How to Complete a Risk Assessment of Adenovirus Experiments in Animals and Cell Culture

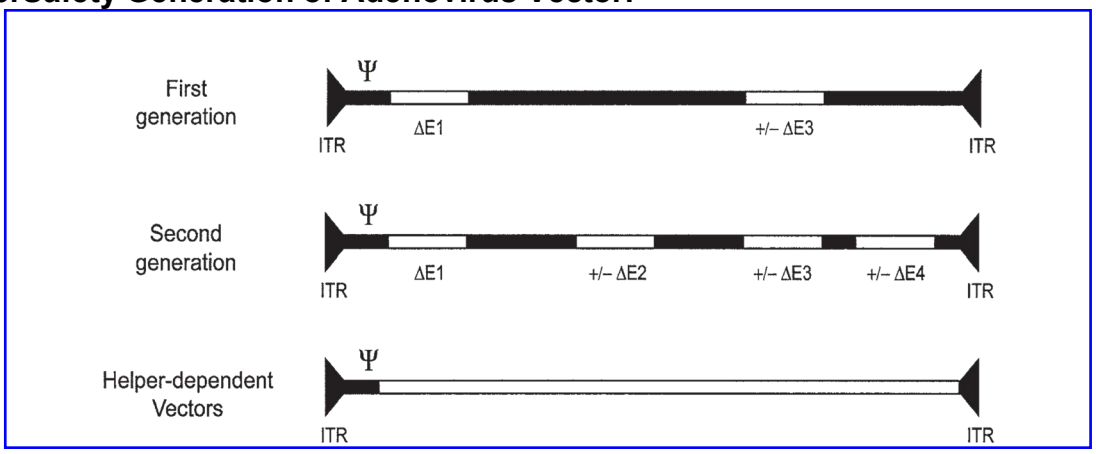
1. **Organism or Agent:** Adenovirus Vectors
2. **Synonym:** *Adenoviridae* family
3. **Characteristics:** A non-enveloped virus, of which there are at least 51 human adenovirus serotypes (genus *Mastadenovirus*) which have been divided into subgroups – (A to F) based on their capacity to agglutinate erythrocytes of human, rat and monkey as well as on their oncogenicity in rodents. The serotypic origin of the E1A gene determines the oncogenic phenotype of adenovirus-transformed cells. Viruses belonging to subgroup A (such as adenovirus 12, Ad12) induce tumours with high frequency and short latency, while viruses from subgroup B (such as Ad3 and Ad7) are weakly oncogenic. Adenoviruses from subgroup C (which includes the well-studied serotypes Ad2 and Ad5), D, E and F are non-oncogenic. All human adenoviruses studied so far can transform primary rodent cells in culture; however, only cells transformed by viruses of subgenus A and B are oncogenic in newborn rodents, paralleling the oncogenic properties of the parental viruses.
4. **Containment Requirements:** Depends on agent: Recommend Biosafety level 2 practices, containment equipment and facilities for all activities involving the manipulation of the virus; 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.

   Manipulations: Depending on the vector, work may need to be performed within a biosafety cabinet, and the use of sharps including needles, blades and
5. 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:** The ASM's Manual of Clinical Microbiology claims 0.25% sodium dodecyl sulfate (SDS) will kill adenovirus, but researchers have usually used between 1-5% SDS. A pH above 12 inactivates the virus, as does

temperatures above 56°C for 10 min. A 2% solution of Virkon has also been shown to kill adenovirus. Vesphene and LpH have also been shown to kill adenovirus, **but only in solution and not on dried surfaces**.

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:** Adenovirus replicates as episomal elements in the nucleus of the host cell & consequently there is no risk of insertional mutagenesis.
12. **Modes of Transmission:** Since adenoviruses are effectively transmitted as aerosols, there is a concern that a person exposed to a mist of adenoviral particles could generate an immune response to any gene inserted. Virus may also be transmitted in the following ways: 1) a skin puncture or injection, 2) contact with mucous membranes (eyes, nose, or mouth), 3) contact with non-intact skin, and 4) low risk exposures include bites and percutaneous contact with body fluids from an animal inoculated with adenovirus.
13. **Length of gene expression:** Adenoviruses integrate into the cell genome only at very low frequency, which results in unstable gene expression [9, 10, 11, 12, 13]. Transgene expression can range up to 14 days.
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 Adenovirus Vector:**



Genome structure of first-generation, second-generation, and helper-dependent vectors. Regions that have been deleted are indicated by open boxes.

17. **Stability in Environment:** Adenoviruses are comprised of a protein shell and a linear double stranded DNA. This makes them fairly resistant to killing by drying. The Public Health Agency of Canada (http://www.phac-aspc.gc.ca/msds-ftss/msds3e.html) has reported that adenovirus type 2 samples have been

recovered from environmental samples for 3-8 weeks after inoculation at room temperature.

18. **Vector concentration, dosage per experiment:** The adenoviruses may be concentrated to 10^{12} viral particles/ml. State your stock vector concentration, and the amount used per experiment or kg.
19. **Vector shedding from humans or animals:** Some adenovirus studies show no observed shedding from intratumoral, intranasal, inhalation, intracoronary, intramyocardial, intravitreous, intraarterial, intrapleural or intramuscular administration.

    Other studies reported on shedding of vector DNA or infectious particles in various excreta, primarily depending on the route and site of administration and the time of analysis. For instance, saliva and nasopharyngeal fluids from some patients treated with a vector by intranasal administration or via inhalation were found to contain vector in a number of studies. In a substantial number of publications describing intratumoral gene therapy in patients with various types of cancer shedding was demonstrated, primarily in blood and related products. In general, shedding in blood was short lasting, peaking during the first hours and disappearing a few days after administration. In one of these studies, vector sequences were found in urine from the majority of treated patients up to day 32 after injection of the vector in prostate tumor cells. The longest duration of shedding was reported in publications on the treatment of cystic fibrosis via inhalation or of lung cancer using intrabronchial or intratumoral administration. After a single treatment, nasopharyngeal fluids like bronchoalveolar lavage, nasal and pharyngeal swabs and saliva were reported to be positive for vector sequences up to 21, 30 and even 90 days. Two publications reported on the analysis of semen from 12 patients with angina pectoris 8 weeks after intracoronary administration and from one patient with prostate cancer at day 14 after injection in a prostate tumor, respectively.
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.