



DUKE NEUROBIOLOGY
SCHOOL OF MEDICINE

Laboratory Biosafety Plan

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Laboratory Biohazards:

- **Biosafety Level 1**
 - Recombinant DNA cloning in *E. coli* K-12 and derivatives
 - Adeno-Associated Vectors

- **Biosafety Level 2**
 - Lentivirus (replication-defective, derived from human immunodeficiency virus and pseudotyped with vesicular stomatitis virus glycoprotein)
 - Rabies Viruses (replication-defective)

Biosafety Level 1:

- 1) Gloves should be worn if the skin on the hands is broken or if a rash is present. Lab personnel must wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
- 2) Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
- 3) Mouth pipetting is prohibited; mechanical pipetting devices are used.
- 4) A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.

- 5) All procedures are performed carefully to minimize the creation of splashes or aerosols. Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.
- 6) Work surfaces where bacteria containing recombinant DNA have been used must be decontaminated at least once each day and after any spill of viable material. Appropriate disinfectants include 1% bleach (500 ppm) and 70% ethanol.
- 7) All bacterial cultures, culture plates and other contaminated items must be decontaminated before disposal by autoclaving or other approved methods.
 - a) Materials to be autoclaved are to be placed in a durable, leakproof container and closed for transport from the laboratory.
 - b) Reusable items such as centrifuge tubes or culture flasks should be decontaminated by soaking in 10% bleach (5000 ppm) or 70% ethanol or 1% Lysol concentrate (500 ppm) for at least 20 minutes and rinsed before standard laboratory washing.
 - c) Liquid waste should be treated with Lysol concentrate at a final concentration of at least 1% (add an approximately equal volume of 2.5%) for at least 10 minutes before disposal.

Biosafety Level 2*:

- 1) When work with infectious agents is in progress access to the laboratory is limited to personnel with appropriate Biosafety Level 2 training.
- 2) Protective laboratory coats, gowns, smocks, or uniforms designated for lab use must be worn while working with BSL2 agents. Open-toed shoes are not permitted. This protective clothing must be removed and left in the laboratory before leaving for non-laboratory areas. All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
- 3) Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Gloves used for BSL-2 work must be treated as contaminated and discarded with other contaminated material. Hands are washed following removal of gloves.
- 4) Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.
- 5) Mouth pipetting is prohibited; mechanical pipetting devices are used.
- 6) The biological safety cabinet must be used whenever possible, including procedures with a potential for creating infectious aerosols or splashes are conducted. Such procedures include opening containers of infectious materials and all cell culture work with BSL2 agents and cell lines. Such materials may be centrifuged or otherwise manipulated in the open laboratory if

* These procedures apply only to non-animal work involving BSL2 agents. Additional precautions and procedures apply to work involving administration of BSL2 agents to animals.

sealed centrifuge tubes, sealed rotor heads or other sealed vessels are used, and if these containers are opened only in a biological safety cabinet.

- 7) Culture flasks and dishes containing high-titer virus should be labeled as a biohazard during incubation or manipulation outside of the biological safety cabinet.
- 8) All procedures are performed carefully to minimize the creation of splashes or aerosols. Face protection (goggles, mask, face shield or other splatter guard) must be used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the biological safety cabinet.
- 9) A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels. BSL2 agents should never loaded into syringes with needles. Plasticware should be substituted for glassware whenever possible.
- 10) Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal.
- 11) Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant (1% bleach [500 ppm] or 70% ethanol) on a routine basis, after work with infectious materials is finished. Overt spills, splashes, or other contamination by infectious materials should be flooded with 10% bleach (5000 ppm) or 70% ethanol before removal. Contaminated equipment must be decontaminated before it is sent for repair or maintenance or packaged for transport.
- 12) All cultures, stocks, and materials contaminated with BSL2 agents must be decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory.
 - a) All disposables contaminated with high concentrations of BSL-2 agents should be rinsed with 10% bleach or 70% ethanol after use and before autoclaving.
 - b) All materials to be autoclaved should be placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
 - c) Reusable items such as centrifuge tubes or bottles should be decontaminated by soaking in 10% bleach or 70% ethanol for at least 20 minutes and rinsed before standard laboratory washing.
 - d) Liquid waste should be treated with bleach at a final concentration of 10% for at least 20 minutes before disposal. **Perform this treatment in the chemical fume hood.**
- 13) Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Disinfectants

Lysol (Lysol Brand Concentrate, Original Scent): Prepare a 1:40 (2.5%) dilution in deionized water. The active ingredient is o-benzyl-p-chlorophenol, present at 5-6% in the concentrate. A

final concentration of 1% (500-600 ppm) is effective against bacteria and most enveloped viruses.

Ethanol (or isopropanol): When used at a final concentration of 70-85% it is effective against bacteria and enveloped viruses. Lysol brand II Disinfectant Spray contains 79% ethanol, so it is also effective. Note that these alcohols are flammable, so should not be used near a flame.

Sodium hypochlorite (household bleach): Sold as a solution containing 5.25% free chlorine. Used at a 1:10 dilution (10% bleach, 5000 ppm), it is useful for disinfecting spilled suspensions of bacteria or viruses and to disinfect liquid waste. **Note that treatment of liquid waste must be performed in a chemical fume hood because of the generation of chlorine gas.** Used at a dilution of 1:100 (1% bleach, 500 ppm), it is useful for routine surface decontamination, although it should not be used for disinfection of biological safety cabinets because of reaction with stainless steel.

Virus Transport Protocol

1. Virus samples are prepared and stored in capped 0.5- to 2.0-ml plastic microfuge tubes. Storage is at -80°C.
2. For transport to other institutional laboratories, tubes containing virus samples must be placed in clean 50-ml plastic centrifuge tubes (or other clean, sealable containers) and caps closed tightly. Samples should be placed on ice or dry ice for transport.
3. Laboratory personnel must wear gloves while placing samples into carrier tubes or containers—these gloves must be discarded into the biohazard waste before leaving the laboratory. Clean gloves should be worn during sample transport.
4. The Principal Investigator of the recipient laboratory assumes all responsibility for safe transport and use of the viruses once the samples leave the Viral Vector Laboratory.