STANDARD OPERATING PROCEDURE #703

USE OF VIRAL VECTORS IN NEUROSCIENCE - RODENTS

1. PURPOSE

This Standard Operating Procedure (SOP) describes the guidelines for the use of Adeno-Associated viral vectors, Lentiviral vectors, G-deleted Rabies viruses, and Herpesviral 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:

- Classified as Risk Group 1 agents by the Public Health Agency of Canada (PHAC).
- Not pathogenic to humans; they cannot replicate without a helper virus (adenovirus or herpesvirus).
- They are defective and cannot replicate and be shed, even in presence of a helper virus.

Lentiviral vectors:

- Classified as human and animal Risk Group 2 (RG2) biological agent by the Public Health Agency of Canada (PHAC).
- Lentiviral vectors are replication defective.
- Risk of exposure is associated to self-injection and droplets in contact with mucosa. Potential consequence is oncogenesis.
- Animals do not support replication of human lentiviruses.

G-deleted rabies viruses:

- Classified as human and animal Risk Group 2 (RG2) biological agent by the Public Health Agency of Canada (PHAC).
- Are not pathogenic to humans.
- The rabies virus has been modified to render it less hazardous and cannot infect mammalian cells.

Herpesviral vectors:

- Classified as human and animal Risk Group 2 (RG2) biological agent by the Public Health Agency of Canada (PHAC).
- Herpes Simplex Virus Type 1 (HSV-1) based vectors are most commonly used.
- Herpesviral vectors are replication defective.

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

The risk assessment of the gene insert, i.e., does the viral vector encode for an oncogenic or toxic protein, is done on a case-by-case basis and is part of the Environmental Health and Safety “Application to Use Biohazardous Materials”, in an Appendix which evaluates the risks of viral vectors, prior to issuing a biohazard certificate.

3. RESPONSIBILITY

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

4. MATERIALS

4.1. Personal protective equipment (PPE):

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

4.2. Class II Biological Safety Cabinet

4.3. Absorbent pads

4.4. 1% Bleach solution (prepared fresh daily)

4.5. Biohazard waste containers/bags

4.6. CSA-approved 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 respirator when not working in a Biological Safety Cabinet (BSC): must be fit-tested and 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 Class II Biological Safety Cabinet (BSC). Refer to McGill University Environmental Health & Safety Standard Operating Procedure (SOP) for Safe Handling of Lentivirus.
- 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.
- 5.1.8. In the event of accidental exposure, complete a McGill University Accident & Incident Report form within 24 hours.

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 viral solutions, 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. When filling the syringe, keep the container with the viral vector solution in a tube holder or rack.

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

5.3.7. 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.

5.3.8. Dispose waste such as PPE, absorbent pads, container, etc. as biohazardous waste.

5.4. Viral vector administration:

5.4.1. Stereotaxic surgical procedures:

5.4.1.1. Refer to SOP 202 – Rodent Stereotaxic Surgery.

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

5.4.1.3. 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

5.5.4. Waste such as PPE and animal bedding can be disposed of as non-regulated or non-infectious Medical Waste.

5.5.5. Dead animals must be double-bagged before disposal by incineration.

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.

5.6.4. All other waste such as PPE and animal bedding can be disposed of as non-regulated or non-infectious Medical Waste.

5.7. Small spills and leakage:

5.7.1. Cover the spill with absorbent material. Apply 1% bleach solution starting at the outer perimeter and working towards the center of the spill. Make sure the absorbent material is completely saturated with disinfectant. Allow to sit for a minimum of 5 minutes.

5.7.2. Wash any surfaces that may have been contaminated with 1% bleach solution.

5.7.3. Dispose waste such as PPE, absorbent pads, etc. as biohazardous waste.

5.8. In case of accidental exposure:

5.8.1. Potential routes of exposures include: inhalation, eye contact, skin absorption, ingestion and unintentional injection.

5.8.2. Report any incident immediately to your supervisor. Complete a McGill University Accident & Incident Report form within 24 hours.

5.8.3. Splash in eyes:

5.8.3.1. Flush eyes with water or normal saline solution for 20 to 30 minutes.

5.8.4. Skin exposure:

5.8.4.1. Immediately flush affected skin with water while removing and isolating all contaminated clothing.

5.8.4.2. Gently wash all affected skin areas thoroughly with soap and water.

5.8.5. Ingestion:

5.8.5.1. Do not induce vomiting.

5.9. Preparation of 1% bleach solution (approximately 10000 ppm chlorine):

5.9.1. Disinfectant solution must be prepared fresh daily as bleach degrades rapidly in water.

5.9.2. Wear personal protective equipment when preparing and using disinfectant solution.

5.9.3. Mix 1 part 5% chlorine bleach with 5 parts water.

5.9.4. Label all storage containers.

6. REFERENCES

6.1. McGill University Biosafety Manual, April 2023.

6.2. Pauwels K, Gijsbers R, Toelen J, Schambach A, Willard-Gallo K, Verheust C, Debyser Z and Herman P, " State-of-the-Art Lentiviral Vectors for Research Use: Risk Assessment and Biosafety Recommendations", Current Gene Therapy (2009) 9: 459. https://doi.org/10.2174/156652309790031120

6.3. NIH Biosafety Considerations for Research with Lentiviral Vectors, 2006.

6.4. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines), April 2019.

6.5. Reuter JD, Fang X, Ly CS, Suter KK, Gibbs D. Assessment of hazard risk associated with the intravenous use of viral vectors in rodents. Comp Med. 2012 Oct;62(5):361-70.

6.6. Schlimgen R, Howard J, Wooley D, Thompson M, Baden LR, Yang OO, Christiani DC, Mostoslavsky G, Diamond DV, Duane EG, Byers K, Winters T, Gelfand JA, Fujimoto G, Hudson TW, Vyas JM. Risks Associated With Lentiviral Vector Exposures and Prevention Strategies. J Occup Environ Med. 2016 Dec;58(12):1159-1166.

6.7. Ghanem A, Conzelmann KK; G gene deficient single-round rabies viruses for neuronal circuit analysis. Virus Research 216 2015 May 23: 41-54.

6.8. Kim, E. J., Juavinett, A. L., Kyubwa, E. M., Jacobs, M. W., & Callaway, E. M. (2015). Three types of cortical layer 5 neurons that differ in brain-wide connectivity and function. Neuron, 88(6), 1253-1267.

6.9. Wickersham IR, Finke S, Conzelmann KK, & Callaway EM (2007). Retrograde neuronal tracing with a deletion-mutant rabies virus. *Nature methods*, *4*(1), 47–49. doi:10.1038/nmeth999

6.10. Wickersham IR, Lyon DC, Barnard RJO, Mori T, Finke S, Conzelmann KK, Young JAT, Callaway EM; Monosynaptic restriction of transynnaptic tracing from single, genetically targeted neurons. Neuron 53, March 1, 2007: 639-647

6.11. Marshel JH, Mori T, Nielsen KJ, Callaway EM. Targeting single neuronal networks for gene expression and cell labeling in vivo. Neuron 67, August 26, 2010: 562-574

6.12. Canadian Centre for Occupational Health and Safety, OSH Answers Fact Sheets Rabies, January 2020.

6.13. Collins DE, Reuter JD, Rush HG, Villano JS. Viral Vector Biosafety in Laboratory Animal Research. Comp Med. 2017;67(3):215–221.

6.14. Berto E, Bozac A, Marconi P. Development and application of replication-incompetent HSV-1-based vectors. Gene Ther. 2005 Oct;12 Suppl 1:S98-102.

SOP REVISION HISTORY

DATE	NEW VERSION
2018.10.15	2. Viral vectors are used to transfect organisms and cell lines with new genes. Adeno-associated viral vectors **are classified as Risk Group 1 agents** and lentiviral vectors are classified as ~~Biosafety Level 1 agents~~ **human and animal Risk Group 2 (RG2) biological agent by the Public Health Agency of Canada (PHAC)**.
2018.10.15	4.2. **Class II** Biological Safety Cabinet
2018.10.15	5.3.1. Handling and preparation of solutions, including preparations of syringes and any procedures with the potential of producing aerosols, must be conducted in a certified **Class II** Biological Safety Cabinet (BSC). **Refer to McGill University Environmental Health & Safety Standard Operating Procedure (SOP) for Safe Handling of Lentivirus https://mcgill.ca/ehs/files/ehs/standard_operating_procedure_lentivirus_2014_1.pdf**
2018.11.14	5.1.2.3. N95 mask when not working in a Biological Safety Cabinet (BSC): must **be fit-tested and** cover the mouth and nose at all times.
2018.11.14	4.6. **CSA-approved** sharps disposal containers
2019.06.19	This Standard Operating Procedure (SOP) describes the guidelines for the use of Adeno-Associated viral vectors, Lentiviral vectors, and **G-deleted Rabies viruses** delivered directly to the central nervous system (CNS) via intracranial injection in rodents.
2019.06.19	**G-deleted rabies viruses:** **• Classified as human and animal Risk Group 2 (RG2) biological agent by the Public Health Agency of Canada (PHAC).** **• Are not pathogenic to humans.** **• The rabies virus has been modified to render it less hazardous and cannot infect mammalian cells.**
2019.06.19	**6. References**
2019.07.04	This Standard Operating Procedure (SOP) describes the guidelines for the use of Adeno-Associated viral vectors, Lentiviral vectors, ~~and~~ G-deleted Rabies viruses**, and Herpesviral vectors** delivered directly to the central nervous system (CNS) via intracranial injection in rodents.
2019.07.04	**Herpesviral vectors:** **• Classified as human and animal Risk Group 2 (RG2) biological agent by the Public Health Agency of Canada (PHAC).** **• Herpes Simplex Virus Type 1 (HSV-1) based vectors are most commonly used.** **• Herpesviral vectors are replication defective.**
2019.07.04	**5.3.5. When filling the syringe, keep the container with the viral vector solution in a tube holder or rack.**
2019.07.04	**6.1. Collins DE, Reuter JD, Rush HG, Villano JS. Viral Vector Biosafety in Laboratory Animal Research. Comp Med. 2017;67(3):215–221.** **6.2. Berto E, Bozac A, Marconi P. Development and application of replication-incompetent HSV-1-based vectors. Gene Ther. 2005 Oct;12 Suppl 1:S98-102.**
2019.10.09	~~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~~
2019.10.09	5.3. Handling **viral solutions**, needles, and sharps: **5.3.7. 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.** **5.3.8. Dispose waste such as PPE, absorbent pads, container, etc. as biohazardous waste.**
2019.10.09	**5.5.4. Waste such as PPE and animal bedding can be disposed of as non-regulated or non-infectious Medical Waste.** **5.5.5. Dead animals must be double-bagged before disposal by incineration.**
2019.10.09	**5.6.4. All other waste such as PPE and animal bedding can be disposed of as non-regulated or non-infectious Medical Waste.**
2019.10.09	~~5.8~~ **5.7.** Small spills and leakage: **5.7.1.** ~~Use absorbent paper to pick up all liquid spill material.~~ **Cover the spill with absorbent material. Apply 1% bleach solution starting at the outer perimeter and working towards the center of the spill. Make sure the absorbent material is completely saturated with disinfectant. Allow to sit for a minimum of 5 minutes.** **5.7.2.** Wash any surfaces that may have been contaminated with 1% bleach solution. **5.7.3. Dispose waste such as PPE, absorbent pads, etc. as biohazardous waste.**
2019.10.09	5.9. Preparation of 1% bleach solution **(approximately 10000ppm chlorine)**:
2019.10.09	5.9.3. Mix 1 part 5% chlorine bleach with ~~100~~ **5** parts water.