

Biosafety Level 2/3 Procedures

For use with Biological Agents such as: **Lentiviral Vectors**
Other Retroviral Vectors
Vaccinia
Rabies
Bacterial Toxins

What is meant by BL2/BL3?

BL2/BL3 means that the containment level of the lab is BL2, but BL3 practices are used to manipulate the organism.

Risks/Special Precautions

Lentiviral vectors have the ability to integrate genetic material into the chromosomes of non-dividing cells. In addition, staff should be aware that there may be a potential for recombinant virus generation with an enhanced host range. Therefore, all experimental materials must be handled so as to avoid aerosol production, accidental inoculation, and exposures to the mucous membranes.

Working with other retroviral vectors also has the potential risk of recombinant virus generation.

The “Biosafety in Microbiological and Biomedical Laboratories” 4th edition published by the CDC and NIH recommends the use of BL3 practices with Rabies when there is a “high potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials.” They also recommend that ALL individuals working in a rabies facility be immunized, whether they work directly with the virus or not. The IBC has adopted this recommendation and also has recommended that all rabies work be performed at the BL2/3 level, at a minimum.

The “Biosafety in Microbiological and Biomedical Laboratories” 4th edition recommends work with Vaccinia be carried out at BL2, **as long as the individuals working with it are vaccinated**. If not, they recommend BL3 conditions. Since not everyone in a lab may work with vaccinia and not everyone may be vaccinated, the IBC recommends that work with vaccinia be performed at the BL2/BL3 level.

IBC Approval

IBC approval must be obtained prior to beginning any experiments with these agents.

Facility (BL2 containment) Requirements

- 1) Access to the lab must be limited and at the discretion of the Principal Investigator (PI). Individuals who are at an increased risk of infection, such as immunocompromised or immunosuppressed individuals, may **not** work in the facility.
- 2) A sign must be posted on the door indicating the biohazards within, the appropriate personal protective equipment (PPE) needed, and an emergency contact number. In addition, a separate sign should be posted warning immunocompromised or immunosuppressed individuals that they should not enter the lab.
- 3) A certified Class II biosafety cabinet must be available.
- 4) A centrifuge with safety cups must be available.

- 5) A dedicated hand washing sink must be available and appropriately marked.
- 6) An eyewash station must be readily accessible within 55 feet of anywhere in the lab. It must be tested once a week to assure adequate water flow. This testing must be documented.
- 7) Bench tops must be impervious to water, moderately heat resistant, and able to withstand the disinfectants commonly used to clean them.
- 8) Lab chairs must be covered in a non-fabric material to make them easily cleanable.
- 9) Carpeting is not permitted on the floor of a lab. The floors should be easily cleaned and any spaces between benches, cabinets, and equipment should be accessible for cleaning.
- 10) A HEPA filter must be placed on the house vacuum lines (between the nozzle and the flask) to protect the line from accidental contamination.
- 11) Fresh 10% bleach must be made up on a daily basis due to the fact that it is inherently unstable and breaks down over time, rendering it ineffective as a decontamination product.

Personal Protective Equipment (PPE) Required

At a minimum, lab coat, gloves, and eye protection must be worn.

Work Practices (BL3 practices) Required

- 1) Use the biological safety cabinet for all manipulations.
- 2) A dedicated incubator must be available and appropriately labeled with a biohazard label.
- 3) Centrifuge rotors must be loaded and unloaded within the biosafety cabinet. When loading the rotor, the centrifuge tubes should be wiped down with 10% bleach before being placed in the rotor. The safety caps must be put on first and then the rotor lid. The outside of the rotor must be wiped down with 10% bleach and the outer pair of gloves replaced before removing the rotor to the centrifuge. The process is reversed for unloading the rotor.
- 4) The use of sharps in the laboratory should be kept to a minimum and eliminated where possible. For example, plastic aspiration pipettes should be used in place of glass Pasteur pipettes.
- 5) If needles/syringes must be used for animal experiments, safety needles must be used to the extent possible. If the safety device proves to be an impediment to the safe performance of the procedure, then standard needles/syringes may be used. HOWEVER, extreme caution must be used to avoid an accidental auto-inoculation.
- 6) In the same manner, if scalpels are needed for harvesting organs, scalpels with safety covers should be tried first. Again, extreme caution should be used to avoid accidentally cutting oneself.
- 7) Undiluted bleach must be pulled into the aspirator flask so the final concentration is 10%. The flask should be disconnected, the opening covered with aluminum foil, and the flask allowed to sit in a secondary container for a minimum of 20 minutes. The contents of the flask can then be poured down the sink with copious amounts of water.
- 8) Serological pipettes and pipette tips must be placed in a tray of 10% bleach which is located in the hood. After completion of the experiment, these items are rinsed in water. The serological pipettes must be placed in an 18 gallon impervious sharps container while the pipette tips are placed in the red bag/black plastic biohazard box system.

- 9) Solid waste must be autoclaved at the end of the day, more often if necessary, and then placed into the red bag/black plastic biohazard box system for disposal.
- 10) The biosafety cabinets are to be wiped down with 10% bleach followed by 70% ethanol prior to beginning work in the cabinet and after work has been completed.
- 11) Spill procedures must be posted.
 - a. For spills inside the biosafety cabinet, leave the cabinet on and remove any contaminated clothing. Wash hands well. Put on clean gloves and/or lab coat. Soak paper towels in straight bleach, and place them on top of the spill. Place a diaper on top of the paper towels, with the plastic side away from the spill. Allow to sit for a minimum of 20 minutes. Clean up the area, disposing of all items in the red bag/ black plastic biohazard box system. Change gloves and wipe down the area with 10% bleach followed by 70% ethanol.
 - b. For spills outside the biosafety cabinet, evacuate the area, close the door, and notify the PI and Biosafety Officer. The PI and Biosafety Officer will direct and help in the clean-up effort.

Transportation of Specimens

When transporting BL2/BL3 organisms or specimens containing these organisms, the primary container must be secured in a secondary container, sealed, and all outer surfaces decontaminated. All containers must be loaded or opened within a biosafety cabinet.

Animal Use

Animals that will be injected with BL2/BL3 organisms must have the procedure performed within the animal **BL3** suite.

Accidental Exposure

If an exposure to these organisms should occur, the PI **must** be notified and an accident report **must** be filled out. The employee must also report to University Health Services for evaluation of the exposure during regular business hours. If the incident occurs after hours or on the weekend, the individual must report to the emergency room. In either case, the employee must tell the attending physician what organism or toxin they were working with and provide the name and emergency contact number for the PI. If an MSDS is available for the organism, it should be provided as well. In addition, Environmental Health and Safety should be notified by calling the accident reporting line at 5-SAFE.