**Lab PI Biosafety Level X Manual**

 ** **

 Both top pictures from epidemic.bio.ed.ac.uk

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bacteria-from-agricultural-soils-versatile-weapons-against-aflatoxigenic-fungi

griggsdakota.com

This lab-specific manual applies to the following BSL-X agents:

1. Agent one

2. Agent two

3. Agent three

4. Agent four

5. Agent five

***PI's Last Name Laboratory*** ***Building(s) and room number(s)***

Note:

This template is provided to assist Principal Investigators (PIs) in the development of a *laboratory-specific* Biosafety Manual with instructions to safely handle and manipulate a particular agent or agents under Biosafety Level 1 or 2 (BSL-1 or BSL-2) laboratory conditions. Developing and maintaining this Manual is **required** for BSL-2 labs, and is **encouraged** for BSL-1 labs. This Manual will become part of the Laboratory inspection process at NDSU and a copy (electronic is preferred) needs to be sent to the [NDSU Safety Office](https://www.ndsu.edu/police_safety/contact). Electronic copies can be sent to the [Associate Director of Environmental Health and Safety](https://www.ndsu.edu/police_safety/environmental_health_and_safety/).

The PI is responsible for including basic background information for each agent, writing an exposure risk, detailing surface decontamination, and writing standard operating procedures for experiments where safety is of an elevated concern (see “Safety SOPs” on page 13). Also, please provide lab-specific information where you see gray text fields throughout this template. This template is simply provided as a starting point. Additions/changes to this template that will render the final manual more useful for the laboratory’s safety needs are strongly encouraged.

In addition to this manual, whenever working with recombinant or synthetic DNA, the NDSU Institutional Biosafety Committee (IBC) requires all labs to adhere to the National Institutes of Health (NIH) [*NIH Guidelines for Research Involving Recombinant DNA Molecules*](http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines)*.* It is also strongly encouraged that you use and follow the guidelines provided the CDC’s [*Biosafety in Microbiological and Biomedical Laboratories, 5th Edition*](http://www.cdc.gov/biosafety/publications/bmbl5/). Many of the requirements provided in these two documents will be utilized in the annual laboratory inspections.

This template was originally developed by the Oregon Health and Science University, and modified/edited to meet the needs of NDSU researchers.

**A loose-leaf binder that can easily accommodate changes or new materials is the recommended means for maintaining and organizing this laboratory-specific Biosafety Level 2 Manual.**

All lab personnel must read the contents of this manual and sign & date below. **This manual must be updated and reviewed by laboratory personnel annually.** By signing this page, lab personnel agree to abide by the safety precautions and procedures discussed herein.

*I have read, understand, and agree to adhere to the biosafety procedures contained within:*

Principal Investigator:

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| --- | --- | --- | --- |
| Typed Name | Title | Signature | Date |
| First, Last | Principal Investigator |  |  |

Laboratory Staff:

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# Lab Contacts and Training

|  |  |
| --- | --- |
| **Principal Investigator:** | **First, Last** |
| Lab Location: | Lab Location |
| Office Phone: | Office Phone |
| 24/7 contact (cell phone/pager): | Enter number |
| **IBC Protocol #(s):** | **Enter number(s)** |
| **IACUC Protocol #(s) (if applicable):** | **Enter number(s)** |

**NDSU Training Courses.** Courses required of all laboratory researchers listed below. Other courses may be added if they relate to the work conducted in your specific lab, for example Animal Care and Use Training or Bloodborne Pathogen Training. Copies of completed training certificates should be included in Appendix IV.

|  |  |
| --- | --- |
| **Lab Personnel** | **Relevant Training Dates** |
| **Name** | **NDSU Baseline Safety Training (annual)** | **NDSU Laboratory Safety Modules 1-6- initial** | **NDSU Laboratory Safety Module 2 (annual)** | **NDSU Laboratory Safety Modules 1, 3-6 (every 3 yrs)** | **Biosafety- CITI** |  |  |
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***Agent(s)*-specific Training.** Laboratory personnel are not allowed to work with agent(s) until they have been trained by the PI who supervises their work, or other designated laboratory personnel with technical expertise. The worker should demonstrate good microbiological skills and an understanding of this manual prior to being permitted to work with agent(s).

# Background

***Describe the known risks to be considered when working with each agent. Include supplemental background information regarding biological traits that are essential to consider prior to experiments with the agent. An example for lentivirus is provided below. Describe the symptoms and signs associated with infection by the agents being worked with in the lab. This section should be designed to be taken with laboratory workers to health care providers should they require care for suspected laboratory acquired infections.***

***You might find it helpful to reference:***

* [***Canadian Pathogen Safety Data Sheets***](http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php)
* [***BMBL, 5th Edition***](http://www.cdc.gov/biosafety/publications/bmbl5/)
* [***CDC A-Z Index:***](http://www.cdc.gov/az/a.html)

*Insert text here*

·~·~·~·~·~·~·~·~·~·~·~·~***Example***·~·~·~·~·~·~·~·~·~·~·~·~

*The major risks to be considered for research with HIV-1 based lentivirus vectors are the potential for generation of replication-competent lentivirus (RCL), and the potential for oncogenesis via random chromosomal integration. The nature of the transgene must also be considered in assessing risk. These risks can be mitigated by the nature of the vector system, and its safety features, or exacerbated by the nature of the transgene insert encoded by the vector (e.g., expression of a known oncogene with a constitutive strong promoter may require heightened safety precautions).*

*The potential for generation of RCL from HIV-1 based lentivirus vectors depends upon several parameters, the most important of which are the number of recombination events necessary to reassemble a replication competent virus genome and the number of essential genes that have been deleted from the vector/packaging system. On this basis, later generation lentivirus vector systems are likely to provide a greater margin of personal and public safety than earlier vectors, because they use a heterologous coat protein (e.g., VSV-G) in place of the native HIV-1 envelope protein, thus reducing the risk of RCL generation. (It should be noted, however, that pseudotyping with coat proteins such as VSV-G may broaden the host cell and tissue tropism of lentivirus vectors, which will be considered in the overall safety assessment by the IBC). Later generation vector systems also separate vector and packaging functions onto three or four plasmids and they include additional safety features such as the deletion of Tat, which is essential for replication of wild-type HIV-1, and altered 3’ LTR that renders the vector “self-inactivating” (SIN). In contrast, earlier vector systems (such as two-plasmid vector systems) may have a higher potential for generation of RCL.*

# Exposure Risk

***Describe the means by which laboratory personnel could be exposed to the agent(s). Include practices that pose potential for exposure, such as those that could create aerosols. An exposure risk example for lentivirus is shown below.***

***If uncertain if your procedures would provide an exposure risk, contact the*** [***NDSU Safety Office***](https://www.ndsu.edu/police_safety/contact/) ***to discuss potential exposure risks. Also, as stated in the example, immunocompromised or medically concerned individuals (including women who are or may become pregnant) are encouraged to self-identify prior to working with BSL-2 agents.***

*Insert text here*

·~·~·~·~·~·~·~·~·~·~·~·~***Example***·~·~·~·~·~·~·~·~·~·~·~·~

*The most probable route of exposure for work with lentivirus would be parenteral (dermal via sharps), absorption through exposed scratches or abrasions on skin, or mucous membrane exposure of the eyes, nose, and mouth. Another possible route is inhalation via aerosols during the use of equipment such as centrifuges or vortex mixers. Care must be taken when pipetting in order to avoid splashing or generation of aerosols. Immunocompromised individuals are encouraged to self-identify prior to working with lentivirus.*

# Inactivation and Surface Decontamination

***Describe the reagents and/or processes used to inactivate the agent(s) and the method to decontaminate surfaces. See the last page of this manual for a suggested disinfectant chart. An example for lentivirus inactivation is below.***

*Insert text here*

·~·~·~·~·~·~·~·~·~·~·~·~***Example***·~·~·~·~·~·~·~·~·~·~·~·~

*Lentiviral particles can be inactivated with a number of reagents, including 10% household bleach\* (final concentration 0.5% sodium hypocholorite), 5% Amphyl (phenolic), and 0.5% Wescodyne (iodophor). This SOP has been written for the use of bleach, but alternative disinfectants can be substituted, as long as they are known to be effective for lentivirus.*

\***A note on bleach:** Household bleach is effective and inexpensive, but it is also volatile and corrosive. Bleach-soaked paper towels should not be autoclaved because autoclaving 1) releases chlorine, a chemical hazard, and 2) will corrode the autoclave over time. 10% (0.5% final concentration sodium hypochlorite) household bleach solutions should be prepared fresh at least weekly. If 10% bleach is used to decontaminate a spill within the Biosafety Cabinet (BSC), once the spill has been absorbed on paper towels and disinfected with 10% bleach, the BSC should be wiped down with 70% ethanol (EtOH) in order to remove residual bleach to prevent corrosion.

# Biosafety Requirements and Procedures

1. Physical Containment. All work with agent(s) must be performed in a properly maintained and resourced BSL-2 laboratory. Appropriate signage must be posted at the entrance to the lab. This sign must include the biosafety level, a biohazard symbol, the name of the agent(s) in use, the name and phone number of the PI or lab supervisor, and required procedures for entering and exiting the lab. (A sign that meets these requirements is available in the Appendix). Additionally, incubators and freezers must bear biohazard warning labels if they contain BSL-2 agents. Doors to the laboratory must be locked when not attended. Laboratory windows that open to the outdoors must be fitted with fly screens.
2. **Safety Equipment.**
	1. **Biosafety Cabinet:** An annually certified Class II Biosafety Cabinet (BSC) must be used to contain any experiments where aerosols or splashes from a BSL-2 agent are concerns. Common techniques that cause aerosolization and splashing include pipetting, centrifuging, grinding, blending, shaking, sonicating, opening containers of infectious materials, inoculating animals intranasally, and harvesting infected tissues from animals or eggs. BSCs must be positioned in a BSL-2 lab such that fluctuations of the room air supply and exhaust do not disrupt the proper airflow within the BSC. The best placement of a BSC is a location with minimal walking paths and away from doors and windows. All BSCs must be certified annually. Note that HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory. The BSC exhaust can also be fed to the laboratory room exhaust via canopy or direct connection. If the blower on the BSC is not left on continuously, it should be turned on and allowed to run for 5 minutes to facilitate several complete exchanges of air before work begins. At the beginning of the work session, plastic-backed absorbent paper can be placed on the work surface (optional), but must not obstruct air flow. The work area should be segregated into clean and contaminated sections, with contaminated material being located at the rear of the cabinet workspace. Discarded material should be added to a small, red biohazard bag within the cabinet. Work with all materials 4-6 inches inside the sash. Keep containers of liquids capped when not in use. At the end of the work session, all items to be removed from the BSC must be decontaminated. The surface of the BSC must be wiped down with 70% EtOH, and the sash lowered.
	2. **Vacuum lines**: Vacuum lines to be used for aspiration must be equipped with an in-line HEPA filter and a vacuum flask (two flasks connected in series are recommended, but not required), containing an appropriate disinfectant in a volume sufficient to provide the recommended final concentration for that disinfectant when the flask is full.
	3. **Centrifuges:** If agent(s) will be concentrated in an ultracentrifuge, rotors must be equipped with features (e.g., sealing o-rings) to minimize the risk of aerosol generation when necessary. Low-speed swinging-bucket centrifuge buckets must be equipped with aerosol-tight safety covers when necessary. Microcentrifuges must have aerosol-tight rotors capable of being removed while sealed so that the rotor can be unloaded in the BSC when necessary.
3. Personal Protective Equipment (PPE). The following PPE must be worn when working with agent(s):

Please check appropriate boxes by double clicking and selecting “checked.”

**[ ]  Gloves** **[ ]  Safety glasses [ ]  N95 Respirator [ ]  Shoe covers**

**[ ]  Latex [ ]  Face shield** **[ ]  Surgical mask [ ]  Medical scrubs**

**[ ]  Nitrile [ ]  Lab coat [ ]  Hair net**

**List other required PPE not mentioned above, optional PPE, and other helpful suggestions to achieve the highest level of personal protection from this agent (ex: use of double gloves, tucking cuffs of lab coat into sleeves, etc.). Add separate sections as necessary if PPE requirements differ for each *agent.*** When working, be cognizant to remove potentially contaminated gloves and replace them with new gloves before touching anything such as the refrigerator, centrifuge, incubator, etc. to prevent contamination or lab work surfaces.

Certain procedures may require additional PPE. Contact the Associate Director, Environmental Health and Safety – 231-6299 if you would like to discuss PPE requirements.

*BSL-2 Personal Protective Equipment recommendations by agent*

*Bacteria: Gloves, Lab coat, Safety glasses when working with bacteria outside a BSC.*

*Viruses: Gloves, Lab coat, Safety glasses when working with virus outside a BSC.*

1. Spill Kit. The lab must have a spill kit readily accessible in the event of a spill. The spill kit should have:
* an easy-to-read outline of the spill response SOP
* gloves
* face protection
* safety glasses or goggles
* clean lab coat or disposable gown
* paper towels to absorb contaminated liquids
* disinfectant appropriate for agents used in the lab (e.g. bleach)
* tongs or forceps to pick up broken glass
* a biohazard waste container large enough to handle wet, contaminated paper towels

***It is a good idea during the annual review of this Manual to take the extra time to practice the spill procedure.***

1. General Procedures for working with agent(s). Standard safe microbiological practices should be employed, conforming to the BMBL, including a prohibition of eating, drinking, food storage, handling of contact lenses, applying lipstick, cosmetics or lip balm, mouth pipetting, and a requirement of appropriate PPE.

Additional practices include the following recommendations:

* 1. Whenever possible, work with agent(s) during normal working hours, to enable adequate response to a severe adverse incident. If the laboratory PI/supervisor determines that it is safe for you to do work outside of normal working hours, employ the buddy system, or schedule a call-in time with someone to ensure safety.
	2. Sharps should be avoided whenever possible in a BSL-2 laboratory. Plastic aspirating pipettes (e.g., Corning cat. # 4975; Falcon cat # 3575; Fisher Cat # 13-675-123) should be substituted for glass Pasteur pipettes if possible. Needles with safety devices are recommended wherever possible. If conventional needles are required, they must *never* be re-capped, and must be disposed of in a rigid, red sharps waste container located near the workspace. *Never* reach into a sharps container to retrieve discarded items. Do not allow a sharps container to become more than ¾ full. Reminder: syringes without needles can be discarded in either a biohazard bag or a biohazard sharps container.
	3. Solid Waste: Everything that contains agent(s) or contacts agent(s)***-***containing solutions or vessels must be deposited into a biohazard waste container and handled as solid biohazard waste according these procedures: {lab specific procedure}.
	4. Liquid Waste should be aspirated into a vacuum flask containing an appropriate disinfectant in a volume sufficient to provide the recommended final concentration for that disinfectant when the flask is full. Be mindful of the discarded liquid level before and after aspirating liquid waste to prevent overfilling. At the end of the work session, aspirate a small volume of concentrated disinfectant through the vacuum tubing, into the vacuum flask. The vacuum flask must sit for a minimum time of 30 minutes prior to drain disposal. Liquid waste that is not aspirated must be treated with disinfectant at the recommended final concentration, allowing a minimum time of 30 minutes to inactivate the agent.
	5. Centrifugation: Centrifuging is a procedure that can create aerosols. When concerned about aerosols, use rotors with aerosol tight lids or buckets, and open rotors or buckets in the BSC. At the end of any procedure that involves centrifuging agent(s), it is good practice to decontaminate all rotors and/or buckets.
	6. Storage: Agent(s) stocks must be in closed, secondary containers in a freezer clearly marked with a biohazard sign or sticker.
1. **Accidents and spills**
	1. Spills
		1. Small spills inside the BSC: First, wait 5 minutes to allow the blower to move aerosols through the HEPA filter. Do not disrupt air flow within the BSC during this time. Check to see if the spill is fully contained within the BSC, if any PPE has become contaminated, or if any breach of containment has occurred (e.g., a splash where droplets have escaped the BSC and fallen on the floor). If there has been a breach of containment, response should be as for a spill outside the BSC. Small spills (<25 ml) can be decontaminated by layering paper towels soaked in appropriate disinfectant on top of the spill, allowing 20 min. for the disinfectant to inactivate the agent, then depositing the paper towels in the biohazard waste bag in the BSC. If using bleach, residual bleach can be wiped off with paper towels sprayed with 70% EtOH, and the towels deposited in the biohazard waste bag. Note: a spill of media or buffer not containing the agent does not represent a biohazard, but paper towels used to wipe it up should still be deposited in the biohazard bag in the BSC.
		2. Large spills inside the BSC (spills over 25 ml, with likely splatter droplets outside the BSC): Large spills should be treated more cautiously. Leave the BSC running. Remove PPE and any contaminated clothing (check the sleeves of your lab coat) and place it in sealable plastic container or a biohazard bag. Notify the PI. If you must leave the room to do so, close the door to the room as you leave - make sure you have removed your gloves before you touch the door knob. If you are absolutely sure that there has been no exposure and no breach of containment, proceed as for a small spill inside the BSC. If there has been overt exposure (e.g., actual contact of bare skin with agent(s)), wash skin with soap and water for 15 minutes, and contact the [NDSU Safety Office](https://www.ndsu.edu/police_safety/contact/). After hours, contact the NDSU Police Department for assistance (701-231-8998). Allow 20 min. for any potential aerosols to settle from the spill. Don clean PPE, cover the spill with paper towels, soak with appropriate diluted disinfectant, starting at the perimeter and working inward toward the center. Allow 20 min. contact time with the disinfectant to inactivate the agent. Deposit soaked towels in biohazard waste. The interior of the BSC should be decontaminated by wiping down the walls, sash, and equipment with disinfectant. Autoclavable equipment (e.g., racks, some pipettors, and tube containers) should be autoclaved, if feasible. If the spill has entered the BSC drain pan, more extensive decontamination must be performed. The drain pan should be emptied into a collection vessel containing disinfectant. A hose barb and flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. The drain pan should be decontaminated, flushed with water and the drain tube removed. After decontamination with corrosive disinfectants (e.g., bleach), remember to wipe down the BSC with 70% EtOH to remove residual chemicals. If no overt exposure has occurred, and the spill was completely contained within the BSC, the Biological Safety Officer does not need to be informed. The PI should review the incident to revise procedures to minimize the risk of recurrence.
		3. Small spills **outside** **the BSC**. A small spill, in this circumstance, is defined as a spill with low potential to aerosolize, presents no inhalational hazard, and no endangerment to people or the environment. As a practical consideration, volumes less than 10 ml fall into this category. First, ascertain the extent of the spill. Simply dropping a 150 mm dish contained inside a closed secondary container does not constitute a spill outside the BSC, since there is no breach of containment—as long as the secondary container stays closed. If other personnel are present, alert them immediately. Keep in mind: spills generate aerosols. Quickly check to ascertain the extent of the spill: Is PPE is contaminated? (Gloves, lab coat, pants cuffs, shoes?). Is bare skin exposed? Has liquid splashed over a large area? If shoes are visibly contaminated, decontaminate them with appropriate disinfectant, then evacuate the room, closing the door. Remove gloves before touching the door knob. Remove any potentially contaminated PPE, place it in a biohazard bag, wash hands and face thoroughly. Post a sign on the door warning personnel not to enter. Allow 30 min. for aerosols to settle. During this time, notify the PI (and the [NDSU Safety Office](https://www.ndsu.edu/police_safety/contact/). After hours, contact the NDSU Police for assistance (701-231-8998). After 20 min., don fresh PPE, re-enter the room, use tongs to remove any sharps in the spill and transfer them to a biohazard sharps container, cover the spill with paper towels, then soak them with disinfectant starting at the periphery and moving inward toward the center. Be sure to check for and decontaminate small splashes beyond the main affected area. Leave the soaked towels in place for 20 min. to inactivate the agent. After the 20 min. inactivation time, transfer soaked paper towels to biohazard waste. Wipe up the residual spill with more paper towels. Give the area a final wipe-down with paper towels using the appropriate disinfectant.
		4. Large spills **outside the BSC**. A large spill, in this circumstance, is defined as a spill that spreads rapidly, presents an inhalational hazard, endangers people or the environment, and/or involves personal injury or rescue and should be handled as an emergency. In practical terms, this might be a spill of more than 10 ml splattering over a large area, thus presenting the possibility of aerosolization and widespread contamination. If other personnel are present, alert them immediately. Keep in mind: spills generate aerosols. Ascertain the extent of the spill: possible overt exposure, splash on shoes or soles of shoes, contamination of PPE. If shoes are contaminated, disinfect them before evacuating the room (if shoes are extensively contaminated, you should remove them as you leave the room). After removing gloves evacuate the room, closing the door as you leave. Remove PPE. Wash hands and face thoroughly. Post a sign on the door warning personnel not to enter. Allow 20 min. for aerosols to settle. During this time, notify the PI. If the spill is too difficult to manage alone, seek help from the [NDSU Safety Office](https://www.ndsu.edu/police_safety/contact/), or the NDSU Police if it is after hours (231-8998). After 20 min. don fresh PPE, re-enter the room. If there is any broken glass associated with the spill, pick it up with tongs or forceps, and transfer it to a biohazardous broken glass container. Cover the spill with paper towels, and soak the towels with appropriate disinfectant, working from the outside toward the center. Allow 20 min. for agent(s) to be inactivated. Pick up soaked paper towels, and transfer to a biohazard bag. Give the area a final wipe-down with paper towels using the appropriate disinfectant. All spills outside of the BSC that involve breach of containment, regardless of exposure, should be reported to the [NDSU Safety Office](https://www.ndsu.edu/police_safety/contact/).
	2. Accidents

Accidents include the release of agent(s) due to equipment failure (e.g. tube failure in the centrifuge), needle-sticks, or other injuries concomitant with a breach of containment of agent(s).

* + 1. Centrifugation. If tube failure is suspected (sudden clunking or automatic shut-down due to imbalance), leave the centrifuge lid closed for 30 min. to allow aerosols to settle. During this time, notify the PI. Open the lid cautiously to check the integrity of the rotor/tubes. If the rotor looks intact, spray the rotor with 70% EtOH, and transport it into the BSC before unloading centrifuge tubes. If a tube has cracked or collapsed within a swinging bucket (e.g., SW28), decontaminate the tube and bucket inside the BSC. (Use your own judgment regarding recovery of agent(s)). If there appears to be a leak or spill inside the centrifuge, decontaminate the centrifuge chamber by cautiously opening the centrifuge, adding paper towels to soak up any contaminated liquids, then liberally spraying disinfectant onto the walls and inside the lid of the centrifuge, so that disinfectant pools at the bottom of the chamber. (e.g., about 0.5-1 liter). Close the centrifuge for 20 min. Clean up the soaked paper towels as for a major spill outside the BSC. In the event of a catastrophic failure in the centrifuge (e.g., swinging bucket coming off the rotor at 22,000 rpm, damaging the centrifuge, and releasing agent(s) into the centrifuge chamber), keep the centrifuge lid closed for 30 min. During this time, notify the PI and if the contamination is too extensive to manage alone, ask the [NDSU Safety Office](https://www.ndsu.edu/police_safety/contact/). Decontamination is similar to a major spill outside the BSC. Lay paper towels inside the centrifuge chamber, and soak with 10% bleach (or other appropriate disinfectant). Spray the inside of the centrifuge jacket with 70% EtOH. Close the lid for 20 min. Clean up following the same procedure as for a major spill outside the BSC.
		2. Sharps should be avoided whenever possible for work with agent(s)production, manipulation, and delivery. However, if there is a needle-stick, briefly bleed the wound (squeeze it to produce a couple of drops of blood), then wash thoroughly with soap and water for 15 min. Report the incident to the PI and the [NDSU Safety Office](https://www.ndsu.edu/police_safety/contact/).
		3. Other accidents might include slips, falls, or collisions with other personnel, leading to spills of agent(s). Additional help may be required in the event of personal injury, in which case assisting personnel must be made aware of the presence of uncontained agent(s) so that they can respond appropriately. In the event of a major spill involving serious personal injury or requiring rescue, call Public Safety (231-8998), the [NDSU Safety Office](https://www.ndsu.edu/police_safety/contact/), and contact the PI.

**Note:** An FAQ document on reporting can be found on the National Institutes of Health Office of [Biotechnologies Activities (OBA)](http://www.osp.od.nih.gov/office-biotechnology-activities/biosafety/institutional-biosafety-committees/incident-reporting) website; or, the [National Institutes of Health](http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines) website and search for Incident Reporting.

# Safety SOPs for the *PI Name* Laboratory when using BSL-2 Agents

***The purpose of this section is to develop SOPs that specifically outline instances during protocols where consideration for safety with a BSL-2 agent is paramount. Detailed, step-by-step protocols describing entire experiments with materials and methods are not necessary. Examples of SOPs where safety is emphasized are bulleted below:***

* ***Propagation of viruses***
* ***Experiments that require PPE in addition to a lab coat and gloves***
* ***How to properly vortex or sonicate a viable BSL-2 agent***
* ***Safety concerning the handling of human or non-human primate primary cell lines or tissues***
* ***Safety when injecting a research animal with a BSL-2 agent***

***Please enter Safety SOPs under separate headings.***

*Insert text here*

# Apendix II: Spill Response Cue Cards

***Cut out cue cards and post in a highly visible work area***

  

**SPILLS INSIDE THE BIOSAFETY CABINET**

 1. Make sure the cabinet continues to operate. Wait 5 min. to allow aerosols to be pulled through the HEPA filter.

 2. Decontaminate the surfaces within the cabinet wearing protective clothing. Gently cover the spill with absorbent paper towels and apply the appropriate disinfectant starting at the perimeter and working towards the center.

 \* Note: Examine drain pan for contents of the spill. Disinfect if needed.

 3. Discard soaked paper towels in a biohazard bag. Wipe up residual fluids. Wipe down surfaces with 70% EtOH, discarding towels in a biohazard bag.

 4. Wash hands thoroughly.

**SPILLS OUTSIDE THE BIOSAFETY CABINET**

**Small Spill (<10 mL, localized to small area)**

 1. Alert personnel in the vicinity.

 2. Check for contaminated clothing, including shoes. Decontaminate if necessary.

 3. Evacuate the room. Close door. Discard potentially contaminated PPE, remove and decon any contaminated clothing. Wash hands.

 4. Notify PI. Wait for 20 minutes to allow for aerosols to settle.

 5. Don fresh PPE: lab coat or gown, gloves, mask, eye protection.

 6. Pick up sharps with tongs & place in biohazard sharps container then cover spill with paper towels.

 7. Soak paper towels with the appropriate disinfectant, from perimeter toward the center.

 8. Allow 20 min. of contact time.

 9. Discarded towels go in biohazard bags.

 10. Wipe down spill area one final time with appropriate disinfectant.

 11. Wash hands thoroughly.

**SPILLS OUTSIDE THE BSC**

**Major Spill (>10 mL, localized to small area)**

 1. Alert personnel in the vicinity.

 2. Check for contaminated clothing, including shoes. Decontaminate if necessary.

 3. Evacuate the room. Close door. Discard potentially contaminated PPE and remove any contaminated clothing. Wash hands thoroughly.

 4. Post warning sign: “DO NOT ENTER: Biological spill!”

 5. Wait 20 min. Meanwhile, notify PI.

 6. If assistance is needed, notify the [NDSU Safety Office](https://www.ndsu.edu/police_safety/contact/)

 7. Don fresh PPE: lab coat or gown, gloves, mask, eye protection.

 8. Re-enter the room, pick up sharps with tongs & place in sharps biohazard

 container, cover spill with paper towels.

 9. Soak paper towels with appropriate disinfectant, from perimeter toward the center.

 10. Allow 20 min. of contact time.

 11. Discarded towels go in biohazard bags.

 12. Wipe down spill area one final time with appropriate disinfectant.

 13. Wash hands thoroughly.

 14. With PI, write up a report and submit to the NDSU Safet Office. Or, alternative, schedule a meeting to discuss the events with the NDSU Safety Office.

**SPILLS INSIDE AN INCUBATOR**

 Decontaminate water pan via autoclave.

 1. Alert personnel in the vicinity.

 2. Evacuate the room. Close door. Discard potentially contaminated PPE and remove any contaminated clothing. Wash hands thoroughly.

 3. Notify PI.

 4. Don fresh PPE: lab coat or gown, gloves, mask, eye protection.

 5. Pick up sharps with tongs & place in biohazard sharps container.

Cover spill with paper towels.

 6. Soak paper towels with appropriate disinfectant, from perimeter toward the center.

 7. Allow 20 min. of contact time.

 8. Discarded towels go in biohazard bags.

 9. Wipe down spill area one final time with appropriate disinfectant.

 10. Wash hands thoroughly.

**SPILLS INSIDE A CENTRIFUGE**

 1. Open lid of centrifuge slowly.

 2. If there has been no breach of containment, spray rotor with 70% EtOH.

 3. If inside of rotor is contaminated, decontaminate in the BSC. As a precautionary measure, decontaminate the centrifuge chamber.

 4. If rotor buckets are damaged, close centrifuge lid.

 5. Alert personnel in the vicinity. Evacuate room.

 7. Wait 30 min. Meanwhile, notify PI.

 8. If assistance is needed, notify the [NDSU Safety Office](https://www.ndsu.edu/police_safety/contact/)

 9. Open lid slowly and add paper towels.

 10. Spray walls of chamber and rotor with 70% EtOH.

 11. Close centrifuge lid for 20 min. contact time.

 12. Finish centrifuge clean-up as for major spill outside the BSC. Transport rotor to BSC.

 13. Open and decontaminate rotor/buckets in the BSC.

 14. Wash hands thoroughly.

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Appendix III: Door Signage

***BSL laboratory signage must be displayed on all entrance(s) to the lab and are available on the Downloadable Forms page under the*** [***University Police and Safety Office***](https://www.ndsu.edu/forms/#univ14) ***section.***

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Appendix IV: Training Certificates

***Following this page, please insert copies of training certificates for each person who has completed a training course listed in the table on page 3.***

**Appendix V: IBC Protocol and Approval**

***Following this page, please insert a copy of the lab’s IBC-approved protocol(s) and a copy of the IBC Approval Letter(s).***

Please note: All work with your BSL-2 agent(s) must be pre-approved by the IBC before experiments can begin.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Practical Requirements** | **Inactivation Efficacy** | **Potential Application** | **Characteristics** |
| **Disinfectants** | Effective Dilution | Shelf Life C (dilute/open) | Sink Disposal | bactericidal | virucidal | fungicidal | sporicidal | tuberculocidal | Skin disinfectant | hard surfaces | stainless steel | liquid for disposal | Leaves Residue | Inactivated by organic matter | Other |
| Category**Appendix VIII: Disinfectant Chart** | Brand |
| Quat. Ammon. Cpds |  | 0.1- 5.0% (1,000-50,000 ppm) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Quatricide TB | N/A (2100 ppm) |  | + | + | + | + |  | + | + | + | + |  |  | + | Parvovircidal |
|  | Quatricide PV | 1:64 (750 ppm) |  | + | + | + | + |  |  | + | + | + |  |  | + | Parvovircidal |
|  | Quatricide | 1:64 (780 ppm) |  | + | + | + | + |  |  | + | + | + |  |  | + |  |
|  | Cetylcide II | 1:64 (740 ppm) | 64 days | + | + | + | + | + |  |  | + | + |  |  | + |  |
|  | Lysol I.C. spray | N/A (1000 ppm) | 2 years | + | + | + | + | + | + |  | + | + |  |  | + |  |
|  | Lysol I.C. Quat | 1:256 (660 ppm) | 2 years | + | + | + | + |  |  |  | + | + |  |  | + |  |
|  | Madacide-FD | N/A (3080 ppm) |  |  | + | + | + | + | + |  | + | + |  |  | + |  |
|  | Morning Mist | 1:64 (780 ppm) |  | + | + | + |  |  |  |  | + | + |  |  |  |  |
|  | Roccal | 1:256 (920 ppm) |  | + | + | + | + | + |  |  | + | + |  | + |  |  |
| Chlorine Cpds**Appendix VIII: Disinfectant Chart** |  | 50,000 ppm A |  |  |  |  |  |  |  |  |  |  |  |  | + |  |
|  | A-1 bleach | 1:10 (5,000 ppm) | 1 week | + | + | + | + | + |  |  | + |  | + |  | + | corrodes metal |
|  | A-1 bleach | 1:5 (10,000 ppm) | 1 week | + | + | + | + | + | + |  | + |  | + |  | + | corrodes metal |
| Iodophor |  | 25 –1600 ppm A |  |  |  |  |  |  |  |  |  |  |  |  | + |  |
|  | Wescodyne | 1:100 (125 ppm) | years | + | + | + | + | + | + | + | + |  | + |  | + | poor residual |
|  | Betadine |  | years | + | + | + | + | + |  | + | + |  |  |  | + | poor residual |
|  | Iodine |  | years | + | + | + | + | + |  | + |  |  |  |  | + | Poor residual |
| Phenolic Compounds |  | 1.0 - 5.0% (10,000-50,000ppm) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Beaucoup | 1:100 (125 ppm) |  |  | + | + | + |  | + |  | + | + |  |  |  |  |
|  | Amphyl | 5.0% |  |  | + | + | + | + | + |  | + | + |  |  |  |  |
|  | Sporicidin | N/A (15,600 ppm) | 6 mos |  | + | + | + | + | + |  | + | + |  | + |  |  |
| Alcohols |  | 70 – 85% (700,000-850,000 ppm) |  |  |  |  |  |  |  |  |  |  |  |  |  | flammable |
|  | Ethyl & Isopropyl |  |  | + |  | + |  | + | + |  |  |  |  | + |  |
|  | Envirocide | N/A (202,000 ppm) | 2 years |  | + | + | + |  | + |  | + | + |  |  |  | Biodegradable |
|  | Opticide3 | N/A (210,000 ppm) | years |  | + | + | + |  | + |  | + | + |  |  |  |  |
|  | lysol brand spray - household | N/A (790,000 ppm) | 2 years |  | + | + | + |  |  |  | + | + |  |  |  |  |
| Aldehydes |  | 0.2 – 8.0% (2,000-80,000 ppm) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Cetylcide G | 1:16 (34,000 ppm) |  |  | + | + | + |  + 10h | + |  | + | + |  | + |  |  |
|  | Metricide Plus 30 | N/A (34,000 ppm) |  |  | + | + | + |  + 10h | + |  | + | + |  | + |  |  |
|  | Cidex OPA | N/A (5,500 ppm) | 2yr (14d) | + | + | + | + |  + 32h | + |  | + | + |  | + |  |  |
|  | Cidex Plus 28 | N/A (< 50,000 ppm) | (28d) |  | + | + | + |  + 32h  | + |  |  |  |  | + |  |  |
| Peroxide Compounds |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Chlorhexidine | Hydrogen peroxide (L) | 3-6% (30,000-60,000 ppm) |  |  | + | + | + |  + (18h) |  | + |  |  |  |  | + |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Novalsan | 1:50 (400 ppm) | 1 yr | + | + | + | + | + |  | + | + | + |  |  |  | non-toxic |