Institutional Biosafety Committee

Testing Requirements for Viral Vectors

This table lists the most commonly used viral vectors, the appropriate biosafety levels that must be employed and the requirements for testing for replication competent virus (RCV). The test methods are indicated; however other assays with similar specificity and sensitivity may be accepted. Please provide the appropriate references or details on the alternative assay methods.

Viral Vector	Risk Group	Biosafety Level		Testing for Replication Competent Virus (RCV) *(Test results must be available upon request.)*	Sensitivity
		In vitro	*In Vivo*		
Adenovirus	2	BL2	ABSL2	Every viral preparation must be tested for RCV by the E1a PCR assay[1] prior to *in vitro* or *in vivo* use. *If the viral preparation has been proven to be negative for RCV, animals directly injected with virus or transduced cells may be housed at ABSL1 after 72 hours.*	Test at a sensitivity limit of < 1 in 10^6 infectious units
Adeno-Associated Virus (with adenovirus helper)	2	BL2	ABSL2	Every viral preparation must be tested for the presence of replication competent adenovirus prior to *in vitro* or *in vivo* use. Viral preparations that have undergone heat-inactivation may be tested for the presence of replication competent adenovirus by plaque assay or cytopathic effect[2]. An E1a PCR assay[1] may be used to test viral preparations purified with a heparin-based sulfate column. *If the viral preparation has been proven to be negative for RCV, animals directly injected with virus or transduced cells may be housed at ABSL1 after 72 hours.*	Test at a sensitivity limit of < 1 in 10^6 infectious units
Adeno-Associated Virus (free of adenovirus helper)	1	BL2	ABSL1	No testing required.	N/A

Herpes Simplex Virus (HSV)	2	BL2	ABSL2	Viral preparations for *in vitro* experiments of short duration (used within 72 hours of viral particle generation): Every six months, a representative viral preparation must be tested to ensure RCVs are not being produced. Every large scale viral preparation for multiple experimental procedures or placement in a laboratory repository must be tested for RCV by plaque assay[3] prior to use. Every viral preparation for direct injection into animals must be tested for RCV by plaque assay[3] prior to use.	Test at a sensitivity limit of 1 infectious unit per mL
Lentivirus	2	BL2 w/BL3 practices	ABSL2	RCV testing **is NOT required for**: 1. Commercially obtained vectors created using 3rd generation (4 plasmid) system, or 2. In-house production using the 3rd generation (4 plasmid) system that fulfills the following criteria: - Transfer plasmid contains chimeric 5' LTR and self-inactivating (SIN) through truncated 3' LTR - The *tat* gene is eliminated, and - The *rev* gene is provided in *trans*. RCV testing **is required for**: 1. All lentiviral vectors when used for human gene transfer experiments. 2. All lentiviral vectors produced in volumes > 10 liters 3. Lentiviral vectors using 1st or 2nd generation systems. When RCV testing is required, the following criteria apply: Viral preparations for *in vitro* experiments of short duration (used within 72 hours of viral particle generation): Every six months, a representative viral preparation must be tested to ensure RCVs are not being produced. Every producer cell line or stably transduced cell line generated or placed in a laboratory repository must be tested for RCV prior to use. If a viral preparation is being administered to animals directly, it must be tested for RCV. If cells are being transduced with the intent of administering the cells to animals, you must either test the viral preparation for RCV before transduction or test the cells for RCV following transduction. Serial transfer and p24 ELISA assay[4] is the most common test method currently recommended by the IBC.	For p24 ELISA, testing should demonstrate no increase in p24 titer over time in serial passages (minimum of 3) of transduced cells, or in conditioned media of transduced cells over multiple time points over a 24-48h period if cells can not be passaged.

				Other methods of testing not listed will be considered, provided the investigator presents sufficient rationale for the use of alternative methods, provides accurate details of the method (including what controls will be used) and cites published experimental validation of the method. If the viral preparation has been proven to be negative for RCV, animals directly injected with **VIRUS** may be housed at ABSL1 after 72 hours. *If the viral preparation has been proven to be negative for RCV, animals injected with **TRANSDUCED CELLS** may be housed at ABSL1.*	
Murine Retrovirus (Ecotropic)	1	BL1	ABSL1	No testing required.	N/A
Murine Retrovirus (Amphotropic or VSV-G Pseudotyped)	2	BL2	ABSL2	Viral preparations for *in vitro* experiments of short duration (used within 72 hours of viral particle generation): Every six months, a representative viral preparation must be tested to ensure RCVs are not being produced. Every producer cell line or stably transduced cell line generated or placed in a laboratory repository must be tested for RCV prior to use. If a viral preparation is being administered to animals directly, it must be tested for RCV. If cells are being transduced with the intent of administering the cells to animals, you must either test the viral preparation for RCV before transduction or test the cells for RCV following transduction. Marker rescue, antibiotic selection, PG3S+L-, PERT or infectivity RT-PCR assays[5, 6, 7, 8] are acceptable test methods. If the viral preparation has been proven to be negative for RCV, animals directly injected with **VIRUS** may be housed at ABSL1 after 72 hours. *If the viral preparation has been proven to be negative for RCV, animals injected with **TRANSDUCED CELLS** may be housed at ABSL1.*	Test at a sensitivity limit of 1 infectious unit per mL

References

[1] *Zhang WW, Kock PE, Roth JA. 1995. Detection of wild-type contamination in a recombinant adenoviral preparation by PCR. Biotechniques 18: 444-447.*

[2] *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.*

[3]*Strathdee CA, McLeod MR. 2000. A modular set of helper dependent simplex virus expression vectors. Mol Ther 5: 479-485.*

[4]*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.*

[5]*Forestell SP, Nando JS, Bohnlein E, Rigg RJ. 1996. Improved detection of replication competent virus. J Virol Methods 60: 171-178.*

[6]*Wilson CA, Ng T-H, Miller AE. 1997. Evaluation of recommendations for replication-competent retrovirus testing associated with the use of retroviral vectors. Human Gene Therapy 8: 869-874.*

[7]*Sastry L, Xu Y, Duffy L, Koop S, Jasti A, Roehl H, Jolly D, Cornetta K. 2005. Product-enhanced reverse transcriptase assay for replication-competent retrovirus and lentivirus detection. Human Gene Therapy 16: 1227-1236.*

[8]*Uchida E, Sato K, Iwata A, Ishii-Watabe A, Mizuguchi H, Hikata M, Murata M, Yamaguchi T, Hayakawa T. 2004. An improved method for detection of replication-competent retrovirus vector products. Biologicals 32: 139-146.*