Replication Competency Testing

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Most viral vectors used today are disabled such that replication competent viruses are not readily formed by any biological process that might occur in normal hosts. The Department of Biosafety encourages the use of such vectors in all relevant applications. In particularly sensitive applications, demonstrating that the viral stock used has no apparent contamination with replication competent vectors is essential. Of course, assays for replication competence will never be perfect or absolute, so the Institutional Biosafety Committee (IBC) asks that one use a current procedure of demonstrated sensitivity and specificity. Below is a summary guide of the current IBC recommendations for common classes of vectors. If another procedure or reference method is used to accomplish the same conclusion, researchers are asked to submitthat procedure and published article with their IBC registration.

In general, the IBC will require use of such an assay whenever viruses or virus-infected cells are used in whole animals. Even more rigorous testing may be required in some instances, such as a vector bearing a pathogenic gene or in human gene therapy, or in any materials that could be released in the environment. Viral testing is not generally required if experiments are conducted entirely in tissue culture.

Virus	Method	Reference
Adenovirus	Test for RCV by PCR for E1a prior to use. Confirmation of absence of RCV must be documented by researcher prior to use in animals.	Dion DL, Fang J, Garver RI. 1996. Supernatant rescue assay vs. polymerase chain reaction for detection of wild type adenovirus-contaminating recombinant adenovirus stocks. J Virol Methods 56:99-107.
Adeno-associated virus (with adenovirus helper)	Test for RCV by PCR prior to use. Confirmation of absence of RCV must be documented by researcher prior to use in animals.	Hehir KM, Armentano D, Cardoza LM, et al. 1996. Molecular characterization of replication-competent variants of adenovirus vectors and genome modifications to prevent their occurrence. J Virol 70:8459-8467.
Adeno-associated virus (Adenovirus-free)	Testing not required.	
Lentivirus	Test for RCV by ELISA assay for p24 antigen. Confirmation of absence of RCV must be documented by researcher prior to use in animals.	Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, Naldini L. 1998 A third-generation lentivirus vector with a conditional packaging system. J Virol 72:8463-8471.
Retrovirus (ecotrophic and amphotrophic)	Test for RCV by amplification in a permissive cell line followed by screening by appropriate detection assay. Confirmation of absence of RCV must be documented by researcher prior to use in animals.	Wilson, C.A., Ng, T. H., and Miller, A. E., 1997. Evaluation of recommendations for replication-competent retrovirus testing associated with use of retroviral vectors. Human Gene Therapy, 8(7): 869-874. Forestell, S.P., Nando, J. S., Bohnlein, E., and Rigg, R. J. 1996. Improved detection of replication-competent retrovirus. J Virol Methods 60: 171-178.
Vaccinia	Not applicable due to use as a replicating vector.	