

# Recommended Biosafety Practices for Handling Prions and Prion-Infected Tissues

Updated May 2015

## 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

Disease (abbreviation)	Natural Host	Prion	Pathogenic PrP Isoform	Biosafety Level
Scrapie	sheep, goats and mouflon	scrapie prion	OvPrP <sup>Sc</sup>	2
Transmissible mink encephalopathy (TME)	mink	TME prion	MkPrP <sup>Sc</sup>	2
Chronic wasting disease (CWD)	mule deer, elk and white tail deer	CWD prion	MdePrP <sup>Sc</sup>	2
Bovine spongiform encephalopathy (BSE)	cattle	BSE prion	BoPrP <sup>Sc</sup>	2/3
Feline spongiform encephalopathy (FSE)	cats	FSE prion	FePrP <sup>Sc</sup>	2

Exotic ungulate encephalopathy (EUE)	nyala, greater kudu and oryx	EUE prion	UngPrP <sup>Sc</sup>	2
Kuru	humans	kuru prion	HuPrP <sup>Sc</sup>	2/3
Creutzfeldt-Jakob disease (CJD)	humans	CJD prion	HuPrP <sup>Sc</sup>	2/3
Gerstmann-Sträussler-Scheinker syndrome (GSS)	humans	GSS prion	HuPrP <sup>Sc</sup>	2/3
Fatal familial insomnia (FFI)	humans	FFI prion	HuPrP <sup>Sc</sup>	2/3

BMBL, 5th ed., 2007

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, 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 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 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 Primary Care Clinic 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 CO<sub>2</sub> in air to form carbonates that neutralize NaOH and diminish its disinfective properties. 10 N NaOH solutions do not absorb CO<sub>2</sub>, 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.

### MSU Resources

Dr. Jamie Sue Willard	Biosafety Officer	(517) 353-1877
Amber Bitters	Biosafety Industrial Hygienist	(517) 432-5262
Brian Smith	Hazardous Waste Coordinator	(517) 432-4454
Paula Guss	Occupational Health Nurse	(517) 353-9137

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Excerpt from CDC/NIH BMBL, 5<sup>th</sup> ed.  
Section III: Laboratory Biosafety Level Criteria

**BIOSAFETY LEVEL 2**

**Biosafety Level 2** builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that 1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures; 2) access to the laboratory is restricted when work is being conducted; and 3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

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

**A. Standard Microbiological Practices**

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries.

Precautions, including those listed below, must always be taken with sharp items. These include:

- a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
  - c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.

7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport:
  - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
  - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include: the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
10. An effective integrated pest management program is required. See Appendix G.
11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

## **B. Special Practices**

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.
3. Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.

7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
  - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
  - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

### **C. Safety Equipment (Primary Barriers and Personal Protective Equipment)**

1. Properly maintained BSCs (preferably Class II), other appropriate personal protective equipment, or other physical containment devices must be used whenever:
  - a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
  - b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
2. Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). Dispose of protective clothing appropriately, or deposit it for laundering by the institution. It is recommended that laboratory clothing not be taken home.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms must be handled outside the BSC or containment device. Eye and face protection must be disposed of with other contaminated laboratory waste or decontaminated before reuse. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-2 laboratory workers should:

- a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
  - b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
5. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

#### **D. Laboratory Facilities (Secondary Barriers)**

1. Laboratory doors should be self-closing and have locks in accordance with the institutional policies.
2. Laboratories must have a sink for hand washing. The sink may be manually, hands-free, or automatically operated. It should be located near the exit door.
3. The laboratory should be designed so that it can be easily cleaned and decontaminated. Carpets and rugs in laboratories are not permitted.
4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
  - a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available.
9. There are no specific requirements on ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct

(hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.

11. A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

### **BIOSAFETY LEVEL 3**

**Biosafety Level 3** is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures.

All procedures involving the manipulation of infectious materials must be conducted within BSCs, other physical containment devices, or by personnel wearing appropriate personal protective equipment. A BSL-3 laboratory has special engineering and design features.

The following standard and special safety practices, equipment, and facility requirements apply to BSL-3:

#### ***A. Standard Microbiological Practices***

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.
4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
  - a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
  - b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.

- c. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
  - d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plasticware should be substituted for glassware whenever possible.
- 6. Perform all procedures to minimize the creation of splashes and/or aerosols.
- 7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
- 8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Depending on where the decontamination will be performed, the following methods should be used prior to transport:
  - a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
  - b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
- 9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. Posted information must include the laboratory's biosafety level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with the institutional policy.
- 10. An effective integrated pest management program is required. See Appendix G.
- 11. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures. Personnel must receive annual updates or additional training when procedural or policy changes occur. Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of child-bearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

## **B. Special Practices**

- 1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
- 2. Laboratory personnel must be provided medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.

3. Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.
  - a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
  - b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.
8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided and appropriate records maintained.
9. Animals and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials must be conducted within a BSC, or other physical containment devices. No work with open vessels is conducted on the bench. When a procedure cannot be performed within a BSC, a combination of personal protective equipment and other containment devices, such as a centrifuge safety cup or sealed rotor, must be used.

### **C. Safety Equipment (Primary Barriers and Personal Protective Equipment)**

1. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
2. Protective laboratory clothing with a solid-front such as tie-back or wraparound gowns, scrub suits, or coveralls are worn by workers when in the laboratory. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated with appropriate disinfectant before being laundered. Clothing is changed when contaminated.
3. Eye and face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials. Eye and face protection must be disposed of with other contaminated laboratory waste or

decontaminated before reuse. Persons who wear contact lenses in laboratories must also wear eye protection.

4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. In addition, BSL-3 laboratory workers should:

- a. Change gloves when contaminated, integrity has been compromised, or when otherwise necessary. Wear two pairs of gloves when appropriate.
- b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
- c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

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

#### **D. Laboratory Facilities (Secondary Barriers)**

1. Laboratory doors must be self closing and have locks in accordance with the institutional policies. The laboratory must be separated from areas that are open to unrestricted traffic flow within the building. Access to the laboratory is restricted to entry by a series of two self-closing doors. A clothing change room (anteroom) may be included in the passageway between the two self-closing doors.

2. Laboratories must have a sink for hand washing. The sink must be hands-free or automatically operated. It should be located near the exit door. If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone. Additional sinks may be required as determined by the risk assessment.

3. The laboratory must be designed so that it can be easily cleaned and decontaminated. Carpets and rugs are not permitted. Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.

- a. Floors must be slip resistant, impervious to liquids, and resistant to chemicals. Consideration should be given to the installation of seamless, sealed, resilient or poured floors, with integral cove bases.
- b. Walls should be constructed to produce a sealed smooth finish that can be easily cleaned and decontaminated.
- c. Ceilings should be constructed, sealed, and finished in the same general manner as walls.

Decontamination of the entire laboratory should be considered when there has been gross contamination of the space, significant changes in laboratory usage, for major renovations, or maintenance shut downs. Selection of the appropriate materials and methods used to decontaminate the laboratory must be based on the risk assessment of the biological agents in use.

4. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment must be accessible for cleaning.

- a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.
5. All windows in the laboratory must be sealed.
6. BSCs must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, heavily traveled laboratory areas, and other possible airflow disruptions.
7. Vacuum lines must be protected with HEPA filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.
8. An eyewash station must be readily available in the laboratory.
9. A ducted air ventilation system is required. This system must provide sustained directional airflow by drawing air into the laboratory from "clean" areas toward "potentially contaminated" areas. The laboratory shall be designed such that under failure conditions the airflow will not be reversed.
  - a. Laboratory personnel must be able to verify directional air flow. A visual monitoring device which confirms directional air flow must be provided at the laboratory entry. Audible alarms should be considered to notify personnel of air flow disruption.
  - b. The laboratory exhaust air must not re-circulate to any other area of the building.
  - c. The laboratory building exhaust air should be dispersed away from occupied areas and from building air intake locations or the exhaust air must be HEPA filtered.
10. HEPA filtered exhaust air from a Class II BSC can be safely re-circulated into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or a direct (hard) connection. Provisions to assure proper safety cabinet performance and air system operation must be verified. BSCs should be certified at least annually to assure correct performance. Class III BSCs must be directly (hard) connected up through the second exhaust HEPA filter of the cabinet. Supply air must be provided in such a manner that prevents positive pressurization of the cabinet.
11. A method for decontaminating all laboratory wastes should be available in the facility, preferably within the laboratory (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).
12. Equipment that may produce infectious aerosols must be contained in devices that exhaust air through HEPA filtration or other equivalent technology before being discharged into the laboratory. These HEPA filters should be tested and/or replaced at least annually.
13. Facility design consideration should be given to means of decontaminating large pieces of equipment before removal from the laboratory.

14. Enhanced environmental and personal protection may be required by the agent summary statement, risk assessment, or applicable local, state, or federal regulations. These laboratory enhancements may include, for example, one or more of the following; an anteroom for clean storage of equipment and supplies with dress-in, shower-out capabilities; gas tight dampers to facilitate laboratory isolation; final HEPA filtration of the laboratory exhaust air; laboratory effluent decontamination; and advanced access control devices such as biometrics. HEPA filter housings should have gas-tight isolation dampers; decontamination ports; and/or bag-in/bag-out (with appropriate decontamination procedures) capability. The HEPA filter housing should allow for leak testing of each filter and assembly. The filters and the housing should be certified at least annually.

15. The BSL-3 facility design, operational parameters, and procedures must be verified and documented prior to operation. Facilities must be re-verified and documented at least annually.

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