How to Complete a Risk Assessment of Adeno Associated Virus Experiments in Animals and Cell Culture

1. **Organism or Agent:** Adeno Associated Virus Vectors
2. **Synonym:** *Parvoviridae* family
3. **Characteristics:** non-enveloped virus (stable) dependent upon helper virus for replication (adenovirus, herpes simplex virus, others)
4. **Containment Requirements:** Recommend BSL-2 containment practices, equipment and facilities for all activities involving virus manipulation. primary containment devices and biological safety cabinets are recommended. Centrifuge safety precautions, secondary containers for transport between incubator and BSC. Keep hands away from the eyes, nose and mouth in order to avoid potential exposure of the mucous membranes; eye goggles or face shields may assist in accomplishing this objective.
5. **Manipulations:** Depending on the vector, work may need to be performed within a biosafety cabinet, and the use of sharps including needles, blades and glassware should be minimized.
6. **Spills:** Allow aerosols to settle; wear protective clothing including an N95 respirator, gently cover spill with paper towel and apply disinfectant, starting at perimeter and working towards the center; allow sufficient contact time before clean-up (30 min).
7. **Biohazardous Waste:** Collect in double red bags and transport in a rigid container.
8. **Approved Disinfectants:**
   a. 0.05% Sodium Hypochlorite (1:10 bleach/water) allow 10 minutes of contact time.
   b. Stabilized prediluted bleach (preferred to lab prepared bleach)
   c. Alcohols are not acceptable disinfectants.
9. **Disposal:** Decontaminate before disposal; steam sterilization, incineration, chemical disinfection.
10. **Storage:** Store in sealed containers appropriately labeled with a biohazard label, description and contact information.
11. **Pathogenicity:** Hazards depend on multiple factors: whether the vector is capable of infecting human cells, whether the vector is replication competent, how many viral genes are contained in the vector, and the specific transgenes present in the vector. The following must be included in the risk assessment: Despite the lack of evidence for AAV pathogenicity, correlations have recently been made between: a report by Russell and Grompe (Nature Genetics, 47(10), 1104-1105, 2015) that chromosomal insertions of AAV serotype 2 seem to activate proto-oncogenes in human hepatocellular carcinoma. This includes replication-incompetent AAV vectors containing foreign promoter-enhancer elements; the occurrence of male infertility and the presence of AAV viral DNA sequences in human semen [Rohde et al., 1999]; the occurrence of miscarriage and the presence of infectious AAV in embryonic material as well as in the

cervical epithelium [Tobiasch et al., 1998; Walz et al.,1997]. A clear association is hard to establish from these studies, given that co-incident evidence of human papillomavirus infection is present in most subjects [Malhomme et al., 1997], and that AAV DNA can be detected in cervical samples in the majority of women [Burguete et al., 1999] but is very dependent on differences in sample collection between studies [Erles etal., 2001]. In favor of a possible causal role of AAV in the occurrence of miscarriage, AAV 2 has been shown to interfere with mouse embryonic development [Botquin et al., 1994]. Furthermore, a significant correlation has been established between the presence of AAV DNA in amnion fluids and premature amniorrhexis and premature labor [Burguete et al., 1999].

12. **Modes of Transmission:** Virus may be transmitted in the following ways: 1) a skin puncture or injection, 2) ingestion, 3) contact with mucous membranes (eyes, nose, or mouth), 4) contact with non-intact skin, and 5) low risk exposures include bites from an animal inoculated with lentivirus, percutaneous contact with body fluids from an animal inoculated with lentivirus and aerosols.
13. **Length of gene expression:** Variable, may be months to years
14. **Communicability:** Replication incompetent vectors: Not communicable.

15. **Medical surveillance and clinical treatment procedure**: No medical surveillance is required. There is no effective medical treatment should accidental exposure occur.
16. **Safety Generation of AAV Vector:** AAV vector where the *rep* gene has been deleted have lost the ability to preferentially integrate and may randomly integrate into host genomes, form episomes or randomly recombine with host genomes. There is a theoretical risk of insertional mutagenesis which may result in an increased cancer risk. Cells and tissues infected with viral vectors may exhibit expression of insert genes affecting their normal function. Oncogenes, immune modulators and toxins may cause increased cancer risk, inflammation and cell death, respectively.
17. **Stability in Environment:** Non-lipid enveloped viruses such as AAV are low resistant to level disinfectants, survive well outside of the laboratory and environment can be easily transmitted via contaminated clothing. AAV particles are resistant to a wide pH range (pH 3-9) and can resist heating at 56^{o}C for 1 hour. AAV can remain infectious for at least a month at room temperature following simple desiccation or lyophilization. Contaminated materials must be chemically decontaminated with a suitable disinfectant or autoclaved. Weaker disinfectants like 70% ethanol, quaternary ammonium compounds (ingredients in Pine Sol©, Lysol and Cavicide©) and phenols should be avoided.
18. **Vector concentration, dosage per experiment:** State your stock vector concentration, and the amount used per experiment or kg.
19. **Vector shedding from humans or animals:** In humans, shedding in blood was rarely observed]. Intramuscular administration resulted in shedding of the AAV vector as measured by PCR in saliva and serum up to 24 and 48 h after injection,

respectively, and no vector was observed in semen obtained after about 2 months. When patients were treated with the same vector by infusion through the hepatic artery, dose-dependent shedding in urine was found during the first post- treatment week. In 6 out of these 7 treated patients, vector DNA was found in semen up to 16 weeks after therapy. In one patient the vector was present in the seminal fluid and not in motile sperm.

After dosing, animals can be housed at ABSL-1 for the duration of the experiment.

20. **Transgene Information:** Discuss effects of transgene on animal or cell line. A good source for understanding the transgene being silenced or over-expressed is GENE CARDS (http://www.genecards.org/). A snapshot of a sample gene card is shown below:

To better understand potential human outcomes from accidental silencing, you can see if information exists in the Mouse Genome Informatics (http://informatics.jax.org/)

You must discuss the potential effects due to accidental worker exposure. If unknown, state that. Is the gene sequence or siRNA specific to an animal, humans or could it affect both.