

Work with Adenoviruses and Adenovirus Vectors in the University of Hong Kong

Guidance

1. Sources of Basic Information

A substantial contribution to the structure and content of this guidance has been made by the adenovirus section in Part 2 of the UK, Health and Safety Executives, Scientific Advisory Committee on Genetic Modification “Compendium of Guidance” which can be found at

<http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp>. Other guidance on containment conditions for work at BSL2 (appropriate for most adenovirus work) can be found in the Centre for Disease Control’s Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th edition

<https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF>

A useful summary of adenovirus properties can also be found on the CDC website at

<https://www.cdc.gov/adenovirus/hcp/clinical-overview.html>

A user manual for the Takarabio Adeno-X™ vector including background and some safety advice is available on the company website

[https://www.takarabio.com/products/viral-transduction/adenoviral-vector-systems/adeno-xt-adenoviral-system-3-\(tet-on%C2%AE-3g-inducible\)](https://www.takarabio.com/products/viral-transduction/adenoviral-vector-systems/adeno-xt-adenoviral-system-3-(tet-on%C2%AE-3g-inducible))

Similarly Agilent have user manuals for the AdEasy™ system which can be found at

<https://www.agilent.com/cs/library/usermanuals/public/240010.pdf>

<https://www.agilent.com/cs/library/usermanuals/public/240009.pdf>

2. Introduction

As soon as the basic tools of molecular biology became available adenoviruses, (Ad) (particularly Ad2 and 5) became the focus of much interest. This was in part due to their relative ease of culture, the fact that they were DNA viruses – DNA was easier to handle than RNA, and they were intermediate in complexity between Φ X174, SV40, Polyoma, and the much larger herpesviruses and poxviruses. Features such as genomic inverted terminal repeats, small Pol III derived non coding RNA’s (VA RNA’s), proteins involved in replication covalently bound to the end of the genome and probably most significantly RNA splicing were all first described in adenoviruses. Today adenoviruses are popular vectors for expressing genes, initially developed for gene therapy applications, but are now also being widely used for vaccination studies since they are able to induce CTL’s against foreign genes expressed by the virus. The following background and guidance focuses on safe working with human adenoviruses, however, many of the principles will also apply to work with animal adenoviruses such as ovine atadenovirus 31, canine adenovirus type 2 and bovine adenovirus serotype 3, all of which are being developed as vectors for gene therapy and vaccination.

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3. Adenovirus Background

3.1 Natural History and Serotypes

Over 100 serotypes of adenoviruses have been described, infecting a wide range of animals including mammals and birds. Of these, there are 57 serotypes of human adenoviruses (Ads), which are divided into seven subgenera (A–G) based on biochemical, immunological, and morphological criteria. Human disease ranges in severity from asymptomatic infections as in the case of Ad12 to mild respiratory infections (Ad2; Ad5), conjunctivitis (Ad8; Ad19; Ad37), gastroenteritis (Ad40; Ad41), and acute respiratory disease in adults (Ad4; Ad7). Interestingly Ad 7 and Ad 12 have also been shown to be tumourigenic in neonatal rats, although this has never been documented in humans. Primary infection generally occurs in childhood via the airborne or faecal-oral routes and some types are capable of establishing persistent asymptomatic infections in tonsils, adenoids, and intestines of infected hosts which can result in virus shedding for months or even years. Immunity is thought to be lifelong and over 90% of individuals are seropositive for Ad2 and Ad5.

Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body. For example after having been extracted with ether and/or chloroform, adenoviruses (unlike HIV or herpesviruses) can still be infective. This fact is significant in any environmental risk assessments that are undertaken.

Risk Group Classification

Wild type human adenoviruses are generally considered as Biosafety Level 2 (BSL2) agents, this means they are of a moderate risk to the individual and a low risk to the community.

WHO risk group 2 (equivalent to BSL2) agent definition:-

A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

3.2 Viral Structure and Replication

The adenovirus virion as typified by Ad 2 and Ad 5 comprises a non-enveloped icosahedral capsid containing a 36 kb double-stranded DNA genome. Adenoviruses can infect dividing and non-dividing cells from a broad variety cell types including those derived from the Chicken, Monkey, Mouse, Pig, Rabbit, Rat and Sheep. The initial interaction between the virus and cell is mediated by the viral fibre protein (product of adenovirus L5 gene) which binds to a widely expressed, 46 kDa member of the immunoglobulin superfamily - the cellular Coxsackie B Adenovirus Receptor (CAR) - so named because the picornavirus Coxsackie B also uses the protein as a receptor. Following virus adsorption, tripeptide Arg-Gly-Asp (RGD) motifs on the penton base interact with cell surface α integrins, stimulating cell signaling which induces actin polymerization resulting in entry of the virion into the host cell within an endosome. It is worth noting that not all serotypes share the same affinity for CAR and some utilise alternate receptors (e.g. group B viruses use CD46) and cell-surface integrins.

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As indicated above the adenovirus serotype 5 fiber protein engages the CAR to bind cells in cell culture. However, both experimentally and clinically it has been found that adenoviruses target liver cells *in vivo* and this has compromised their potential efficacy in gene therapy trials. Paradoxically, following intravascular delivery, CAR is not used for liver transduction. Recent data has shown that coagulation factor (F) X directly binds Ad5 hexon, via an interaction between the FX Gla domain and hypervariable regions of the hexon surface. Liver infection by the FX-Ad5 complex is mediated through a heparin-binding exosite in the FX serine protease domain. This unanticipated function for hexon in mediating liver gene transfer *in vivo* illustrates a general observation that natural infection and its risks may be quite different from risks encountered in clinical applications or in laboratory acquired infection.

Once in the host cell the endosome acidifies leading to release of the virion and with the help of cellular microtubules the virus is transported to the nuclear pore complex. In the process the adenovirus particles disassemble and viral DNA is released which enters the nucleus via the nuclear pore and initiates replication.

3.3 Virus Gene Expression

Adenovirus infection is separated by the DNA replication process into two phases: an early and a late phase. In both phases primary transcripts are generated which then become alternatively spliced to generate monocistronic mRNAs. Early transcription occurs 6 to 8 hours after infection, generating early proteins from four major regions, E1, E2, E3 and E4. The early genes are responsible for expressing mainly non-structural,

regulatory proteins which perform three main functions:-

- 3.3.1** To alter the expression of host proteins that are necessary for DNA synthesis (e.g. E1A disrupts cell-cycle regulation by binding to key regulators of transcription and mitosis. This results in the expression of the pro-apoptotic factors, including p53, which is bound and inactivated by an E1B protein);
- 3.3.2** To activate other virus genes (e.g. the virus-encoded DNA polymerase, mediated by E2 derived proteins); and
- 3.3.3** To avoid premature death of the infected cell by the host-immune defenses (blockage of apoptosis, blockage of interferon activity, and blockage of MHC class I translocation and expression).

Once the early transcripts have produced sufficient virus proteins, replication machinery and replication substrates, replication of the adenovirus genome can occur. A terminal protein that is covalently bound to the 5' end of the adenovirus genome acts as a primer for replication. The viral DNA polymerase then uses a strand displacement mechanism, to replicate the genome.

The late phase of the adenovirus life cycle commences with DNA replication. Late transcription is directed by the strong Major Late Promoter (MLP) 4 to 6 hours after the onset of early transcription and produces 30,000 nucleotide primary transcripts which are alternatively spliced resulting in 5 families of transcripts and the expression of the structural proteins L1, L2, L3, L4 and L5. The lytic cycle

lasts for 24 - 48 hours (depending on subtype and target cell) generating up to 1×10^5 viral particles per infected cell and virus stocks are often generated with titres in the range of 10^{10} to 10^{11} pfu/ml.

Biosafety Level 2 (BSL2) should be adopted as a minimum requirement for conditionally replicating adenoviruses (CRAds) because although attenuated there remains the risk that lytic infection could occur at unforeseen sites and recombination resulting in a replication competent viruses or wild-type virus is a possibility. Only in cases where the risk assessment clearly shows BSL2 to be unwarranted or where *in vivo* safety data is available can they be handled at BSL1.

Once the viral components have been replicated the virus is assembled into its virion and released from the cell as a result of virally induced cell lysis (aided by one of the E3 proteins - the so called Adenovirus Death Protein!).

4. Adenovirus vector systems

4.1 Conditionally Replicative Vectors

Conditionally replicating adenoviruses (CRAds) are capable of undergoing the full viral lytic cycle in permissive cells but are restricted in their growth on normal cells. For example, E1B-deleted vectors replicate preferentially in cells that do not express p53 or have a disrupted p53 pathway (which encompasses most malignant cell types). Consequently tumours with disrupted p53 can be selectively lysed by infection with this type of CRad. Indeed an E1B (and partial E3) deleted adenovirus, H101, developed by Shanghai Sunwaybio, has been

used extensively to treat human tumours in China and is now licensed for clinical use.

Alternatively, the E1A promoter can be replaced by a tissue-specific or inducible promoter allowing the virus to replicate only in a targeted cell type or in response to known stimuli. Further modifications at the level of mRNA stabilization are also possible. For example the E1A gene fused to the 3' untranslated region (UTR) of the COX2 gene allows activated RAS/P-MAPK-specific stabilization of its mRNA. Induction of activated RAS (as found in some tumour types) supports replication, whereas matched cells in which activated RAS/P-MAPK is not expressed are very poor substrates for viral replication both *in vitro* and *in vivo*.

4.2 Disabled Vectors

"First Generation" Vectors

The vast majority of disabled Ad viruses used as vectors have a deletion in the E1 early gene region. The E1A and E1B are usually supplied *in trans* using a complementing cell line that contains the E1 genes integrated into the genome of the cell e.g. HEK293 or PerC6. The packaging sequences are retained in the viral genome so that viable, disabled, recombinant progeny can be generated. Since adenoviruses have a strict packaging limit (105% of the wt genome size), the E3 cassette is also commonly deleted since it is dispensable for growth *in vitro*, allowing up to 8Kb of foreign genes to be inserted. This includes systems such as Ad-X™ from Takarabio, and the Ad Easy™ system from Agilent.

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"Second Generation" Vectors

These Ad vectors also have much of the E2 cassette deleted, increasing its packaging capacity and further disabling the virus by removing its capability to replicate and process viral DNA. This deletion also virtually eliminates the possibility of a recombination event that might result in replication competent viruses (RCV) thereby improving safety.

"Third Generation", or "Gutless" Vectors

Generally this type of vector retains only the packaging sequences from adenovirus and therefore has the largest capacity for inserted genetic material. These vectors require extensive complementation in trans from a helper virus and therefore risks associated with the helper must be considered in detail.

Adenovirus vector strains that pose a much-reduced risk of harm compared to the wild-type virus e.g. the disabled, E1-E3 deleted, viruses might be handled at Biosafety Level 1. The risk assessment must demonstrate that the virus is sufficiently attenuated to warrant the lower classification. Furthermore, the likelihood of a reversion event must be low and the stock should be demonstrably free of any replication competent virus.

Hazards arising from the insertion of sequences or phenotypic alterations might require additional containment measures. However, it is likely that RCV free E1-E3 deleted viruses containing marker genes such as β -galactosidase, luciferase or GFP could be safely handled at BSL-1 while E1-E3 deleted recombinants expressing biologically active molecules would be handled at BSL-2.

5. Hazards Associated with Inserted Genetic Elements

Inserted genetic elements include (but are not limited to) natural, synthetic or hybrid genes, DNA coding for various RNA molecules involved in gene control and expression such as ribozymes, shRNA, microRNA, and other elements such as RNA stabilising regions, ribosome binding sites, gene expression "insulators", modified promoters etc. The risk assessment should take into consideration the potential effects of the expressed product.

The factors to consider include:-

Expression Characteristics

This will be dependent on the cell type and the regulatory sequences used to control expression. For example, many Ad recombinants use of the Human cytomegalovirus major immediate-early promoter/enhancer which would be expected to direct high-level expression in a many different cell types. Where tissue-specific expression is required promoters active in the target tissue can be used e.g. long PSA and osteocalcin in prostate cells have been used to drive HSV-tk expression in Ad based gene therapy vectors designed to treat prostate cancer. It should be remembered that this type of promoter generally has some basal activity in non-target cells. The specific details of the construct should also be analysed carefully, for example the remnants of the adenovirus E1 promoter (which overlaps with essential viral packaging sequences) might overcome any restriction imposed by a cell-type specific promoter on genes cloned into the E1 region of the virus. It is advised that promoter characteristics are thoroughly assessed where possible using non-hazardous reporter genes in

low-risk virus-free cell culture systems before generating a recombinant virus.

In most transduced tissues, expression from Ad vectors is transient due to clearance of the virus by the immune system and lasts only one to two weeks. However, in some “immune privileged” tissues expression may be longer, persisting for a year or more.

Integration into Host DNA

Integration into the host genome represents the only significant mechanism by which long-term expression can be maintained by disabled Ad vectors. This is relatively rare, occurring at a frequency of approximately 1 in 10⁸ pfu in human primary cell cultures. The effects of integration in relation to the properties of the insert should be considered. Gutless vectors are primarily maintained episomally and may therefore integrate at lower frequencies.

Biological Properties of the Gene Product

The expected activities or toxicity of the gene products incorporated into the vector should be assessed. For example, a cytokine or a bacterial toxin would represent greater risk of harm than reporter genes such as Green Fluorescent Protein (GFP), Luciferase and β -galactosidase. Properties of the gene products with respect to individual cell types should also be considered.

6. Alteration of Phenotype

6.1 Tissue Tropism

Adenoviruses can infect a wide variety of cell types, although individual serotypes have more restricted tropisms. For a variety of reasons several strategies have been employed to broaden the tropism of Ad5-based gene transfer

vectors. The approaches can be divided in those that involve direct genetic modification of the capsid proteins and those that rely on conjugating adenovirus with adapter molecules. Conditionally replicating adenoviruses (CRAd) carrying foreign peptides in the fiber have exhibited an expanded tropism. Also CRAd carrying chimeric fibers generated by exchanging parts of the Ad5 fiber protein with that of different adenovirus serotypes displayed augmented infectivity on CAR-deficient cancer cells. This approach has some limitations as insertions in the fiber are restricted by structural restraints e.g. retaining proper trimerisation of the fiber and pseudotyped viruses are limited by the targeting repertoire of naturally occurring adenovirus serotypes.

The adapter molecule approach is illustrated by one paper that describes the use of a bispecific single-chain antibody (scFv) that contains an anti-epidermal growth factor receptor (EGFR) domain and an anti-adenovirus fiber knob domain. This molecule could be used to treat adenovirus preparations allowing the virus to bind to cells that express EGFR but have only low levels of CAR its normal receptor. A disadvantage of this two-component approach is that the targeting moiety is not an integral part of the adenovirus capsid. However if the gene encoding the adapter protein is incorporated into a CRAd genome the targeting property of the recombinant will be retained upon in situ replication and spread. Thus in any risk assessment of this type of recombinant the susceptibility of additional tissues to infection should also be considered.

6.2 Immunogenicity and Pathogenicity

Deletions in the viral vector or the genetic insert may alter the immunogenic or pathogenic nature

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of the virus. For example, proteins derived from the E3 cassette (which is often deleted in adenoviral vectors) are involved in immune evasion strategies *in vivo* such as down regulation of MHC on the infected cell surface. Their deletion, whilst facilitating the clearance of virus by the host immune system, might result in an increased inflammatory response and increased pathogenicity. There is evidence for this increased pathogenicity in cotton rats when E3 is deleted, however this may not be the case in other species. Likewise, insertion and expression of cytokines which modulate the immune response e.g. IL-4 may have a similar effect.

6.3 Recombination and Complementation

One concern when using Ad vectors is the occurrence of replication competent viruses (RCV) in a population of replication-deficient viruses. RCV can emerge as a result of a rare double crossover through overlapping sequences present in the recombinant adenovirus and the genome of HEK293 cells. This event results in the replacement of the transgene by E1 region. Once this happens, the adenovirus could replicate, without the need of a complementing cell line. The probability of acquisition of sequences from a complementing cell line or helper virus can be minimised if there are no overlapping sequences. For example, HEK293 cells carry 11% of the adenovirus genome containing the E1 cassette; this includes at least 800 bp of sequence present within most E1-deleted adenovirus vectors, providing the potential for recombination that restores the E1 region in the virus. In contrast, PerC6 and similar cell lines have been engineered to express the minimal E1A and E1B genes from heterologous promoters, and thus have no sequence overlap with most newer E1-deleted

vectors, greatly reducing the frequency of generating replication-competent virus.

The presence of RCV can be detected by PCR and quantitated by plaque assay on non-complementary cells. According to some companies manufacturing Ad vectors <1 plaque in about 10⁴ viruses is considered safe to use. To avoid the occurrence of RCV, viruses should be produced and amplified in low passage packaging cells. Serial passage should be avoided as any replication competent virus present may have a selective advantage over the defective recombinant.

The possibility of recombination that might result in harmful sequences being transferred between related viruses should also be considered. This could take place between a vector and a wt adenovirus or viral sequences present in a cell. For example it has been known for some time that >20% of normal healthy adults have E1A sequences present in their respiratory epithelium*. In most adenovirus vectors the site of insertion of the foreign genetic material is also the site of disablement. i.e. E1 has been replaced by the foreign element. Thus, any homologous recombination in infected cells that restores E1 sequences to the vector thereby allowing replication will also delete the insert and vice-versa. Inserts cloned into other areas of the viral genome could be maintained in the event that E1 sequences are restored, resulting in a recombinant RCV.

* Eissa NT, Chu CS, Danel C and R.G. Crystal (1994) Evaluation of the respiratory epithelium of normals and individuals with cystic fibrosis for the presence of adenovirus E1a sequences relevant to the use of E1a-adenovirus vectors for gene therapy for the respiratory manifestations of cystic fibrosis. Human Gene Therapy

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A means for monitoring for the presence of RCV in disabled virus stocks should be in place, where appropriate. Permissive, non-complementing cell lines should show signs of productive infection (cytopathic effect, plaque formation) in the presence of RCV and they could be used to test stocks of a disabled recombinant virus. However, such assays may not be completely reliable as disabled viruses are often cytopathic themselves. The use of molecular detection methods (for example quantification of E1 sequences in a purified virus preparation using quantitative PCR) would represent a more reliable method of RCV detection.

7. Operational Considerations

Adenovirus vectors are generally constructed by molecular cloning of two overlapping plasmids containing distinct regions of the viral genome. These plasmids are either ligated together prior to transfection or are co-transfected into a complementing cell line whereby viable viral genomes are generated by homologous recombination. Alternatively whole Ad genomes are cloned and manipulated in *E.coli* as bacterial artificial chromosomes and in *S. cerevisiae* as a yeast artificial chromosomes, the genomic DNA purified and the recombinant adenovirus rescued in a complementing cell line. Other systems, particularly the gutless vectors, require the use of “helper viruses”. The hazards associated with these methods should be considered in addition to those associated with the proposed recombinant virus.

Adenoviruses are often purified by ultracentrifugation on caesium chloride gradients and high-titre, concentrated virus extracted using a hollow needle. Care should be taken to ensure

that centrifugation vessels are properly sealed. Needles should be used with extreme care, only when absolutely necessary and never be re-sheathed but rather disposed of directly.

8. Control Measures and Monitoring Procedures

8.1 Use of Class II Biological Safety Cabinets

Many adenovirus vectors will be considered low risk GM activity class 1 and can be handled at containment-level 1. This means that virus preparations could be handled on the open bench. However, adenoviruses are robust and transmitted effectively in aerosols and droplets, even if disabled or attenuated. Therefore measures might be required to control aerosol generation and airborne dissemination.

Most work with adenoviruses will take place within a microbiological safety cabinet. As a general point the reasons are primarily twofold:-

8.1.1 To keep the materials under study free from bacterial (or adventitious viral) infection – sterile technique is still required to avoid cross contamination from gloved hands etc.

8.1.2 To protect the worker against *airborne* virus generated by the manipulations being carried out. Injection of animals in a cabinet is not usually necessary because there is a low risk of generating aerosols during animal work. However, where the risk assessment shows that exposure to airborne adenovirus represents a hazard, the use of a cabinet might be required as a control measure. For example, when purifying adenoviruses, using ultra- centrifugation on

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caesium chloride gradients it is prudent to open the rotor in a Class 2 biosafety cabinet.

8.2 Guidelines for the Safe Handling of Adenovirus and Vectors

Control measures will depend on the nature of the risks with particular experiments. The experiments must be risk assessed before work commences to ensure appropriate safety measures are employed. Below is a brief outline of factors to consider:-

8.2.1 Transport of adenovirus:

Transport all material in a double-sealed leakproof container.

Label the container with a biohazard symbol, the name of the agent, the amount, and the Principal Investigator's name and telephone number.

8.2.2 Waste:

Decontaminate all cultures, stocks, and other biological wastes before disposal using approved decontamination methods, such as autoclaving. Before decontamination outside of the laboratory the biological materials should be placed in a sealed, durable, leak-proof container for transport.

The most effective decontamination is given (with a minimum of 15 minutes contact time) by:-

- 1% Sodium hypochlorite
- 2% Glutaraldehyde
- 5% Phenol

OR

- Autoclaving for 30 minutes at 121°C or 250°F (15 lbs per square inch of steam pressure)

8.2.3 For general laboratory work and tissue culture:

- A biological hazard sign indicating the use of adenovirus should be placed outside laboratory or tissue culture room and on the biological safety cabinet.
- Laboratory coats, gloves, and safety glasses or goggles must be worn.
- Where virus is handled laboratory coats etc used inside the laboratory should not be worn outside of the laboratory.
- Materials containing adenovirus should be handled inside biological safety cabinets capable of protecting the product and personnel, whenever possible.
- *Wear a respirator (N95 NIOSH classification TC-84A) for which the wearer has been fit tested, to protect against exposures from aerosols during spills of virus containing materials when handling adenovirus-containing cultures outside of containment equipment.*
- When performing centrifugation procedures, use sealed rotors with primary and secondary containment. Open rotors in a biological safety cabinet.
- Avoid vacuum lines but if carrying out large numbers of manipulations these can be used. As a minimum protect them with disinfectant traps and filters. If aspirated liquid waste is 2/3 full, aspirate sodium

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hypochlorite through the suction tube so that the final concentration is appropriate, allow it to soak for at least 15 minutes, and empty entire contents down the drain.

8.2.4 Biological safety cabinets:

- Materials should not be stored inside the biological safety cabinet (BSC). Take only what is needed to perform the procedure(s) and place it in the BSC upon initiation of the procedure. Upon conclusion of the procedure(s), remove everything from the BSC.
- Serological pipettes and pipette tips should be decontaminated with an appropriate anti-viral agent such as a 1:10 dilution of household bleach (final concentration 0.525%), for at least 15 minutes prior to discarding in solid biohazard waste. For this purpose, a beaker containing the virucide can be kept inside the BSC while experimental procedures are being performed.
- Upon conclusion of procedures in the BSC spray all work surfaces with a virucide and then with 80% ethanol. Allow the surface to air dry.

8.2.5 Sharps policy:

Adopt a stringent sharps policy particularly handling needles i.e. use sharps only when absolutely necessary, no re-sheathing, limiting multiple uses of the syringe, disposal directly to sharps bins, appropriate restraint/anesthesia if injecting animals etc. It is worth noting that harvesting from caesium chloride gradients is a high risk operation because of the high titres of virus

present and the need to harvest the virus band with a hollow needle.

8.2.5 Emergency procedures:

Clear procedures for emergencies must be documented in advance of the work being carried out. Details for different scenarios are appropriate e.g. dropping the stock vial of virus inside or outside of a hood, accidental injection or spilling /dripping virus containing material in a hood or onto the bench or even contaminating an animal while injecting.

8.3 University Administrative Procedures

- (i) Complete risk assessment form [RA4](#). Worked examples of several adenovirus vectors can be found on the University Safety Office website under the Biosafety microsite at <http://hku.hk/safety>.
- (ii) Return the completed assessment to the University Biological Safety Officer via e-mail (paulhunt@hku.hk or safety@hku.hk) or by internal post. The proposal, which is treated as confidential information, will be reviewed by the University Biosafety committee and will be approved if deemed appropriate. It is the intention of the committee to carry out the review within 5 working days but in some circumstances such as holiday periods or if large numbers of proposals are submitted it may take longer.
- (iii) It is advisable for departments to develop their own local code of practice (COP), sometimes referred to as a standard operating procedure (SOP), for the areas where they will work with adenovirus or

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adenovirus vectors. The type of information that might be included in a COP are an introduction, general procedures, the operation of the laboratory, any local rules, procedures for safe handling of waste, any

staff health issues, what testing and maintenance is required and emergency procedures. An SOP is likely to contain more detail in terms of the specific experimental protocols to be adopted.
