1. PURPOSE

This Standard Operating Procedure (SOP) describes the guidelines for the use of Adeno-Associated viral vectors and Lentiviral vectors delivered directly to the central nervous system (CNS) via intracranial injection in rodents.

2. CONSIDERATIONS

Viral vectors are used to transfect organisms and cell lines with new genes. Adeno-associated viral vectors and Lentiviral vectors are classified as Biosafety Level 1 agents.

Adeno-associated viruses are not pathogenic to humans; they cannot replicate without a helper virus (adenovirus or herpesvirus). Adeno-associated viral vectors are defective and cannot replicate and be shed, even in presence of a helper virus.

Lentiviral vectors are replication defective. Risk of exposure are associated to self-injection and droplets in contact with mucosa. Potential consequence is oncogenesis. Animals do not support replication of human lentiviruses.

The use of viral vectors must be described in an approved Animal Use Protocol (AUP).

3. RESPONSIBILITY

Principal investigator (PI) and their research staff, animal care staff, veterinary care staff.

4. MATERIALS

4.1. Personal protective equipment (PPE):

4.1.1. Gloves
4.1.2. Earloop or N95 Mask
4.1.3. Gown, disposable gown or lab coat
4.1.4. Safety glasses or goggles

4.2. Biological Safety Cabinet

4.3. Absorbent pads
4.4. 1% Bleach solution (prepared fresh daily)
4.5. Biohazard waste containers/bags
4.6. Sharps disposal containers

5. PROCEDURES

5.1. General precautions:

5.1.1. Pregnant or breast-feeding women should not work with viral vectors.

5.1.2. Personal protective equipment requirements when working with, preparing or administering viral vectors:

5.1.2.1. Gloves: should be inspected for tears frequently and changed as needed. Stretch gloves over the cuff of the gown to cover any exposed skin.

5.1.2.2. Mask: must cover the mouth and nose at all times

5.1.2.3. N95 mask when not working in a Biological Safety Cabinet (BSC): must cover the mouth and nose at all times

5.1.2.4. Gown or lab coat
5.1.2.5. Disposable, single-use, gown when not working in a Biological Safety Cabinet (BSC).

5.1.2.6. Safety glasses or goggles when not working in a Biological Safety Cabinet (BSC).

5.1.3. Handling and preparation of solutions, including preparations of syringes and any procedures with the potential of producing aerosols, must be conducted in a certified Biological Safety Cabinet (BSC).

5.1.4. Work areas should be protected from spills by placing an absorbent pad with an impervious backing (absorbent material facing up).

5.1.5. Administration of viral vectors should be performed in a certified BSC whenever possible.

5.1.6. Areas where viral vector solutions are prepared or administered must be cleaned and decontaminated with 1% bleach solution immediately following each procedure.

5.1.7. Thoroughly wash hands after handling or administering viral vectors.


5.2. Transport and storage precautions:

5.2.1. Solutions must be transported in unbreakable containers.

5.2.2. Transport only the amount required for the number of animals to be injected at that time.

5.2.3. All containers containing viral vector solutions must be clearly labeled and adequately stored when not in use.

5.2.4. Keep containers tightly closed.

5.2.5. Dispose of empty containers by incineration.

5.3. Handling needles and sharps:

5.3.1. Needles and sharps used with viral vectors must be disposed of immediately in a sharps container for autoclaving or incineration.

5.3.2. Never bend or recap needles.

5.3.3. Safety needles should be used whenever possible.

5.3.4. Handle needles only if necessary, using forceps.

5.3.5. Maintain a safe distance between fingers and needles or sharps.

5.4. Viral vector administration:

5.4.1. Stereotaxic surgical procedures:

5.4.1.7. Refer to SOP 202 – Rodent Stereotaxic Surgery.

5.4.1.8. Use holder attached to stereotaxic frame for holding the syringe and injection.

5.4.1.9. Clean surgical site with swabs or gauzes prior to closing.

5.5. Animal Handling and Husbandry:

5.5.1. Standard animal housing procedures should be followed.

5.5.2. All animal handling should be conducted in a certified Biological Safety Cabinet (BSC) whenever possible.

5.5.3. All cages housing animals that have been treated with viral vectors must be clearly labeled with the following information:

5.5.3.1. Name of agent

5.5.3.2. Date of administration

5.6. Collection of CNS tissue following euthanasia:

5.6.1. Euthanize animals as per the approved AUP. Refer to SOP 301.

5.6.2. Perfuse brain with fixative solution (e.g., paraformaldehyde) before collection, whenever possible.

5.6.3. Use scissors and forceps, and rounded bone rongeurs. Avoid scalpel blades.
5.7. Waste disposal:
   5.7.1. Concentrated viral vector stock solutions may be deactivated by adding an equal volume of 10% bleach solution, allow for 20 minutes contact time. Dispose in laboratory sink drain.
   5.7.2. Dead animals must be double-bagged before disposal by incineration.
   5.7.3. All other waste such as PPE and animal bedding can be disposed of as non-regulated or non-infectious Medical Waste.

5.8. Small spills and leakage:
   5.8.1. Use absorbent paper to pick up all liquid spill material.
   5.8.2. Wash any surfaces that may have been contaminated with 1% bleach solution.

5.9. In case of accidental exposure:
   5.9.1. Potential routes of exposures include: inhalation, eye contact, skin absorption, ingestion and unintentional injection.
   5.9.3. Splash in eyes:
       5.9.3.1. Flush eyes with water or normal saline solution for 20 to 30 minutes.
   5.9.4. Skin exposure:
       5.9.4.1. Immediately flush affected skin with water while removing and isolating all contaminated clothing.
       5.9.4.2. Gently wash all affected skin areas thoroughly with soap and water.
   5.9.5. Ingestion:
       5.9.5.1. Do not induce vomiting.

5.10. Preparation of 1% bleach solution:
   5.10.1. Disinfectant solution must be prepared fresh daily as bleach degrades rapidly in water.
   5.10.2. Wear personal protective equipment when preparing and using disinfectant solution.
   5.10.3. Mix 1 part 5% chlorine bleach with 99 parts water.
   5.10.4. Label all storage containers.