to avoid release and exposure of biohazardous materials. For instance pipetting liquid biohazardous agents should always be conducted in a certified biosafety cabinet. Needles should never be recapped due to the high risk of punctures.
- Emergency response planning for biohazardous releases or spills should be prepared by the Principal Investigator and be included in the training of the laboratory workers and others in the building who may be affected. Refer to Chapter 6 for Emergency Response Information.