

# *Environmental Health and Safety Update*

## Adenovirus Containment Level

Clarification Email  
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The following information is in follow-up to a phone conversation on July 22, 2002 regarding the containment levels for Adenovirus-based vectors that have been rendered replication incompetent. The specific question was the appropriateness of classifying replication-defective adenoviruses as Risk Group 1 (RG1) agents, thereby allowing manipulations of these vectors to occur at Biosafety Level 1 (BL1).

Section III-D-3 of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) - April 2002 states the following:

Section III-D-3. Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems

Caution: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions that permit a productive infection. In such cases, serious consideration should be given to increasing physical containment by at least one level.

Note: Recombinant DNA or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family [see Section V-J <[http://www4.od.nih.gov/oba/rac/guidelines\\_02/](http://www4.od.nih.gov/oba/rac/guidelines_02/)>, Footnotes and References of Sections I-IV) being considered identical (see Section V-K <[http://www4.od.nih.gov/oba/rac/guidelines\\_02/](http://www4.od.nih.gov/oba/rac/guidelines_02/)> are considered defective and may be used in the absence of helper under the conditions specified in Section III-E-1 <[http://www4.od.nih.gov/oba/rac/guidelines\\_02/](http://www4.od.nih.gov/oba/rac/guidelines_02/)>, Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus.

Section III-D-3-a. Experiments involving the use of infectious or defective Risk Group 2 viruses (see Appendix B-II <[http://www4.od.nih.gov/oba/rac/guidelines\\_02/APPENDIX\\_B.htm](http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_B.htm)> Risk Group 2 Agents) in the presence of helper virus may be conducted at BL2.

Section III-E-1 states:

Section III-E-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus

Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family being considered identical [see Section V-J <[http://www4.od.nih.gov/oba/rac/guidelines\\_02/](http://www4.od.nih.gov/oba/rac/guidelines_02/)> Footnotes and References of Sections I-IV]) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III-D-3 <[http://www4.od.nih.gov/oba/rac/guidelines\\_02/](http://www4.od.nih.gov/oba/rac/guidelines_02/)>, Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper

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Virus in Tissue Culture Systems, should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.

Therefore, replication defective adenoviral vectors that are deleted to 2/3 of their genome size may be manipulated at BL-1 provided that no contaminating helper virus (or replication competent adenovirus -- RCA) is present in the vector preparation. At the present time, the only adenoviral vectors that could fall in this category are the internally deleted adenoviral vectors - the so-called "gutless" vectors. To date no one has approached the NIH Office of Biotechnology Activities formally requesting that the Risk Group assignment for adenovirus-based vectors be diminished.

In fact the only vector system that has been given special consideration in this regard is the Murine Retroviral vector system; this provision is specifically mentioned under Appendix B-V-1 as follows:

### Appendix B-V-1 Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

Again, it is important to stress that although Murine Retroviral vectors can be manipulated at BL1, the vector preparation must be shown to be free of replication competent retrovirus (RCR). Thus, viral preparations must be handled at BL2 in the production phase until subsequent analyses demonstrate the absence of (detectable) RCR, at which point the vector preparations may be handled at BL1.

I have performed a risk assessment of the use of E1-deleted adenoviral vectors (a RG2 agent) in my laboratory, and I have determined that these vectors are safe. Can my IBC allow me to operate at BL1?

No.

According to Section III-D of the NIH Guidelines:

### Section III-D. Experiments that Require Institutional Biosafety Committee Approval Before Initiation

Prior to the initiation of an experiment that falls into this category, the Principal Investigator must submit a registration document to the Institutional Biosafety Committee which contains the following information: (i) the source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the NIH Guidelines. For experiments in this category, the registration document shall be dated, signed by the Principal Investigator, and filed with the Institutional Biosafety Committee. The Institutional Biosafety Committee shall review and approve all experiments in this category prior to their initiation. Requests to decrease the level of containment specified for experiments in this category will be considered by NIH (see Section IV-C-1-b-(2)-(c) <[http://www4.od.nih.gov/oba/rac/guidelines\\_02/](http://www4.od.nih.gov/oba/rac/guidelines_02/), Minor Actions).

Section III-D-1-a. Experiments involving the introduction of recombinant DNA into Risk Group 2 agents will usually be conducted at Biosafety Level (BL) 2 containment. Experiments with such agents will usually be conducted with whole animals at BL2 or BL2-N (Animals) containment.

The IBC cannot change the Risk Group level attributed to a biological organism or the containment level associated with that risk group. This is a decision that only NIH can make. However, the IBC can require that containment procedures and practices be INCREASED based on the risk assessment of a planned project.

Note, however, that according to Section III-D-2-(a) of the NIH Guidelines:

Section III-D-2-a. Experiments in which DNA from Risk Group 2 or Risk Group 3 agents (see Section II-A <[http://www4.od.nih.gov/oba/rac/guidelines\\_02/](http://www4.od.nih.gov/oba/rac/guidelines_02/)>, Risk Assessment) is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment. Experiments in which DNA

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from Risk Group 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BL4 containment shall be used. The Institutional Biosafety Committee may approve the specific lowering of containment for particular experiments to BL1. Many experiments in this category are exempt from the NIH Guidelines (see Section III-F <[http://www4.od.nih.gov/oba/rac/guidelines\\_02/](http://www4.od.nih.gov/oba/rac/guidelines_02/)>, Exempt Experiments). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH/OBA approval (see Section III-B-1 <[http://www4.od.nih.gov/oba/rac/guidelines\\_02/](http://www4.od.nih.gov/oba/rac/guidelines_02/)>, Experiments Involving the Cloning of Toxin Molecules with LD50 of Less than 100 Nanograms Per Kilogram Body Weight) or shall be conducted under NIH specified conditions as described in Appendix F <[http://www4.od.nih.gov/oba/rac/guidelines\\_02/APPENDIX\\_F.htm](http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_F.htm)>, Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates.

The IBC can at its discretion, reduce the containment level of certain experiments if recombinant DNA is being introduced into NON-PATHOGENIC ORGANISMS - the preparation and cloning of vector shuttle plasmids is a pertinent example. However, the IBC cannot reduce the containment level of a known pathogen (i.e., the adenoviral vector that contains more than 2/3 of its genome being a RG2 agent in this case).

Why can't replication defective vectors be used at lower containment levels?

Scientific experience has demonstrated that simple vector systems can rescue wild-type viruses.

For retroviral vectors this was particularly true when murine cells were used in the vector propagation process. It turns out that the mouse genome is replete with endogenous retroviral sequences. Through a process of genetic recombination in the packaging cell line, this could lead to the rescue of wild-type retrovirus. Such an event was documented in 1994 and it resulted in a number of non-human primates developing lymphomas.

See:

Vanin EF, Kaloss M, Broscius C, Nienhuis AW.  
Characterization of replication-competent retroviruses from nonhuman primates with virus-induced T-cell lymphomas and observations regarding the mechanism of oncogenesis. *Virol.*  
1994 Jul;68(7):4241-50.  
PMID: 8207799 [PubMed - indexed for MEDLINE]

Similarly, Adenoviruses are equally as recombinogenic, and the use of 293 cells in conjunction with E1-deleted adenoviral vectors leads to a systematic rescue of wild type adenovirus by a process that involves only a single recombination event. In fact, the rescue of wild-type adenovirus using an E1-deleted vector and 293 cells is probably more likely than the rescue of wild-type retrovirus in murine cells. Thus a laboratory scale preparation of E1-deleted adenoviral vector is highly likely to be contaminated with wild-type adenovirus though the level of contamination may be below the limits of PCR detection.

Other cell lines have since been developed to reduce the likelihood of rescue by reengineering the vector cell line to require at least a double recombination event for wild-type rescue. However, illegitimate recombination events are possible and testing for wild-type is a requirement for pre-clinical and clinical applications.

See:

Fallaux FJ, Bout A, van der Velde I, van den Wollenberg DJ, Hehir KM, Keegan J, Auger C, Cramer SJ, van Ormondt H, van der Eb AJ, Valerio D, Hoeben RC.  
New helper cells and matched early region 1-deleted adenovirus vectors prevent generation of replication-competent adenoviruses.  
*Hum Gene Ther.* 1998 Sep 1;9(13):1909-17.  
PMID: 9741429 [PubMed - indexed for MEDLINE]

How, when and what kind of containment should be used with adenoviral vectors?

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In most instances, other than those discussed above, adenoviral vectors must be manipulated under BL2 conditions. The practices, equipment and facilities required for BL2 containment are described in Appendix G-II-B-2 of the NIH Guidelines.

The primary containment equipment in a BL2 lab is the class II biosafety cabinet.

According to Appendix G-II-B-3:

### Appendix G-II-B-3. Containment Equipment (BL2)

Appendix G-II-B-3-a. Biological safety cabinets (Class I or II) (see Appendix G-III-L <[http://www4.od.nih.gov/oba/rac/guidelines\\_02/](http://www4.od.nih.gov/oba/rac/guidelines_02/)>, Footnotes and References of Appendix G) or other appropriate personal protective or physical containment devices are used whenever:

Appendix G-II-B-3-a-(1). Procedures with a high potential for creating aerosols are conducted (see Appendix G-III-O <[http://www4.od.nih.gov/oba/rac/guidelines\\_02/](http://www4.od.nih.gov/oba/rac/guidelines_02/)>, Footnotes and References of Appendix G). These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.

It is highly recommended that any manipulations of liquids in open vessels be conducted in a biosafety cabinet. It is clear that not all procedures (such as centrifugation) can be conducted in a biosafety cabinet. In that case the use of primary and secondary containment barriers should be adopted (these include air tight pressure resistant centrifuge canisters) or other engineering approaches to prevent the generation of aerosols, as stated below:-

Appendix G-II-B-3-a-(2). High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

By far the riskiest aspect of working with adenoviral vectors (and other RG2 agents) is associated with the use of hypodermic syringes. In such cases the NIH Guidelines are very clear:

Appendix G-II-B-2-j. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, before discard or reuse.

A hypodermic needle is a piece of equipment that has a high potential for aerosol generation (not to mention auto-inoculation). Consequently, the injection of mice should be performed in a class II biosafety cabinet.

I hope this response is helpful. Please let us know if we can provide any additional assistance.

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