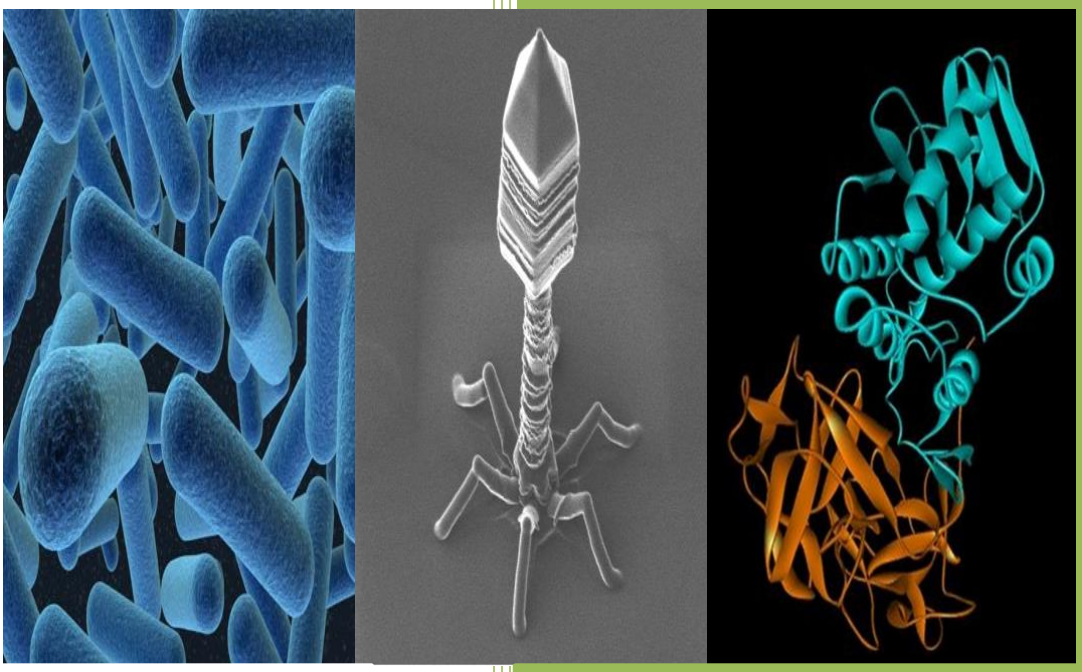
NORTHERN ARIZONA UNIVERSITY | Office of Regulatory Compliance

2017

PI Name
Biosafety Level 2 Laboratory Manual



This lab-specific manual applies to the following BSL2 agents:

1. Agent one
2. Agent two
3. Agent three
4. Agent four
5. Agent five

Template provided by:

Northern Arizona University

Environmental Health & Safety

Revised June 2017

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 2 (BSL2) laboratory conditions. The PI is responsible for including laboratory-specific safety practices, addressing hazards in their laboratory, and for adding laboratory-specific standard operating procedures for common protocols with the agent(s). Please provide lab-specific information where you see gray text fields. Also enter lab-specific protocols beginning on page 14 for work with the agent(s), items needed in the appendices, training dates for lab personnel, and cut out and display the spill procedure cards. Additions to this template are encouraged and should be sent to the Biosafety Officer for review.

In addition to this manual, the National Institutes of Health (NIH) and the OHSU Institutional Biosafety Committee (IBC) require the lab to follow BSL2 procedures as outlined in *Biosafety in Microbiological and Biomedical Laboratories, 5th Edition* (http://www.cdc.gov/biosafety/publications/bmbl5/). For research involving recombinant DNA, the lab must also follow the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (https://osp.od.nih.gov/biotechnology/nih-guidelines/).

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. 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:

Typed Name	Title	Signature	Date
First, Last	Principal Investigator		

Laboratory Staff:

Typed Name	Job/Student Title	Signature	Date
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Table of Contents

Lab Contacts and Training

Principal Investigator:	
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)**
IRB Protocol #(s) (if applicable):	**Enter number(s)**

Lab Personnel	**Relevant Training Dates**					
Name	**Biosafety and/or Bloodborne Pathogens Training**	**BBP Hepatitis B Vaccination Status (if applicable)**	**NIH Guidelines rDNA training**	**CITI Responsible Conduct of Research**	**Dangerous Goods Shipping**	**Chemical Hygiene**
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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 a designated technical expert. The worker should demonstrate good microbiological skills and an understanding of this manual and all applicable protocols prior to being permitted to work with agent(s).

Background

Describe the known risks to be considered when working with this 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.

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***

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·~·~·~·~·~·~·~·~·~·~·~·~·~·**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 specific laboratory practices that pose potential for exposure, such as those that could create aerosols. An exposure risk example for lentivirus is shown below.

Describe any and all medical surveillance that is provided for laboratory personnel (e.g. physical before beginning work with pathogen(s), vaccinations and prophylaxis that are available, etc)

If uncertain if your procedures would provide an exposure risk, contact a Biosafety Officer (see Appendix I for contact information). Also, as stated in the example, immunocompromised individuals are encouraged to self-identify with Employee/Student Health prior to working with lentivirus.

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 with Employee/Student Health 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 Appendix VIII for a disinfectant chart. An example for lentivirus inactivation is below. Contact a Biosafety Officer/Specialist if assistance is needed.

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 prior to each work session, when needed. 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.

Biosafety Requirements and Procedures

1. **Physical Containment.** All work with agent(s) must be performed in a BSL-2 laboratory. When agent(s) is/are present, a sign must be posted at the entrance to the lab. This sign must include the biosafety level (BSL-2), 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 Appendix III). Additionally, incubators and freezers must bear biohazard warning labels. (Please contact a Biosafety Officer to request biohazard stickers). Doors to the laboratory should be lockable and self-closing. Laboratory windows that open to the outdoors must be fitted with fly screens. A room suitable solely for tissue culture (if applicable to the research) with negative airflow and a closing door, equipped with a Class II Biosafety Cabinet, must be present (see below for more information on BSCs).

2. **Safety Equipment.**

   a. **Biosafety Cabinet:** An annually certified Class II Biosafety Cabinet (BSC) must be used to contain any experiments that may generate an aerosol or splash. 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 the BSL2 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. Note that HEPA filtered exhaust air from a Class II BSC can be safely re-circulated back into the laboratory, but only if the BSC is certified annually. Alternatively, the BSC exhaust can be fed to the laboratory exhaust via canopy or direct connection. If the blower on the BSC is not left on continuously, it should be turned on and run for 5 minutes to allow 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. When double-gloving (optional), remove the outer pair of gloves and deposit in a solid waste bag before removing hands from the BSC. 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.

   b. **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.

   c. **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. Low-speed swinging-bucket centrifuge buckets must be equipped with aerosol-tight safety covers. Microcentrifuges must have aerosol-tight rotors capable of being removed while sealed so that the rotor can be unloaded in the BSC.

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.). Remove potentially contaminated gloves and replace them with new gloves before touching anything outside the BSC, such as the refrigerator, centrifuge, or incubator.

Certain procedures may require additional PPE. Contact a Biosafety Officer as appropriate.

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.
Toxins: Gloves, Lab coat, Safety glasses when working with toxin outside a BSC or when using powder form of toxin.

Please edit the information below to reflect current handling and disinfection practices for your agent(s). Contact a Biosafety Officer if you have any questions or concerns.

4. **Spill Kit.** The lab must have a spill kit, or the components of such, readily accessible in the event of a spill. The spill kit should have:
    - an easy-to-read outline of the spill response SOP,
    - gloves,
    - surgical masks,
    - safety glasses or goggles,
    - clean lab coat, disposable gown or clean scrubs and spare slip-on shoes (Crocs are not recommended because they do not fully enclose the feet) in case clothing not covered by lab coat becomes contaminated,
    - paper towels to absorb contaminated liquids,
    - disinfectant (e.g., 10% bleach),
    - tongs or forceps to pick up broken glass,
    - a biohazard waste container large enough to handle wet, contaminated paper towels.
5. **General Procedures for working with agent(s).** Standard BSL2 practices should be employed, conforming to the BMBL and NAU Biosafety Manual (see link below) 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. NAU Biosafety Manual link:

    http://nau.edu/uploadedFiles/Administrative/Research/Compliance/_Forms/Biosafety-Manual.pdf

    Additional practices include the following recommendations:

    a. Whenever possible, work with agent(s) during normal working hours, to enable adequate response to a severe adverse incident.

    b. Sharps should be avoided whenever possible in a BSL-2 laboratory. 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. *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 biohazardous sharps container, but must never be discarded in regular trash.

    c. Solid Waste: Everything that contacts agent(s)-containing solutions or vessels must be decontaminated or contained before being removed from the biosafety cabinet. Solid waste can

be collected in a biohazard bag inside the Biosafety Cabinet. Pipette tips can be collected in a disposable plastic or cardboard box, and the box closed and deposited into the biohazard bag (inside the Biosafety Cabinet!) at the end of the work session. At the end of the work session, the biohazard bag must be closed, sprayed with 70% EtOH (or other appropriate disinfectant), and deposited into a biohazard waste container. When the biohazardous waste container(s) are full, please create a service ticket through the orange "Service Request" button on the EH&S website, http://nau.edu/research/compliance/environmental-health-and-safety/.

d. 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. For agent(s) work, engineer a way to anchor the end of the vacuum tubing to the outside of the sash or frame of the Biosafety Cabinet. 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. A simple 500 ml bottle containing disinfectant may be suitable to collect non-aspirated liquid waste.

e. Centrifugation: Centrifuge tubes should be prepared and sealed in the biosafety cabinet. This includes methods to ensure tubes are properly balanced (unless the balance tube contains no infectious material). Fixed angle rotors should be loaded in the BSC as well, and the entire rotor sprayed with 70% EtOH before removal of the rotor from the BSC. For ultracentrifugation with swinging bucket rotors, individual buckets can be prepared in the BSC, securely closed, wiped down with 70% EtOH, and then transported to the centrifuge in the respective rack for those buckets . When safety cups are used (for low-speed centrifugation to clarify viral supernatants), the aerosol-tight safety cups must be loaded, closed, wiped down with 70% EtOH (or other appropriate disinfectant) prior to removal from the BSC; they must also be unloaded in the BSC. After centrifugation, the centrifuge lid must be opened cautiously and the rotor quickly visually inspected for a failure which could have generated aerosols in the centrifuge chamber. The rotor and chamber must be misted with 70% EtOH (or other appropriate disinfectant), and the rotor (or swinging buckets/safety cups) transported into the BSC for further work. At the end of the procedure, rotors and/or buckets must be decontaminated.

f. Vortexing must be done inside the BSC.

g. If tissue culture dishes are used for agent(s) production, they must be transported to an incubator (clearly marked with a warning label to indicate that agent(s) is/are present) in a secondary, closed container in case liquid media sloshes out of the dishes during transport (see Accidents and Spills). A Tupperware-type container will work, and the lid of the Tupperware container can be removed or left ajar once the container is in the incubator, to enable gas exchange. To remove the tissue culture dishes from the incubator, close the Tupperware container with the lid before taking the dishes out of the incubator.

h. Storage: Agent(s) stocks must be in leak-proof secondary containers in a -80°C freezer, that is clearly marked with a warning label to indicate that agent(s) is/are present.

6. **Accidents and spills**

a. Spills

i. Small spills inside the BSC: First, lower the sash for 5 minutes to allow the blower to move aerosols through the HEPA filter. 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 (≤50 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. Small spills inside the BSC that do not involve an exposure do not require notification of the IBC or Biosafety Officer, but do require notification of the PI, who will direct further training (e.g. retraining on pipetting techniques, or organization of materials and instruments in the BSC) to minimize the risk of recurrence. 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 be deposited in the biohazard bag in the BSC.

ii. Large spills inside the BSC (spills over 50 ml, with likely splattering of droplets outside the BSC): Large spills should be treated more cautiously. Leave the BSC running. Remove gloves (or outer gloves, if double-gloved) before touching the door knob. Close the door to the room as you leave, remove PPE and any contaminated clothing (check the sleeves of your lab coat), and place it in sealable plastic container or a biohazard bag. Everyone in the room at the time of the spill should thoroughly wash their hands, using disinfectant soap. Notify the PI. 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 campus Biosafety Officer (see Appendix I for contact information). Allow 20 min. for any potential aerosols to settle. Don clean PPE, re-enter the room, cover the spill with paper towels, soak with appropriate diluted disinfectant, starting at the perimeter and working inward toward the center. Allow 20 min. 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 Biosafety Officer/Specialist does not need to be informed. The PI should review the incident to revise procedures to minimize the risk of recurrence.

iii. 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 is 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 (or outer gloves, if double-gloved) 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 campus Biosafety Officer (see Appendix I for contact

information). After 20 min., don fresh PPE, re-enter the room, 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. Leave the room during this time. 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.

iv. 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 (or outer gloves, if double-gloved), 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 and the Biosafety Officer (see Appendix I for contact information). If the spill is too difficult to manage alone, seek help from the Biosafety Officer. After 20 min. don fresh PPE, re-enter the room, 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. 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. 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, must be reported to a Biosafety Officer.

b. 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).

i. 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 contact the Biosafety Officer/Specialist (see Appendix I for contact information). If the contamination is too extensive to manage alone, ask the Biosafety Officer for assistance. Decontamination is similar to a major spill outside the BSC. Lay paper towels inside the centrifuge chamber, and

soak with 10% bleach. Spray the inside of the centrifuge jacket with 70% EtOH. Close the lid for 20 min. Clean up as for a major spill outside the BSC.

ii. 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 Biosafety Officer.

iii. 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 a Biosafety Officer (see Appendix I for contact information), and contact the PI.

iv. Follow-up of exposures or injuries involving agent(s)*:* Follow-up of laboratory injuries or documented, overt exposures to agent(s) will be conducted through NAU Campus Health.

The working hours for Campus Health are 8:00AM-5:00PM, Monday-Friday, and 11:00AM-2:00PM on Saturday. Flagstaff Medical Center is always available for life-threatening injuries, serious injuries, or overt exposures such as needle-sticks outside normal working hours.

In the event of an exposure or injury the following steps should be followed:

1. Remove soiled clothing and wash exposed area with soap and water, if appropriate.
2. Administer first aid as appropriate to the exposure.
3. Immediately notify attending physician/supervisor of exposure.
4. Employee shall present at the NAU Health Center, ER, or Urgent Care for assessment and initial prophylactic treatment, if applicable. If employee goes to a private physician, download the Workers Compensation Physician Information Sheet from http://hr.nau.edu/sites/default/files/files/workers_comp_info_sheet.doc
5. Following the incident, the supervisor must report all employee-related injuries by:
    I. Fill out the Report of Significant Exposure to Bodily Fluids
    II. Fill out the Supervisor's Report of Injury Form (SRI) and send to NAU HR
    III. Call State of Arizona, Workers' Compensation Early Claims Reporting Services at 1-800-837-8583 prior to the end of the shift on the day of the occurrence.
6. For Blood/Body Fluid Exposures: Following the incident, the health care provider shall immediately make available to the affected employee a copy of all the employee's records relating the treatment and follow up, and if and when available, results regarding the HIV, HBV, and HCV status of the source, to the extent permitted by the law.

Specific Protocols for the *PI Name* Laboratory when using *BSL-2 Agent(s)*

Please enter laboratory-specific protocols in this section under separate headings for each BSL-2 agent in use. An example protocol is provided below.

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

(specific to this lab or the agents in use)

Production of Lentivirus

Day 1 - Transfection

Materials:

$3x10^7$ 293FT cells (about 3 confluent T75 flasks)
Two $150cm^2$ tissue culture dishes
15 µg pLenti 6.4 EmGFP
45 µg equimolar mixture of pLP1, pLP2, and pLP/VSVG (ViraPower Mix)
180 µl Lipofectamine 2000
12 ml Opti-Mem

Procedure:

1. Add Lipofectamine to 6mL Opti-Mem. Mix by inverting 5 times.
2. Add DNA to 6mL Opti-Mem. Mix by inverting 5 times.
3. Wait 5 minutes, then combine the 2 mixtures. Mix by inverting 5 times.
4. Allow 20 minutes for DNA/Lipofectamine complex to form. Meanwhile …
5. Trypsinize 293FT cells and resuspend in media WITHOUT Geneticin
6. Add $3x10^7$ cells in 15mL media to each dish.
7. Add 6mL DNA/Lipofectamine/Opti-Mem to each dish. Mix by gentle rocking.
8. Incubate at 37°C, 5% CO_2.

Day 2 - Change Media

Remove media and replace with 17mL fresh media WITHOUT Geneticin in each dish.

Day 3 - Virus Production

Day 4 - Harvest and Concentration by Ultracentrifugation

*** All handling of virus must be done in biological safety cabinet ***

Procedure:

1. Sterilize 2 Beckman ultracentrifuge tubes under UV in tissue culture hood for 30 min.
2. Cool SW28 rotor in ultracentrifuge to 4°C.
3. Collect media in a 50-ml conical tube.
4. Centrifuge at 500g for 10 min at 25° C to remove cells and large cell debris.
5. Filter supernatant through 0.44µm filter into ultracentrifuge tube
6. Layer 4ml of 20% sucrose in 20 mM HEPES into the bottom of ultracentrifuge tube
7. Centrifuge in SW-28 for 2 hours at 22,000 rpm and 4° C.
8. Discard supernatant and invert tube to dry. There should be a miniscule pellet.
9. Re-suspend the pellet in 100 µl of PBS by gently pipetting up and down. Avoid the formation of bubbles.
10. Place the ultracentrifuge tube into a 50-ml conical tube and close the lid. Wrap lid with parafilm. Incubate the tubes at 4°C for 2 h. Vortex gently every 20 min.
11. Centrifuge at 500g for 1 min at 25°C to collect the virus-containing liquid.
12. The virus may now be titered and/or frozen in aliquots at -80°C. Screw-cap microfuge tubes are recommended, since snap-cap tubes may release aerosols upon opening.

Reference: Kutner RH, Zhang X-Y, and Reiser J (2009) Production, concentration and titration of pseudotyped HIV-1-based lentiviral vectors. Nature Protocols 4:497-505.

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Appendix I: Contact Information

NAU Campus Contacts

EMERGENCY

NAU Police	928-523-3000 or 911

ENVIRONMENTAL HEALTH & SAFETY

Shelley Jones	928-523-7268	office
Biosafety Officer	928-523-0050	fax
Director of Biological Safety	480-248-0741	cell
	shelley.jones@nau.edu	
Janelle Runberg Baron	928-523-4782	office
Assistant Biosafety Officer	480-516-1072	cell
	janelle.runberg@nau.edu	
Emily Kaufman	928-523-1746	office
Health and Safety Specialist	928-533-7442	cell
	emily.kaufman@nau.edu	
Jim Biddle	928-523-6109	office
Manager of Industrial Hygiene &	928-220-1728	cell
Environmental Programs	jim.biddle@nau.edu	
Garett Hall	928-523-1146	office
Environmental Compliance	928-607-3059	cell
Technician	matthew.freyer@nau.edu	
Michael Kelly	928-523-5903	office
Assistant Chemical Hygiene Officer	928-308-6507	cell
Hazardous Waste Supervisor	michael.j.kelly@nau.edu	
Sarah Ells	928-523-3961	office
Loss Prevention Coordinator	928-607-6857	cell
EH&S Program Coordinator	928-523-1607	fax
Training & Manuals	sarah.ells@nau.edu	

FACILITIES SERVICES

Facilities office	928-523-4227

MEDICAL SERVICES

Campus Health Services	928-523-8995
Flagstaff Medical Center	928-779-3366 or 911

SHIPPING/RECEIVING

Campus Supply	928-523-1108

Appendix 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.

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 room air exchanges to clear aerosols through room exhaust.
5. Don fresh PPE: lab coat or gown, gloves, mask, eye protection.
6. Cover spill with paper towels.
7. Soak paper towels with the appropriate disinfectant, from perimeter toward the center.
8. Allow at least 15 min. of contact time (use a contact time that is appropriate for the disinfectant and the organism). Work can continue during contact time.
9. Discarded towels go in biohazard bags. Pick up sharps with tongs & place in sharps container.
10. Wipe down spill area one final time with appropriate disinfectant.

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 and a Biosafety Officer.
6. If assistance is needed, discuss with Biosafety Officer.
7. Don fresh PPE: lab coat or gown, gloves, mask, eye protection.
8. Re-enter the room, cover spill with paper towels.
9. Soak paper towels with appropriate disinfectant, from perimeter toward the center.
10. Allow at least 15 min. of contact time (use a contact time that is appropriate for the disinfectant and the organism). Work can continue during contact time.
11. Discarded towels go in biohazard bags. Pick up sharps with tongs & place in sharps container.
12. Wipe down spill area one final time with appropriate disinfectant.
13. With PI, write up a report and submit to the Biosafety Officer.

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. Cover spill with paper towels.
6. Soak paper towels with appropriate disinfectant, from perimeter toward the center.
7. Allow at least 15 min. of contact time (use a contact time that is appropriate for the disinfectant and the organism). Work can continue during contact time.
8. Discarded towels go in biohazard bags. Pick up sharps with tongs & place in sharps container.
9. Wipe down spill area one final time with appropriate disinfectant.

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 and a Biosafety Officer/Specialist (4-0655, 4-2580).
8. If assistance is needed, discuss with Biosafety Officer.
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. With PI, write up a report and submit to Biosafety Officer

Appendix III: BSL-2 Door Signage

Please complete the fields in the sign on the next page. Display a copy of the sign on the main entrance(s) to the lab.

Room No.:

BSL-2 Laboratory

BIOHAZARD

Authorized Personnel Only

Biosafety Level 2 Biological Agents:	Enter agents here
Special Procedures, PPE, or Precautions for Entry/Exit:	Add information here

Principal Investigator		**Emergency Contact (must be 24/7)**	
Name	**Phone**	**Name**	**Phone**
Type name	Enter phone #	Type name	Enter phone #

Appendix IV: Risk Assessments

Insert completed Health Hazard Assessments for organisms here. Contact EH&S if you need a form.

If you have requested and received approval from the Biosafety Officer for a laboratory protocol that deviates from the BMBL, then add the protocol deviation(s) here:

Appendix V: IBC Protocol and Approval

Please insert a copy of the IBC-approved Biological Agent Registration Form. Also include a copy of the IBC Approval Letter.

Please note: All work with your BSL2 agent(s) must be pre-approved by the IBC before experiments can begin.

Appendix VI: IACUC Protocol and Approval

Please insert a copy of the IACUC-approved protocol(s). Also include a copy of the IACUC Approval Letter.

Appendix VII: IRB Protocol and Approval

Please insert a copy of the IRB-approved Biological protocol(s). Also include a copy of the IRB Approval Letter.

Appendix VIII: Inventory Records Example

Please refer to the following table for guidance when creating an inventory spreadsheet. Please indicate in this section the procedure for updating and verifying the accuracy of the inventory spreadsheet. Please contact the Biosafety Officer with any questions or concerns.

Sample Label/ID	Genus & Species	Building	Room #	Storage location: freezer/fridge /cabinet	Storage Location: box/shelf/ container	Sample Type
Bp1013	*Bordetella pertussis*	SLF, Building 17	112	-80 Freezer #1	B. pertussis Box 4	Cryovial Glycerol Stock
Cd0075	*Corynebacterium diphtheriae*	SLF, Building 17	112	Refrigerator #2	Tube rack 5, shelf 2	Slant