BSL-2+ Safety Manual

Version 1.1

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Contents

Scope

To provide a general overview of the facilities and procedures for all personnel working in BioLabs BSL2+ designated spaces.

Introduction

The BSL-2+ facilities involve moderate to high-risk agents and therefore requires a strict adherence to BSL-2 containment with BSL-3 work practices and procedures. It is important that all personnel that work in a BSL-2+ facility understand and adhere to the proper procedures and techniques outlined in this manual. Failure to adhere to appropriate practices and procedures may endanger others.

Information About Agents In Use.

Unfixed human cells from blood, body fluid, or other tissues can be used in the BSL-2+ spaces. All untested human samples should be considered potentially infectious for HIV, hepatitis viruses and other bloodborne pathogens, which can infect humans through exposure of mucosal membranes to aerosol, broken skin, or aerosol inhalation. BioLabs requires all personnel to observe universal precautions when handling live human cells. Since hepatitis B virus is a bloodborne pathogen, immunization is strongly recommended.

Retroviral and lentiviral transfected cells may be used in this space and are to be prepared and handled according to BioLabs Guidelines. The retroviral and lentiviral vectors must be replication-defective; however, they may be inserted with genes that could be oncogenic.

BSL-2+ Personnel Requirements

All individuals working in a BSL-2+ space must be trained according to the compliance policies of BioLabs. Personnel will receive training as necessary for activities covered within this manual and any additional training due to procedural or policy changes. Additionally, the Lab Operations Manager or designee will conduct training for all personnel in the area, covering the potential hazards associated with the work, the necessary precautions to prevent exposures, exposure evaluation procedures, and the standard operating procedures of a BSL-2+ laboratory. Resident companies are responsible for training their employees concerning the specific hazards of the agents they will be working with and the proper laboratory techniques to use to avoid injury and illness. New resident company employees must be trained prior to assignment in the lab.

Hygiene And Good Laboratory Practices

Basic hygiene

It is mandatory that all personnel wear personal protective equipment (lab coats, safety glasses, and gloves) when handling human specimens or working with any BSL-2+ materials. When cleaning the inside of a Class II biosafety cabinet, a face protection shield or goggles, and a surgical mask and double gloves must be worn. All personnel must wash their hands after removing gloves. Eating, drinking, storing food, handling contacts, and applying cosmetics are not permitted in the laboratory. Food must not be stored in refrigerators or freezers in the laboratory.

All unfixed specimens should be handled in a Class II safety hood using universal precautions. Mandatory laboratory practices include use of mechanical pipetting, use of plastic instead of glass, minimizing the use of sharps, labeling equipment with appropriate biohazard stickers, and minimizing work with infectious substances on the open bench.

Safety Training

No one is permitted to work with BSL2+ materials without having prior training by the BioLabs Safety Officer or designee in addition to all other required safety training; training records are to be maintained by the BioLabs Safety Officer. The worker should demonstrate good microbiological and tissue culture technique and an understanding of this guidance prior to being permitted to work with BSL2+ materials.

Exposure Risk

The most probable route of exposure for this work would be dermal via sharps (needle-sticks), absorption through exposed scratches or abrasions on skin, or mucous membrane exposure of the eyes, nose, and mouth. Another route would be inhalation via aerosols depending on the use of equipment such as centrifuges or vortex mixers. Care must be taken when pipetting to avoid splashing or generation of aerosols. Immunocompromised individuals should not work with BSL2+ materials.

Inactivation And Surface Decontamination

Viral particles can be inactivated with a number of reagents, including (final concentrations) 20% bleach*, 5% Amphyl (phenolic), and 0.5% Wescodyne (iodophor). This manual has been written for the use of bleach, but alternative disinfectants can be substituted, provided they are known to be effective for the BSL-2+ material in use.

**A note on bleach - Bleach is effective, inexpensive, but volatile and corrosive. Bleach-soaked paper towels should not be autoclaved because autoclaving releases chlorine, a chemical hazard, and could corrode the autoclave over time. 20% bleach solutions should be prepared fresh prior each work session. If 20% bleach is used to decontaminate a spill within the Biosafety Cabinet (BSC), once the spill has been absorbed on paper bowels and disinfected with 20% bleach, the BSC should be wiped down with 70% Ethanol (EtOH) to remove residual bleach. All bleach-soaked items should be packaged separately and then discarded in regular solid waste.*

Biosafety Level 2+ Area Access

Only authorized personnel should enter the BSL-2+ area. Authorization is granted once the guidance document for BSL-2+ work has been read and a declaration signed whereby the user declares that they have read and understood the BSL-2+ Safety Manual.

Work with BSL-2+ materials is only permitted during regular business hours (8:00am to 5:00pm Monday to Friday) to ensure worker safety in the event of an accident. The BSL-2+ area is a multi-use space and therefore it is extremely important that all BSL-2+ processes and procedures are followed to ensure everyone’s safety.

Procedures for Working with BSL-2+ Materials

Physical Containment

In general, all work with viral vectors must be performed in a BSL-2+ laboratory. This includes but is not limited to a room suitable for tissue culture with negative pressure and a closing door and equipped with a certified Class II Biosafety Cabinet (BSC), and a dedicated tissue culture incubator. During work with viral particles, a warning sign must be posted on the door alerting personnel of the presence of the hazardous material being used. 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 required), containing 20% bleach. Alternatively, using pipet aspiration and pooling supernatants for bleach sterilization in place of vacuum aspiration for virus-contaminated waste can be utilized if virus will be concentrated in a centrifuge, 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.

Personal Protective Equipment (PPE)

The following PPE must be worn when working with BSL-2+ vectors: double gloves, a lab coat (preferably disposable), eye protection, and face mask. A surgical mask and eye protection (goggles) or face shield is required any time there is a risk of a splash of BSL-2+ materials to the face outside the BSC. It is required that double gloves be worn when working directly with BSL-2+ materials, with particular attention to ensuring bare skin at the wrists is covered. Another suggestion is the use of gloves with longer than standard wrists and tucking the cuffs of the lab coat sleeves into the gloves or disposable sleeves. Remove potentially contaminated gloves and replace them with new gloves before touching anything outside the BSC, such as the refrigerator, centrifuge, or incubator.

Spill Kit

The lab must have a spill kit, or the components of such readily accessible in the event of a spill. This comprises: an easy-to-read outline of the spill response requirements; gloves, masks, goggles; clean lab gown or lab coat, 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., 20% bleach); tongs or forceps to pick up broken glass; a waste container large enough to handle wet, bleach-soaked paper towels.

Decontamination Of Solid Waste

Solid wastes generated while using BSL-2+ material such as unknown human and non-human primate cells or lenti- or retroviral-transfected cells (e.g., pipettes tips, tubes) will be deposited into a beaker containing 1:10 dilution of household bleach to be located within the designated BSC(s). Waste materials must soak in bleach for at least 15 minutes before removing from the BSC. Bleach-decontaminated waste will be strained in the sink. Decontaminated waste and used gloves will be disposed of in biohazardous waste. If the facility autoclaves biohazardous waste, then the solid waste must be rinsed with water before being placed in biohazardous waste.

Exit Procedures

Before leaving the work area, all solid and liquid waste is to be disposed of properly. All equipment exposed to potentially biohazardous materials will be disinfected and returned to their correct place in the lab. Gloves and masks must be removed and disposed of in the biohazard solid waste container before leaving the lab. Uncontaminated, regular lab coats must be placed in the laundry hamper. Disposable lab coats are placed in the biohazardous waste. Regular lab coats with significant contamination must be disposed of in biohazardous waste.

Lentiviral Vectors

Background

Lentiviruses are members of the viral family *Retroviridae* that are characterized by their use of viral RT (reverse transcriptase) and IN (integrase) for stable insertion of viral genomic information into the host genome. Unlike other retroviruses, lentiviruses have the capability of infecting both dividing and non-dividing cells. Lentiviruses include viruses such as human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV) and human T-lymphotropic virus (HTLV) that can infect humans. Transmission occurs by penetration of the skin by puncture or absorption through scratches, cuts abrasions or other lesions and through mucous membranes of the eye, nose, and mouth. The infections by these viruses are characterized to be chronic and persistent. Lentiviral vectors offer several attractive properties as gene-delivery vehicles. Lentiviral vectors based on HIV-1 are the most currently used in basic biology and translational studies such as stable transgene overexpression and persistent gene silencing.

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. 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-l 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.

General Procedures for Working with Lentivirus

Standard BSL2 practices should be employed, conforming to CDC publication, "Biosafety in Microbiological and Biomedical Laboratories", including a prohibition of eating, drinking, food storage, handling of contact lenses, applying lipstick or lip balm, mouth pipetting, and a requirement of appropriate PPE. Additional practices include the following recommendations:

- Work with lentiviral vectors must be done during normal weekday working hours, to enable adequate response to a severe adverse incident.
- Biosafety Cabinet - 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 can begin. At the beginning of the work session, plastic-backed absorbent toweling can be placed on the work surface (optional), but not obstructing air flow. Alternatively, the stainless-steel work surface can be wiped down with 70% EtOH. When double-gloving, remove the outer pair of gloves and deposit in a biohazard 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.
- Sharps should be avoided whenever possible. Plastic aspirating pipets should be substituted for glass Pasteur pipets. If needles are required, they must never be re-capped, and must be disposed of in a rigid red sharps waste container. 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.
- Solid Waste - Everything that contacts virus-containing solutions or vessels must be decontaminated or contained before exiting the biosafety cabinet. Solid waste can be collected in a biohazard bag inside the Biosafety Cabinet. Pipet tips can be collected in a disposable plastic box (e.g., an empty P-1000 box), and the box closed and deposited into the biohazard bag (in the Biosafety Cabinet!) at the end of the work session. At the end of the work session, the biohazard bag will be closed, sprayed with 70% EtOH, and deposited into a biohazardous waste container.
- Liquid Waste is normally aspirated into a vacuum flask containing 1/10 volume concentrated bleach, or 1/20 volume Amphyl, or 1/40 volume Wescodyne. A common practice is to anchor the end of the vacuum tubing to the outside of the sash or frame of the Biosafety Cabinet. For lentiviral work, liquid waste shall be pooled and then be treated with bleach, to a final concentration of at least 20%, in the hood, allowing a minimum time of 30 minutes to inactivate virus. A 500 ml bottle with 100 ml concentrated bleach may be suitable to collect virus-containing, non-aspirated liquid waste.
- 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 (e.g., SW28), 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 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 immediately visually inspected for a failure which could have generated aerosols in the centrifuge chamber. The rotor and chamber must be misted with 70% EtOH, 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.
- Vortexing must be done in the BSC.
- If tissue culture dishes are used for Lentiviral production, they must be transported to an

incubator (clearly marked with a warning label to indicate that lentivirus is present) in a secondary, closed container in case liquid media sloshes out of the dishes during transport (see Accidents and Spills). A Tupperware-like container will work, and the lid of the Tupperware-like 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.

- Storage of lentiviral stocks must be in leak-proof secondary containers (i.e., freezer boxes) in a - 80°C freezer clearly marked with a warning label to indicate that lentivirus is present.
- Animal Work - Injections of lentiviral particles into rodents do not present a potential hazard other than autoinoculation during injection. Injected rodents can be housed at ABSL1. However, some experiments may call for transduction of human cells *in vitro*, followed by injection of the human cells into rodents. Because the human cells could allow replication of RCL which might conceivably be present, these animals should be housed in ABSL2.

Accidents and Spills

All spills possibly containing BSL-2+ materials, regardless of containment breach, shall be reported to the BioLabs Safety Officer 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.

In the event of a spill with the possibility of aerosols forming outside of the BSC (this includes spills/accidents in centrifuges), additional PPE must be worn: a face mask and goggles (included in the spill kit) must be worn to prevent possible contamination of mucous membranes.

Minor Spills in 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 (<25 ml) can be decontaminated by layering paper towels soaked in l0% bleach on top of the spill, allowing 20 minutes for the bleach to inactivate virus, then depositing the paper towels in the solid waste bag in the BSC (not the biohazard bag).
- Residual bleach can be wiped off with paper towels sprayed with 70% EtOH, and the towels deposited in the solid waste bag.

Note - a spill of media or buffer not containing virus is not a biohazard per se, but paper towels used io wipe it up should be deposited in the biohazard bag in the BSC.

Major spills in the BSC

Over 25 ml, with likely splattering of droplets outside the BSC) should be treated more cautiously.

- Leave the BSC running and evacuate all personnel from the room; remove gloves or outer gloves, if double-gloved, before touching the doorknob.
- 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 containers or a biohazard bag.
- Everyone in the room at the time of the spill should thoroughly wash their hands and face, using disinfectant soap.
- Post a warning sign on the door of the lentiviral room advising personnel not to enter.
- Notify a supervisor and the BioLabs Safety Officer.

- If you are absolutely sure that there has been no exposure and no breach of containment, proceed as for a small spill in the BSC.
- If there has been overt exposure (e.g., actual contact of bare skin with virus), wash skin with soap and water for 15 minutes, and contact BioLabs Safety Officer.
- Allow 30 minutes for possible aerosols to settle.
- Don clean PPE, re-enter the room, cover the spill with paper towels, soak with 20% bleach (or 5% Amphyl, or 2.5% Wescodyne), starting at the perimeter and working inward toward the center.
- Allow 20 minutes to inactivate the virus.
- Discard soaked towels in solid waste.
- The interior of the BSC should be decontaminated by wiping down the walls, sash, and equipment with disinfectant (20% bleach and then 70% EtOH).
- Autoclavable equipment (e.g., racks, some pipettors, and tube containers) should be autoclaved, if feasible.
- If the spill has inundated the BSC drain pan, more extensive decontamination must be carried out:
    - 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 with 20% bleach, 5% Amphyl or 2.5% Wescodyne, flushed with water and the drain tube removed.
    - Again, after decontamination with corrosive disinfectants, remember to wipe down the BSC with 70% EtOH to remove residual chemicals.

Note: Extensive spills might require a complete decontamination of the BSC and all internal workings using paraformaldehyde. In this case, please contact the BioLabs Safety Officer to have this scheduled.

Minor spills outside the BSC

A minor spill 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 l50mm dish contained inside a closed Tupperware container does not constitute a spill outside the BSC, since there is no breach of containment—as long as the Tupperware 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:
    - If PPE is contaminated, (gloves, lab coat, pants cuffs, and especially shoes!), bare skin is exposed, or if liquid has splashed over a large area.
    - If shoes are visibly contaminated, decontaminate them with 20% bleach, or other disinfectant, then evacuate the room (remove gloves, or outer gloves, if double- gloved, before touching the doorknob), closing the door.
- 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 minutes for aerosols to settle.
- During this time, notify a supervisor and the BioLabs Safety Officer.
- After 30 minutes, don fresh PPE, re-enter the room, cover the spill with paper towels, then soak them with 20% bleach (or 5% Amphyl, or 2.5% Wescodyne), starting at the periphery and moving inward toward the center.

- Be sure to check for and to decontaminate small splashes beyond the main affected area.
- Leave the soaked towels in place for 20 minutes to inactivate virus.
- Leave the room during this time.
- After the 20 minutes inactivation time, transfer soaked paper towels to solid waste.
- Wipe up the residual spill with more paper towels.
- Give the area a final wipe-down with paper towels sprayed with 70% EtOH.

Major spills outside the BSC

A major spill 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. Dropping a tightly closed Tupperware container with six 150 mm dishes of HEK293T cells producing Lentiviral particles does not constitute a spill as long as the Tupperware container does not come open—just carry the container back to the BSC prior to decontamination.

- 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 30 minutes for aerosols to settle.
- During this time, notify a supervisor and the BioLabs Safety Officer.
- If the spill is too difficult to manage alone, seek help from the BioLabs Safety Officer.
- After 30 minutes don fresh PPE, re-enter the room, cover the spill with paper towels, and soak the towels with 20% bleach (or 5% Amphyl, or 2.5% Wescodyne), working from the outside toward the center.
- Allow 20 minutes for virus 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 container for decontamination.
- Pick up soaked paper towels, and transfer to solid waste.
- Wipe up residual spill with more paper towels.
- Give the area a final wipe-down with paper towels sprayed with 70% EtOH.

Note: Spill Response Cue Cards can be found in Appendix A.

Accidents

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

Centrifugation

- In the event of a tube failure (sudden clunking or automatic shut-down due to imbalance):
  - Leave the centrifuge lid closed for 30 minutes to allow aerosols to settle.
  - During this time, notify a supervisor.
  - 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, decontaminate the tube and

bucket inside the BSC (use your own judgment regarding recovery of viral pellets).
    - 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 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 minutes.
    - Clean up the soaked paper towels as for a major spill outside the BSC.
- In the event of a catastrophic failure in the centrifuge which damages the centrifuge and possible release of virus into the centrifuge chamber:
    - Keep the centrifuge lid closed for 30 minutes.
    - During this time, notify a supervisor and contact the BioLabs Safety Officer.
    - If the contamination is too extensive to manage alone, ask the BioLabs Safety Officer for assistance.
    - Decontamination is similar to a major spill outside the BSC.
    - Lay paper towels inside the centrifuge chamber, and soak with 20% bleach (or 5% Amphyl, or 2.5% Wescodyne).
    - Spray the inside of the centrifuge jacket with 70% EtOH.
    - Close the lid for 20 minutes to inactivate virus.
    - Clean up as for a major spill outside the BSC.

Sharps

Sharps should be avoided whenever possible for work on lentiviral production 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 minutes. **Report the incident to a supervisor and the BioLabs Safety Officer immediately.**

Other Accidents

Other accidents might include slips, falls, or collisions with other personnel, leading to spills of virus. 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 virus so that they can respond appropriately. **In the event of a major spill involving serious personal injury or requiring rescue, call Emergency Services at 911, the BioLabs Safety Officer, and a supervisor.**

Follow-up of exposures or injuries involving Lentiviral particles

Follow-up of laboratory injuries or documented, overt exposures to lentiviral particles will be conducted by the BioLabs Safety Officer. In the event of an exposure or injury the following steps should be followed:

- Determine the severity of the injury/exposure. If it is life-threatening, call EMS immediately (911). They will help you decide whether to go directly to the ER or wait for emergency responders.
- If the injury/exposure is non-life-threatening, call the BioLabs Safety Officer.
- If you are injured/exposed and need to leave the lab for treatment, delegate responsibility to a colleague for any clean-up or decontamination that may be necessary. Have the colleague document what happened.
- Monitoring after potential exposure to a potentially infectious agent (e.g., a needlestick with uncharacterized primary human cells or blood) will be conducted by a physician.
- All work-related injuries should be reported to the BioLabs Safety Officer.

Special considerations for stereotactic injections

The nature of this experimental procedure precludes the use of a BSC, therefore, the risk of exposure to recombinant viral particles will be minimized by the following steps:

- During the injection procedure the immediate area will be isolated from the rest of the lab by means of closed and appropriately signed doors.
- A warning sign will be posted during procedures involving lentivirus. Warning signs are available from the BioLabs Safety Officer.
- Lentivirus will be transported to the work area in closed secondary containers.
- PPE will comprise lab coat, gloves, surgical mask (optional), and eye protection (optional) to accommodate use of the microscope during the procedure.
- All contaminated or potentially contaminated waste must be decontaminated prior to disposal. Transport of solid waste to be autoclaved must be in a sealed biohazard bag. Sharps containers must be sealed.
- After injection of animals, the injection site should be cleansed with 70% ethanol and then animals should be placed into a secondary container without bedding. Once the injection site is dry, animals can be returned to original cage(s) and returned to the ABSL-1 animal facility. The secondary container should also be cleaned with 70% ethanol. Cages should be identified as containing lentivirally transduced animals, so that vivarium personnel will handle them last.

History

DATE	AMENDMENT	INITIAL
09/23/2022	Original BSL-2+ Safety Manual Document	JL
10/10/2022	Updated bleach concentrations to 20% and PPE requirements	JL

Appendix I

Spills Inside The BSC
A. Cleanup of a Small Spill (< 25ml)
1. Make sure the cabinet continues to operate. Wait 5 minutes to allow aerosols to be drawn through the HEPA filter.
2. Decontaminate the surfaces within the cabinet, wearing protective clothing, gently cover the spill with absorbent paper towel and apply 20% bleach (or 5% Amphyl, or 2.5% Wescodyne) starting at the perimeter and working towards the center; allow sufficient contact time (20 minutes) before cleanup.
3. Discard soaked paper towels in a solid waste can in the BSC. Wipe up residual mess. Wipe down surfaces with 70% EtOH, discarding towels in a solid waste can.
4. Lower sash for 5 minutes with blower on to circulate air through the HEPA filter.
5. With supervisor, write up a report to submit to the BioLabs Safety Officer.
B. Cleanup a Large Spill (> 25ml)
1. Evacuate the room, breathing as little as possible of any aerosols.
2. Close the door to the room.
3. Remove PPE and any contaminated clothing and place it in sealed plastic containers.
4. Post warning sign: DO NOT ENTER—lentivirus spill!
5. Everyone in the room at the time of the spill should thoroughly wash their hands and face, using disinfectant soap.
6. Wait 30 minutes for aerosols to settle. Meanwhile, notify supervisor. If the spill has escaped the BSC, proceed as for a spill outside the BSC.
7. Proceed with clean-up as for minor spill; decontaminate all equipment supplies, or surfaces that were potentially contaminated.
8. If a large quantity is spilled, the entire cabinet, including fans, filters, airflow plenums, will need to be decontaminated.
9. With supervisor, write up a report to submit to the BioLabs Safety Officer.

Spills Outside the BSC
A. Clean-up of minor spill (< 10ml, localized to a small area)
1. Alert personnel in the vicinity.
2. Ascertain extent of spill - check shoes! Decontaminate if necessary.
3. Evacuate the room. Close door. Discard potentially contaminated PPE and remove any contaminated clothing. Wash hands and face thoroughly.
4. Post warning sign: DO NOT ENTER—Lentivirus spill!
5. Wait 30 minutes. Meanwhile, notify supervisor.
6. Don fresh PPE: lab coat or gown, gloves, mask, eye protection.
7. Re-enter the room, cover spill with paper towels.
8. Soak paper towels with 20% bleach, from perimeter toward the center.
9. Allow 20 minutes contact time.
10. Continue clean-up: soaked towels go in biohazard bags. Pick up sharps with tongs.
11. Wipe up residual mess with paper towels. Discard in solid waste can.
12. Wipe down spill area with 70% EtOH.
13. With supervisor, write up a report to submit to the BioLabs Safety Officer.
B. Clean-up of major spill (> 10ml, splattered over a large area)
1. Alert personnel in the vicinity.
2. Ascertain extent of spill - check shoes! Decontaminate if necessary.
3. Evacuate the room. Close door. Discard potentially contaminated PPE and remove any contaminated clothing. Wash hands and face thoroughly. If eyes have been exposed, flush in eye station.
4. Post warning sign: DO NOT ENTER—Lentivirus spill!
5. Wait 30 minutes. Meanwhile, notify supervisor and BioLabs Safety Officer.
6. If assistance is needed, discuss with BioLabs Safety 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 20% bleach, from perimeter toward the center.
10. Allow 30 minutes contact time.
11. Continue clean-up: soaked towels go in solid waste can. Pick up sharps with tongs.
12. Wipe up residual mess with paper towels. Discard in solid waste can.
13. Wipe down spill area with 70% EtOH.
14. With supervisor, write up a report and submit to the BioLabs Safety Officer.

Special Situations
A. In the incubator
1. In the case of contamination of the water pan - add bleach to 20% final for 30 minutes and discard.
2. Decontaminate incubator as for a minor spill outside the BSC.
3. After decontamination procedure, follow regular incubator decontamination procedure.
4. With supervisor, write up a report to submit to the BioLabs Safety Officer.
B. In the Centrifuge (with no breach of containment)
1. Spray rotor with 70% EtOH, unload rotor in BSC.
2. If inside of rotor is contaminated, decontaminate in the BSC.
3. As a precautionary measure, decontaminate the centrifuge chamber.
4. With supervisor, write up a report to submit to the BioLabs Safety Officer.
C. In the Centrifuge (with breach of containment)
1. Alert personnel in the vicinity and evacuate the room.
2. Post Warning Sign: DO NOT ENTER--Lentivirus Spill!
3. Wait 30 minutes. Meanwhile, notify supervisor and BioLabs Safety Officer.
4. If assistance is needed, discuss with BioLabs Safety Officer.
5. Open lid slowly, add paper towels, flood chamber with disinfectant to saturate paper towels.
6. Spray walls of chamber and rotor with 70% EtOH.
7. Close centrifuge lid for 20 minutes contact time.
8. Finish centrifuge clean-up as for major spill outside the BSC.
9. Transport rotor to BSC. Open and decontaminate rotor/buckets in the BSC.
10. With supervisor, write up a report and submit to the BioLabs Safety Officer.

Appendix II

Biosafety Considerations for Research with Lentiviral Vectors

Recombinant DNA Advisory Committee (RAC) Guidance Document

Background: The use of lentiviral vectors has been increasing because the vector system has attractive features; however, such research also raises biosafety issues. The NIH Office of Biotechnology Activities has received frequent questions regarding the appropriate containment for lentiviral vectors, particularly those derived from HIV-1. Because the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) do not explicitly address containment for research with lentiviral vectors, the RAC was asked to provide additional guidance for institutional biosafety committees (IBCs) and investigators on how to conduct a risk assessment for lentiviral vector research. At the March RAC 2006 meeting (webcast), the RAC offered the following findings and recommendations.

Risks of lentivirus vectors: The major risks to be considered for research with HIV-1 based lentivirus vectors are:

- Potential for generation of replication-competent lentivirus (RCL).
- Potential for oncogenesis.

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.

General criteria for risk assessment of lentivirus vectors: Decisions about containment should take into account a range of parameters/considerations including:

- The nature of the vector system and the potential for regeneration of replication competent.
- Virus from the vector components.
- The nature of the transgene insert (e.g., known oncogenes or genes with high oncogenic potential may merit special care).
- The vector titer and the total amount of vector.
- The inherent biological containment of the animal host, if relevant.
- Negative RCL testing (see section below).

General containment considerations: Either BL2 containment or enhanced BL2 containment is often appropriate in the laboratory setting for research involving the use of advanced lentivirus vector systems that have multiple safety features and that segregate vector and packaging functions onto four or more plasmids. Enhanced BL2 containment may include in addition to attention to sharps (and use of safety needles where feasible), the use of personal protective equipment intended to reduce the potential for mucosal exposure to the vector. In most such research, these levels of containment are also expected to be appropriate even when producing large volumes of HIV-1 vectors (>10 L).

The appropriate containment level for specific lentivirus vector research is, of course, determined following a complete risk assessment and local IBC review. The following sections discuss some considerations which should form an important part of the biosafety assessment for research involving lentivirus vectors.

Potential for generation of replication competent lentivirus (RCL) from HIV-1 based lentivirus vectors: 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 for 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 (However, the use of the certain coat proteins, such as VSV-G, may broaden the host cell and tissue tropism of lentivirus vectors, which should also be considered in the overall safety assessment by the IBC),
- They separate vector and packaging functions onto four or more plasmids and
- They include additional safety features (e.g., they do not encode Tat, which is essential for replication of wild-type HIV-1).

In contrast, earlier vector systems (such as two-plasmid vector systems) may have a higher potential for generation of RCL.

RCL testing: The National Gene Vector Laboratory (NGVL) has produced over 60 liters of HIV-1 vector and has screened supernatant and cells from different vector systems, using different assays, without detecting RCL (K. Cornetta, personal communication of unpublished data). This suggests that the frequency of RCL generation using lentivirus vectors is very low. It may not, however, be zero. There is a need for continued investigation of RCL generation using lentivirus vectors, in order to inform and advance the field of lentivirus vector technology.

The FDA requires that lentiviral vector stocks used in human clinical trials be tested for RCL. Individual research laboratories conducting preclinical research often use only small volumes (e.g., a few milliliters) of lentivirus vectors expressing lower risk transgenes such as GFP. While these laboratories are not mandated to characterize vector stocks, such testing should be encouraged. However, RCL testing requires expertise with the appropriate assays and such expertise may not be available in laboratories that do not work regularly with infectious lentiviruses. In such laboratories, the use of a positive control may increase risk to the investigator as compared to use of the test material. IBCs may make containment assignments without requiring such testing by undertaking a risk assessment that considers the nature of the specific vector system being used and overall past experience with the system.

Animal studies: Some animals, such as wild-type mice, cannot support replication of infectious HIV-1. As a result, the potential for shedding of RCL from such animals is very low (even if RCL were present in the original vector inoculum). IBCs may consider the biosafety issues associated with animal husbandry and housing after the initial injection separately from the initial inoculation itself. In general, the initial delivery of vector should be performed under Biosafety Level 2 for Animals (BL2-N) or under enhanced BL2-N containment (see "General containment considerations"), so as to minimize the risk of autoinoculation by the investigator. However, it may be permissible to reduce the containment level at some point following vector delivery. For example, if there is no expectation of infection (see below), the site of inoculation has been thoroughly cleansed, and the bedding changed, it may be acceptable to consider reducing

containment from BL2-N to BL1-N within a few days (the specific time period can be specified by the local IBC and may vary anywhere from 1-7 days depending on local and experimental considerations). Animals engrafted with human cells or animal hosts that are permissive for HIV-1 replication constitute a special case, in light of their potential to support replication of infectious HIV-1. Use of lentivirus vectors in these animals requires a higher level of containment.

Other lentivirus vectors: Some non-human lentivirus vectors (e.g., FIV, SIV, EIAV, etc.) are also in use. Of these, the most frequently encountered are feline immunodeficiency virus (FIV) vectors. In the Appendix B-V of the NIH Guidelines, a containment level appropriate for Risk Group 1 agents is recommended for use of certain animal viral etiologic agents not associated with disease in healthy human adults. However, replication-defective vectors in which a heterologous envelope (such as VSV-G) is used for vector packaging may require BL2 containment in the laboratory setting, since these vectors have the potential to transduce human cells, and thus have the potential to cause insertional mutagenesis. Under circumstances in which mice are not permissive hosts for FIV replication, BLN-1 containment may be acceptable for mouse housing and husbandry when dealing with mice that have received FIV vectors (subject to the considerations noted above).

Summary: A comprehensive risk assessment and determination of containment for research with lentiviral vectors should consider the nature of the vector system, transgene insert, and type of manipulations involved. For many experiments, either BL-2 or enhanced BL-2 will be appropriate. Examples of biosafety considerations and risk assessments for three different scenarios are included below.

Examples of Biosafety Considerations

Vector Considerations

- Potential for generation of RCL.
  - Vector and packaging functions separated onto multiple plasmids.
  - Deletion of viral genes.
- Viral Env used in packaging system.
  - Non-native Env (decrease potential for generation of RCL).
  - Coat protein that increases species or cell type tropism of parent virus (e.g., VSV-G).
- Safety modifications (e.g., no expression of Tat).

Transgene Considerations

- Oncogene.
- Non-oncogene.

Vector Generation Considerations

- Laboratory scale.
- Large scale.

Animal Research Considerations

- Permissive host.
- Non-permissive host.
- Animal engrafted with permissive cells.

- Vector Administration (e.g., injection).
- Housing and husbandry.

Practices, Containment Equipment and Training Considerations

- Training in use of PPE.
- Availability of safety equipment (e.g., sealed centrifuge rotor cups).
- Laboratory-specific safety and spill cleanup protocols.
- Availability of on-site occupational health support in the event of accident.

Biosafety Considerations and Risk Levels

Biosafety Considerations	**Higher Risk**	**Lower Risk**
Vector Design	• Vector packaging functions on two plasmids • Expression of viral genes	• Vector and packaging functions separated onto multiple plasmids • Deletion of viral genes
Transgene	• Oncogene	• Non-oncogene
Vector Generation	• Large scale	• Laboratory scale
Animal Hosts	• Permissive host • Animals engrafted with human cells	• Non-permissive host
Animal Manipulation	• Vector administration (e.g., use of sharps during injection	• Housing and husbandry (no use of sharps)

Example Scenarios

Example One: *in vitro* study A:

Use of a 4-plasmid derived lentivirus vector encoding siRNA against Lck in primary human T cells.

Considerations

- What is the amount of vector to be produced? A = LOW (100 ml).
- What is the nature of the vector? A = 4-Plasmid System.
- What is the nature of the insert? A = non-oncogenic.

Tentative Safety Assessment = BL2

(Note that the use of primary human cells would require BSL2 containment, independent of the vector, as well as use of Universal Precautions and compliance with the OHSA standard for Bloodborne Pathogens)

Example Two: *in vitro* study B:

Use of a 2-plasmid derived lentivirus vector encoding luciferase in a human cell line (A549 cells).

Considerations

- What is the amount of vector to be produced? A = LOW (100 ml)
- What is the nature of the vector? A = 2-Plasmid System (non-commercial)
- What is the nature of the insert? A = Non-Oncogenic

Tentative Safety Assessment = BL2 enhanced

BSL2 "enhanced" stipulations might include:

- Avoidance of needles and sharps, where possible.
- Use of a containment hood for all work with the vector (including the loading and unloading of centrifuge rotors, which should have an aerosol-tight seal).
- Use of personal protective equipment [PPE] designed to prevent a mucosal exposure/splash to the face and exposure of hands (especially in persons with broken skin or open cuts).
- A requirement for an in-person consultation between biosafety staff and lab personnel prior to initiation of experiments.

Example Three: *in vivo* study A

Use of a 4-plasmid derived lentivirus vector encoding brain-derived neurotrophic factor (BDNF) in mouse brain.

Considerations

- What is the amount of vector to be produced? A = LOW (100 ml).
- What is the nature of the vector? A = 4-Plasmid System.
- What is the nature of the insert? A = non-oncogenic.
- What is the nature of the animal host? A = Non-permissive for HIV-1.

Tentative Safety Assessment = BL2-N for lab work and initial injection of mice (which would probably be done using a stereotactic frame); after 1-7 days, animals could be moved to BL1-N containment.

Added explanation:

- Even though BDNF is a growth factor for neurons, it has no known oncogenic activity for skin or blood cells that might be the target of a potential needle stick. Hence, this insert would not automatically trigger a requirement for increased biocontainment.
- Stereotactic injection frames cannot easily be placed into a laminar flow hood and may use a syringe or pulled glass pipette for inoculation; they may also use a pump to ensure a slow rate of delivery of the agent. BL-2 containment does NOT require the use of a biosafety cabinet and is therefore compatible with the use of a stereotactic frame, even if that frame is not contained within a laminar flow cabinet.

Additional points to consider:

- An in-person consultation between biosafety staff and lab personnel prior to initiation of experiments may be a useful stipulation.
- One might also impose additional biosafety enhancements during the injection process, perhaps by requiring use of additional PPE above and beyond the stipulated requirements associated with BL2/BL2-N. See Example 2 for examples of such stipulations.

Relevant Sections from the NIH Guidelines

General Considerations

Section II-A. Risk Assessment

Section II-A-3. Comprehensive Risk Assessment

BL2 containment is recommended for activities involving all blood-contaminated clinical specimens, body fluids, and tissues from all humans, or from HIV – or HBV-infected or inoculated laboratory animals. Activities such as the production of research-laboratory scale quantities of HIV or other bloodborne pathogens, manipulating concentrated virus preparations, or conducting procedures that may produce droplets or aerosols, are performed in a BL2 facility using the additional practices and containment equipment recommended for BL3. Activities involving industrial scale volumes or preparations of concentrated HIV are conducted in a BL3 facility, or BL3 Large Scale if appropriate, using BL3 practices and containment equipment.

Section III-D. Experiments That Require Institutional Biosafety Committee Approval Before Initiation

Section III-D-3. Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems.

Recombinant DNA or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see Section V-J, Footnotes and References of Sections I-IV) being considered identical (see Section V-K, Footnotes and References of Sections I-IV), are considered defective and may be used in the absence of helper under the conditions specified in Section III-E-1, Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus.

Section III-E. Experiments That Require Institutional Biosafety Committee Notice Simultaneous with Initiation

Section III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus.

Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical [see Section V-J, Footnotes and References of Sections I-IV]) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III-D-3, Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems, should be used. The DNA may contain fragments of the genome of viruses from more than one Family, but each fragment shall be less than two-thirds of a genome.

Animal Studies

Section III-D-4-a.

Recombinant DNA, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study (see Section V-B, Footnotes and References of Sections I-IV). Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under Section III-D-4-b, Experiments Involving Whole Animals. For experiments involving recombinant DNA-modified Risk Groups 2, 3, 4, or restricted organisms, see Sections V-A, V-G, and V-L, Footnotes and References of Sections I-IV. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with plant or animal pathogens (see Section V-G, Footnotes and References of Sections I-IV).

Classification of Human Etiologic Agents on the Basis of Hazard

Appendix B-III-D. Risk Group 3 (RG3) - Viruses and Prions

Retroviruses

Human immunodeficiency virus (HIV) types 1 and 2

BSL2 Facilities

Appendix G-II-B-3. Containment Equipment (BL2)

Appendix G-II-B-3-a. Biological safety cabinets (Class I or II) (see Appendix G-III-L, Footnotes and References of Appendix G) or other appropriate personal protective or physical containment devices are used whenever:

Appendix G-II-B-3-a-(1). Procedures with a high potential for creating aerosols are conducted (see Appendix G-III-O, Footnotes and References of Appendix G).

These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.

Appendix G-II-B-3-a-(2). High concentrations or large volumes of organisms containing recombinant DNA molecules are used.

Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Appendix G-II-B-4. Laboratory Facilities (BL2)

Appendix G-II-B-4-a. The laboratory is designed so that it can be easily cleaned.

Appendix G-II-B-4-b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-B-4-c. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-B-4-d. Each laboratory contains a sink for hand washing.

Appendix G-II-B-4-e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix G-II-B-4-f. An autoclave for decontaminating laboratory wastes is available.