ISU Biosafety laboratory Manual for BSL2

[Laboratory Room number and Building]

*List all lab spaces in use for project*

Laboratory Supervisor/Principal Investigator:

Enter name here

Section 1.0 Biosafety Level 2

Section 2.0 Responsibilities

Section 3.0 Agents and Projects Covered under this Manual

Section 4.0 Medical Surveillance Program

Section 5.0 Procedures for BSL-2

Section 6.0 Principal Investigator Certification

[Date]

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| --- | --- |
| **Principal Investigator/Instructor:**  |  |
| **Laboratory Location:**List bldg. & room number(s) |  | **Protocol ID(s):** |  |

End of PI name and Lab location table

# Biosafety Level 2

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.

# RESPONSIBILITIES

## Principal Investigator/Laboratory Supervisor/Instructor

1. Will assure that all research and support personnel obtain required training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, the exposure control/medical surveillance plan, and the incident reporting procedures.
2. Will assure that all research personnel are proficient in standard and special microbiological practices before working with BSL2 agents.
3. Will assure that documentation of training is maintained in the laboratory and available for inspection.
4. Will assure that biosafety procedures are incorporated into standard operating procedures for the laboratory and that the laboratory maintains written policies and procedures for handling of other potentially infectious materials (OPIM) or biohazardous agents.
5. Will assure that personal protective equipment and necessary safety equipment is provided and used.
6. Will assure that all laboratory personnel and support personnel are compliant with the relevant regulations, guidelines, and policies.
7. Will submit an incident report form to the IBC concerning reportable incidents as outlined in the ISU IBC Biosafety Handbook.
8. Will review and update the Laboratory Biosafety Manual at least annually and more frequently if procedures and practices change.
9. List any additional PI/Laboratory Supervisor/Instructor responsibilities in this laboratory (block will expand as needed):

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## Research Personnel

1. Will participate in and complete all required training.
2. Will follow biosafety procedures and practices outlined in this manual and the ISU Biosafety Handbook.
3. Will report incidents of exposure or accidents as outlined in the ISU IBC Biosafety Handbook to the Principal Investigator/Laboratory Supervisor/Instructor.
4. Will comply with all aspects of the exposure control/medical surveillance plan for the agents covered by this biosafety laboratory manual.
5. Will review this Biosafety Laboratory Manual at least annually and more frequently if procedures and practices change.
6. List additional research personnel responsibilities in this laboratory:

(block will expand as needed):

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## Practices, Equipment and Facility Requirements for BSL-2

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

### 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.
6. These include:
7. 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.
8. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
9. Non-disposable sharps must be placed in a hard walled container for transport to a processing area for decontamination, preferably by autoclaving.
10. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, with tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
11. Perform all procedures to minimize the creation of splashes and/or aerosols.
12. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
13. 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:
14. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
15. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
16. 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, with required procedures for entering and exiting the laboratory. Agent information should be posted in accordance with institutional policy.
17. An effective integrated pest management program is required. (See Appendix G of BMBL)
18. 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 childbearing 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.

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

### Safety Equipment (Primary Barriers and Personal Protective Equipment)

1. Properly maintained BSCs, other appropriate personal protective equipment, or other physical containment devices must be used whenever:
	1. 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.
	2. 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, and 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:
5. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.
6. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
7. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.
8. Eye, face and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

### 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.
	1. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
	2. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
4. 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 liquid disinfectant traps.
8. An eyewash station must be readily available.
9. There are no specific requirements for 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 recirculation back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturers recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a 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).

# AGENTS AND PROJECTS COVERED UNDER THIS MANUAL

Insert the content provided on the applicable protocol form(s) for the materials in use under this laboratory manual. Remember to mark Not Applicable or No for material types that are not in use.

1. Infectious Agents

[ ]  No, will not be used

[ ]  Yes, Infectious agents will be used.

1. If *Yes,* Insert the Section 4. Materials table(s) from the protocol form Section B. Use of Infectious Agents, Toxins and Select Agents in Research for each infectious agent intended for use under this biosafety lab manual.
2. rDNA or synthetic DNA Materials

 [ ]  Not Applicable

 [ ]  Yes, rDNA or synthetic DNA Materials will be used.

1. If *Yes*, insert the table prepared for Protocol form *Section A Use of Recombinant or Synthetic Nucleic Acid Molecules in Research* Question #5., the Materials table(s) for each infectious agent intended for use under this biosafety manual.
2. Human or Nonhuman Primate Materials
3. [ ]  Not Applicable, will not be used

 [ ]  Yes, Human or Nonhuman Primate Materials will be used.

1. If *Yes*, insert the table(s) prepared for Question 2. Use of human and non-human primate cell lines or tissues not involving rDNA table(s) from the Protocol form *Section C Human and Non-Human Primate Blood, Cell Lines, or Other Potentially Infectious Materials (OPIM)* for each material intended for use under this biosafety manual.
2. Biological Toxins

 [ ]  Not Applicable, will not be used

 [ ]  Yes, Biological toxins will be used.

1. If *Yes*, insert the Section 4. Materials table(s) from the protocol form *Section B. Use of Infectious Agents, Toxins and Select Agents in Research* for each biological toxin intended for use under this biosafety lab manual.
2. Select Agents

 [ ]  Not Applicable, no select agents will be used

 [ ]  Yes, Select Agents will be used.

1. If *Yes*, insert the Section 4. Materials table(s) from the protocol form *Section B. Use of Infectious Agents, Toxins and Select Agents in Research* for each select agent intended for use under this biosafety lab manual.

# MEDICAL SURVEILLANCE PROGRAM

## General

All personnel are to be instructed that their health status may have an impact on their susceptibility to infection, and if required, their ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel, and particularly women of childbearing age, will be provided with information regarding immune competence and conditions that may predispose them to infection. Personnel that have conditions that would render them more susceptible to infection, or who are pregnant, will be encouraged to self-identify to these issues to the PI/Laboratory Supervisor/Instructor and their personal physician such that appropriate counseling and guidance can be provided.

## Bloodborne and Airborne Pathogens and OPIM \*

\*In accordance with the OSHA Bloodborne Pathogens standard which included OPIM

Bloodborne pathogens are defined as pathogenic microorganisms that are present in human blood and that can cause disease in humans. Occupational exposure is defined as reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infectious materials that may result from the performance of an employee's duties (from OSHA BBP). These pathogens include but are not limited to hepatitis B (HBV) and human immunodeficiency virus (HIV). Other potential infections materials (OPIM) includes the following:

(1) human body fluids: cerebrospinal, synovial, pleural, pericardial, peritoneal, amniotic, semen, vaginal secretions, saliva in dental procedures; all body fluids, and secretions; all body fluids in situations when it is difficult to differentiate between body fluids;

(2) Any unfixed tissue or organ (other than intact skin) from a human living or dead;

(3) HIV-containing cell or tissue culture, organ culture, and HIV or HBV-containing culture medium or other solutions; and

(4) blood, organs or other tissues from experimental animals infected with HIV or HBV.

For the purposes of this manual, all human cell lines, as well as nonhuman primate blood, unfixed tissues, body fluids, cells, and cell lines, will also be considered to have the potential of carrying bloodborne pathogens.

Airborne pathogens are disease-causing microorganisms that spread from person to person in the form of droplet nuclei in the air. Airborne pathogens can be viral, bacterial or fungal in nature. Meningitis, influenza, pneumonia, and tuberculosis are examples of diseases transmitted through the air. Personnel receiving bloodborne pathogens training will also receive training on airborne pathogens.

Will any personnel working under this biosafety laboratory manual be working with *any* of the potentially infectious materials listed above?

 [ ]  No, none will work with potentially infectious materials listed

 [ ]  Yes, there will be personnel working with potentially infectious materials listed above

**If *Yes*, all personnel**

1. must complete CITI Bloodborne Pathogen training on an annual basis. Documentation training completion must be maintained with this manual. The PI or Lab Supervisor must provide copies to the IBC or EH&S upon request.
2. will be provided information about the Hepatitis B vaccine to include: efficacy of the vaccine, its safety, method of administration, benefit of administration, benefits associated with vaccination. Also, personnel will be encouraged to obtain the vaccine from their personal physician.
3. in the instance of an exposure, the Lab Supervisor/PI/Instructor must report an exposure to the ISU Biosafety Officer or to EH&S. The employee will report to their personal physician or an emergency department for post exposure evaluation and follow-up.
4. must familiarize themselves with the following exposure incident information:
5. An exposure incident is defined as a specific eye, mouth, other mucous membrane, non-intact skin, or parenteral contact with blood or other potentially infectious materials that resulted from the performance of an employee's duties.
6. If exposed to blood or other potentially infectious materials, first determine if it meets the definition of an exposure incident. Blood or fluids splashed onto intact skin are not exposure incidents, but require skin to be washed immediately.
7. Exposure to saliva that is not visibly contaminated with blood does not constitute an exposure incident. If it is determined that an exposure incident has occurred, the exposed employee will immediately report the incident to his/her supervisor or the PI.

## rDNA

In general, the risk of exposure and illness from working with rDNA is very low; however, risks do exist and all personnel working with these reagents must be aware of these potential risks.

The viral supernatants produced by transfecting packaging cell lines with recombinant viral DNA could, depending on the DNA insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant viruses even if they are replication defective.

The potential risks include:

* exposure to unknown pathogens from the cell culture systems used to propagate/package the vectors,
* anticipated or unknown effects from expression of a foreign gene or overexpression of an endogenous gene,
* anticipated or unknown effects from suppression of expression of an endogenous gene, or insertional mutagenesis.

These issues are especially of concern when viral vectors are used to express a known or suspected oncogene or cell cycle regulatory proteins, or when they are used to suppress the expression of endogenous tumor suppressors.

If an exposure incident as defined at section 3.2.4 occurs, the same procedures for reporting and follow-up is to be followed. In addition, the PI/Instructor/Lab Supervisor must complete an IBC incident report form and submit it to the Institutional Biosafety Committee. The report must be submitted within one week of the incident.

## Agent Specific Medical Surveillance Plans

For all BSL-2 pathogens used in the laboratory and covered by this manual, list the signs and symptoms of illness from these pathogens, the usual sequelae of the disease, the natural, as well as, laboratory routes of transmission, and indicate the actions that employees should take if personnel display these signs and symptoms.

If risk assessment by IBC determines it is necessary, a medical surveillance plan may need to be developed in coordination with the IBC and the Biosafety Officer.

If an exposure incident as defined under section 3.2 occurs, the same procedure for reporting and follow-up is to be followed. In addition, the principal investigator must complete an IBC incident report form and submit it to the IBC. The report must be submitted within one week of the incident.

# PROCEDURES FOR BSL-2

## General Signage for BSL-2 materials or agents

1. A Universal biohazard symbol will be posted at the entrance to the laboratory. The biohazard label will read “Biohazard” “Low Risk.”
2. A Laboratory Door Sign will be posted at the laboratory entrance.
The sign must include the following information:
	1. Room, Building, Department, and Date of sign completion.
	2. Room Use/Description such as "Research Laboratory".
	3. The types of potential hazards present in the laboratory.
		1. For Biological Hazards, use the terminology “Low Risk”.
		2. The specific type of agent in use will not be listed.
	4. Emergency Contact information [list here the same personnel that are posted on the door placard]: *field will grow to permit entries*

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## Entry and Exit Procedures

1. Access to this laboratory is restricted to those personnel approved by the principal investigator

(list name of PI/Lab Supervisor/Instructor) when work with BSL-2 agents is in progress.

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1. Does entry into this laboratory requires participation in medical surveillance plan?

[ ]  Yes [ ]  No

1. Does entry into this laboratory require vaccination against some infectious agent(s)

[ ]  Yes [ ]  No

* 1. If Yes, then list the agents in box below:

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1. Good Laboratory Practices must be followed at all times.
2. Eating, drinking, chewing gum, smoking, handling contact lenses, or applying cosmetics is prohibited in this laboratory.
3. All food for human consumption must be stored outside the laboratory area in cabinets or refrigerators designated for this purpose.
4. Mouth pipetting is prohibited. Mechanical pipetting devices must be used.
5. Personal items such as coats, boots, bags and books should not be stored in the laboratory.
6. All procedures will be conducted such that the creation of splashes and aerosols are minimized. Whenever possible, all procedures that generate aerosols will take place in a biosafety cabinet.
7. No animals or plants may enter this laboratory unless used specifically for the research being performed and approved by the IACUC. If animals are used in the context of an IBC protocol, use in the laboratory must be approved by the IBC.
8. Laboratory coat, gown, smock, sleeve protection, or uniform designated for laboratory use must be worn while working with BSL-2 agents.
9. Appropriate PPE must be worn when handling BSL-2 agents.
10. Inside biosafety cabinet, the following PPE must be used:
11. Gloves must be worn when handling or working with BSL-2 agents in the biosafety cabinet.
12. Gloves must be changed when contaminated, integrity has been compromised, or when otherwise necessary.
13. Two pair of gloves may be required for some procedures.
14. Outside biosafety cabinet on open bench, the following PPE must be used:
	1. Gloves must be worn when handling or working with BSL-2 agents outside the biosafety cabinet.
	2. Gloves must be changed when contaminated, integrity has been compromised, or when otherwise necessary.
	3. Two pair of gloves may be required for some procedures.
	4. Eye Protection and Face protection (goggles, mask, face shield, or other splatter guard)
	must be worn if procedure may produce aerosols (splashes or sprays).
	5. Respiratory Protection (N95 or PAPR) must be worn if risk analysis determines that procedure/agent requires this protection.
15. Upon completion of work with BSL-2 agents, the following procedures must be done.
	1. Remove and discard gloves in biohazard waste. Disposable gloves may not be washed or reused.
	2. Wash hands.
	3. Remove Laboratory coat, gown, smock, or uniform before leaving laboratory for non-laboratory areas such as cafeteria, library, or administrative offices. For disposable protective clothing, place in biohazardous waste. For reusable protective clothing, hang in designated area in laboratory for reuse, or place in designated area for laundering by the institution. Protective clothing should not be taken home.
	4. Eye and face protection must be disposed of with contaminated waste or decontaminated after use.
	5. Wash hands before exiting.

## 5.3 Procedures for Working in Biosafety Cabinet in PI’s Laboratory

1. List the Make, Model with building and room location with last certification date for the BSC to be used for the work covered by this laboratory manual:

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| --- | --- | --- | --- |
| Make | Model | Location (Bldg/Room) | Cabinet Last Certified |
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1. Biosafety cabinet is operated: [ ]  Continuously [ ]  As Needed
	1. If "As Needed" is checked, please indicate the minimum time cabinet blower is on

a. prior to beginning work: minutes blower is run.

b. after work in completed: minutes blower is run.

1. Other activities (e.g., rapid movement, open/closing room doors, etc.) in the room are minimized when operations are being conducted in the biosafety cabinet to avoid disrupting the cabinet air barrier.
2. PPE as outlined in section 4.2 will be worn when working in the biosafety cabinet.
3. Before beginning work, stool height will be adjusted such that personnel’s face is above the front opening. The sash should be set at the recommended height in order for proper cabinet operation and user protection. The cabinet user should adjust their shoulder height to be level with the lower edge of the sash.
4. Closure of the drain valve under the work surface will be done prior to beginning work so that all contaminated materials are contained within the cabinet should a large spill occur.
5. Wipe down the interior of the cabinet with an appropriate surface disinfectant(e.g.,10% commercial bleach solution, 70% alcohol, or similar non-corrosive antimicrobial agent)
6. Materials needed for work in the biosafety cabinet will be placed in the cabinet prior to beginning work to avoid disruption of airflow. Materials will be placed as far back in the cabinet as is practical.
	1. All operations within the cabinet will be performed on the work surface at least four (4) inches from the inside edge of the front grille.
	2. If plastic-backed absorbent toweling is placed on work surface it will be placed such that it does not cover front or rear grille openings.
	3. The front grille will not be blocked with research notes, discarded plastic wrappers, pipetting devices, etc.
7. The number of arm-movement disruptions across the air barrier of the cabinet will be minimized.
8. Will any aspirator suction flasks be used? [ ]  No [ ]  Yes
	1. If *Yes*, then **two flasks will be connected in series**, and they will be pre-filled with appropriate disinfectant such that the final concentration is sufficient to kill the microorganisms. A filter (either 0.3 µM or HEPA) will be placed in-line along with a second flask to prevent overflow into building vacuum system.
	2. List disinfectant and final concentration

(check the relevant boxes and add detail if "other" is selected):

[ ]  Not Applicable

[ ]  10% final concentration of household bleach

[ ]  Other – List agent and concentration: Click here to enter text.

**Contact time must be at least 30 minutes and then liquid waste should be handled
as described in 4.5. NOTE: Disposal must be done on at least a daily basis.**

1. Horizontal pipette discard trays containing an autoclave bag or an appropriate chemical disinfectant will be used within the cabinet. Upright pipette collection containers placed on the floor outside the cabinet or autoclavable biohazard collection bags taped to the outside of the cabinet should not be used. The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection.
2. Active work should flow from the clean to contaminated area across the work surface. Bulky items such as biohazard bags, discard pipette trays and suction collection flasks must be placed to one side of the interior of the cabinet.
3. Use of glass Pasteur pipettes is discouraged. Glass pipettes should be replaced with safer alternatives (i.e., plastic) as recommended by the World Health Organization Biosafety Manual (WHO, 2003). Contact the EH&S for more information on safer alternatives.
4. Will open flame be used in the biosafety cabinet (BSC)? [ ]  No [ ]  Yes

If *Yes* describe use:

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1. Will an UV light be used in BSC? [ ]  No [ ]  Yes

If *Yes* describe use:

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1. Upon completion of work, the interior surfaces of the cabinet will be wiped down a disinfectant:

(check relevant boxes and list details as asked):

[ ] 10% final concentration of household bleach followed by 70% ethanol to
 remove bleach residue.

[ ]  Chlorine Dioxide. List final concentration: Click here to enter text.

[ ]  Other – List agent and concentration: Click here to enter text.

1. Gloves and disposable PPE will be removed disposed of as biohazard waste and hands will be washed.

## 5.4 Handling of Sharps

1. Sharps, such as needles, scalpels, contaminated glass pipettes, and broken contaminated glassware will be handled in the following manner:
	1. Whenever possible, use of sharps with potentially hazardous material will be avoided. Plasticware will be substituted for glassware whenever possible.
	2. The handling of sharps will be minimized. Needles will not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
	3. Used disposable needles and syringes will be carefully placed in a puncture- resistant containers used for sharps disposal. Sharps containers will not be beyond ¾ full.
	4. Broken glassware will not be handled directly. It will be removed using a brush and dustpan, tongs, or forceps and properly disposed of glassware waste.
2. Disposal of **biohazardous sharps**. When sharps containers are ¾ full, contact Click here to enter text. at phone extension enter phone # here. to arrange for disposal.
3. Will **Non-disposable** sharps be used in this lab? Yes [ ]  No [ ]
4. List the types of non-disposable sharps used:

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4.1 If *Yes*, once **non-disposable sharps** are contaminated with infectious material,

[ ]  They are placed in a hard-walled container for transport to a processing area for decontamination or

[ ]  They are decontaminated in the manner described below:

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## 5.5 Liquid Waste Decontamination and Disposal

1. The following method will be used for decontamination of liquid biohazardous waste:

[ ]  10% final concentration of household bleach

[ ]  Other – List agent and concentration: Click here to enter text.

[ ]  Not Applicable

**Contact time must be at least 30 minutes.**

2. Following decontamination, liquid may be disposed of down the sink and the sink rinsed with water.

## 5.6 Solid Waste Decontamination and Disposal

Solid waste that has been in contact with potentially infectious materials will be disposed of in the following manner:

**A. Low Risk Waste** (BSL-1 Waste, BSL-2 waste from human or nonhuman primate cells or cell lines and tissues, and containing replication incompetent viral vectors).

NOTE: ALL waste generated by the laboratory within a specific location must qualify as low risk. IF not, ALL waste generated in that location much be treated as moderate risk.

1. Autoclaved and disposed – All biohazardous waste is placed in biohazard bag, placed in autoclavable container (i.e., polypropylene tray), and transported to autoclave on a cart. Waste is autoclaved in the tray and then placed in black plastic bag and discarded in standard waste.
2. Commercial. All biohazardous waste is placed in biohazard bag inside solid biohazard container. EHS approved commercial biohazard disposal company retrieves and disposes biohazard waste in compliance with State and Federal regulation.

**B. Moderate Risk Waste** (BSL-2 waste containing any replication competent infectious agent or BSL-1 waste generated in the same location as moderate risk waste).

1. Autoclaved and disposed – All biohazardous waste is placed in biohazard bag, biohazard bag is placed in autoclavable container (i.e., polypropylene tray), and transported to autoclave on a cart. Waste is autoclaved in the tray and then placed in black plastic bag and discarded in standard waste. (This is only for those laboratories that do not have commercial waste disposal).
2. Autoclaved and then commercial disposal. All biohazardous waste is placed in biohazard bag, biohazard bag is placed in autoclavable container (i.e., polypropylene tray), and transported to autoclave on a cart. Waste is autoclaved in the tray and then placed inside solid biohazard container. EHS approved commercial biohazard disposal company retrieves and disposes biohazard waste in compliance with State and Federal regulation.

## 5.7 Decontamination of Work Surfaces

1. Work surfaces will be decontaminated after completion of work and immediately cleaned after any spill or splash of potentially infectious material.

Check the box indicating the method that will be used for routine decontamination of work surfaces (complete description as applies):

[ ]  10% final concentration of household bleach made daily followed by 70% ethanol to remove bleach residue.

[ ]  Chlorine Dioxide. Final concentration: Other – List agent and concentration:

1. All equipment which comes into contact with biohazardous material must be decontaminated before repair, maintenance, or removal from the laboratory.

## 5.8 Spill Clean-up

1. Spills will be handled in accordance with ISU policies as provided by the Environmental Health and Safety Office (EHS). This may include training provided by EHS. All personnel will be familiar with these procedures.
2. Spills greater than **1 liter** are considered large spills and must be immediately reported to the EH&S (208-282-2310).
3. Spills that result in potential exposure of lab personnel to BSL2 agents or that occur outside of the biosafety cabinet must be reported to the PI/Lab Supervisor/Instructor. The PI/Lab Supervisor/Instructor must complete an IBC incident report form and submit to the Institutional Biosafety Committee via the biosafe@isu.edu email account.
The report must be submitted within one week of the incident.

## 5.9 Transport of BSL-2 Agents

1. The following potentially infectious materials will be transported between the listed locations (add rows as needed):

|  |  |  |
| --- | --- | --- |
| Material(s) | Location/From | Destination/To |
|  |  |  |

1. What will be the primary containment for these materials/agents? List in box below:

|  |
| --- |
|  |

1. The primary containment will be placed in a secondary containment, which is non-breakable and sealed.

## 5.10 Shipping and Receiving

1. If potential infectious materials will be shipped from the facility, EHS must be contacted for guidance, x2310. All appropriate local, state and federal (U.S. Department of Transportation) regulations must be followed. For air or international shipments, International Air Transportation Association (IATA) rules must be followed.
2. Personnel responsible for shipping will complete the appropriate training for packaging, labeling and shipping of all infectious materials. Contact EHS for training on shipping dangerous goods. Documentation of this training must be maintained with this manual and provided to the IBC or the Biosafety Officer upon request.
3. For receiving only, the ISU policies on receiving potentially infectious material must be followed. Please contact EHS for guidance prior to receiving any potentially infectious materials, whether through purchase or by colleague exchange.

## 5.11 Laboratory Specific Procedures

Describe **any** laboratory specific procedures required when handling biohazardous material.

Examples of procedures needed include, but are not limited to, centrifugation, sonication, shakers, etc.

1. Centrifuge will be used: [ ]  Not Applicable [ ]  Applicable

If Applicable, then describe the containment in the box below. List location – building and room number. Include whether sealed rotors are used:

|  |
| --- |
|  |

1. Shaker use: [ ]  Not Applicable [ ]  Applicable

If Applicable, describe the containment in the box below. List location – building and room number.

|  |
| --- |
|  |

1. Sonicator use: [ ] Not Applicable [ ]  Applicable

If Applicable, describe the containment in the box below. List location – building and room number.

|  |
| --- |
|  |

## 5.12 Procedures for Visitor

1. All visitors to the laboratory must comply with the ISU’s *VISITORS TO CAMPUSLABORATORIES PROCEDURES POLICY* to ensure that they are informed of thepotential hazards and receive the appropriate training.
2. **All volunteers** must sign an Acceptance of Risk, Waiver and Release.

## 5.13 Safe Use of Autoclaves

There are many different types of autoclaves used on the ISU campus. It is the responsibility of the supervisor to ensure that all authorized individuals are properly trained on the use of the autoclave(s) used by laboratory personnel.

1. Will Autoclaves be used by this laboratory's personnel? [ ]  Yes [ ]  No

If*Yes*, list the location with the point of contact responsible to maintain the autoclave in the table below:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Machine brand | Location (room & building) | Person responsible for arranging maintenance | Date Last Maintained | Service Vendor |
|  |  |  |  |  |
|  |  |  |  |  |

1. Training on the use of autoclaves will consist of the following (a. through h.). Maintain training records in the laboratory and make them available for review by EH&S and IBC upon request.
2. Appropriate PPE requirements such as the use of heat resistant gloves, lab coats, and safety eye and face protection.
3. A discussion of the types of items that can and cannot be autoclaved.
4. Proper packaging of biohazardous wastes for autoclaving.
5. Methods for loading materials into an autoclave and unloading procedures.
6. The use of test strips and biological indicators for quality control.
7. Autoclave operational procedures including emergency shutdown precautions.
8. How to dispose of autoclaved waste.
9. Record keeping
10. Maintenance and Testing of Autoclaves
	1. Department or person responsible for autoclave must properly maintain and service the machine.
	2. Department or person responsible for autoclave must occasionally quality tested to ensure proper sterilization procedures are met and decontamination of biohazardous waste is complete.

# Principal Investigator Certification

I hereby certify that I reviewed these practices and procedures and affirm they represent the current operating practices in the laboratory. I agree to submit modifications to this manual as additional personnel are trained and certified for work in this laboratory on my project(s).

Signature of PI/Instructor/Laboratory Supervisor Date

Annual Review:

Signature of PI/Instructor/Laboratory Supervisor Date

## Personnel Certification

We, the undersigned, have reviewed these practices and procedures, have been trained in the appropriate methods and practices for handling potential infectious material and agree to follow the stated practices and procedures. We understand that we must review and document compliance with these practices and procedures on an annual basis.

|  |  |  |
| --- | --- | --- |
| Name | Signature | Date |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |