

**UNIVERSITY OF ROCHESTER**  
**ENVIRONMENTAL HEALTH & SAFETY**

<b>Policy No.: BS013</b>	<b>Approved by: UR IBC</b>
<b>Title: Viral Vector Requirements for Laboratories</b>	<b>Date: December 29, 2021</b>
<b>Revision No.: 3</b>	<b>Page 1 of 6</b>
<b>Prepared by: Sonia Rosenberger</b>	

**I. PURPOSE**

The NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) require that Principal Investigators (PIs) seek and obtain Institutional Biosafety Committee (IBC) approval for all viral vectors. As part of the approval process, PIs must submit to the IBC information required by the NIH Guidelines, including the proposed Biosafety Level.

To assist PIs, this document summarizes IBC requirements for viral vectors currently approved at the University. Note: this document does not apply to human studies.

**II. PERSONNEL AFFECTED**

University of Rochester personnel who generate or use viral vectors and their supervisors  
Environmental Health and Safety staff who audit labs

**III. DEFINITIONS**

*Biosafety Level (BSL)* refers to a set of work practices, equipment and facility design appropriate for working with infectious agents. Specific requirements are in BS020 - UR Biosafety Level Requirements for BSL1, ABSL1, BSL2, ABSL2, BSL2+ or ABSL2+.

*G form:* IBC Grant or Project Registration Form, includes the project description, a list of the vectors to be used, and the proposed Biosafety Level(s). For viral vectors, the G form in conjunction with the VV form meets the requirements of the NIH Guidelines.

*NIH Guidelines:* NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

*Replication-defective or Replication-deficient:* describes viral vectors that cannot make additional copies of themselves

*Transduction:* the process by which foreign nucleic acids (DNA, RNA) are introduced into a cell by a viral vector

*UR IBC:* University of Rochester Institutional Biosafety Committee, composed of faculty, staff, and community members; required by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules

*Viral Vector:* Partial viruses that deliver foreign genes into cells

*VV form:* IBC Viral Vector form, one for each viral base, includes the source of the vectors, the plasmids to be used, production methods, and the nucleic acid inserts that will be expressed.

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#### **IV. RESPONSIBILITIES**

It is the responsibility of the Principal Investigator and the Laboratory Supervisor to obtain IBC approval prior to starting work with viral vectors.

It is the responsibility of each person working with viral vectors to use them as approved by the IBC.

It is the responsibility of Environmental Health and Safety to periodically audit labs to ensure personnel are working at the appropriate Biosafety Level.

#### **V. PROCEDURES**

A. Most vectors are designed to be replication-defective. However, since no design system is perfect, replication-competent virus may be present (unless assayed). Therefore, the Biosafety Level is generally the same as the base virus.

B. Special concern is applied to those vectors or modifications that could:

- extend the vector's host range (e.g. VSV-G pseudotyping allows entry into all cell types), tissue tropism, or enhance environmental stability
- result in a replication-competent virus (vector design; using a helper virus)
- integrate the vector into a portion of the genome leading to insertional mutagenesis
- integrate an oncogene (e.g. RAS) or silence a tumor suppressor (e.g. p53) in the genome of a person (including in an exposure event)
- generate a gene drive or selfish genetic element if injected into a person (i.e. a higher chance of a gene being inherited than by Mendelian genetics) – e.g. cassettes encoding Cas9 and sgRNA into a cut site or located adjacent to one another in the genome
- deliver a gene encoding a toxin with a low LD<sub>50</sub>

C. Replication-competent vectors are evaluated on a case-by-case base.

D. See the Appendix for a table of viral vectors and BSLs currently approved by the IBC.

E. To obtain IBC approval, submit G and VV forms. For forms, additional instructions and upcoming meeting schedule, visit [www.safety.rochester.edu/homepages/ibchome.html](http://www.safety.rochester.edu/homepages/ibchome.html).

#### **VI. REFERENCES**

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**VII. APPENDICES/FORMS**

Vectors/Biosafety Levels currently approved by the IBC

**VIII. REVISION HISTORY**

Date	Revision No.	Description
11/13/2011	New	Adenoviral Vector Requirements
06/26/2018	1	Consolidate BS014 (Biosafety Precautions for Replication-Incompetent Human Lentiviruses), BS026 (Biosafety Precautions for Replication-Incompetent Human Feline Immunodeficiency Virus) and the IBC guidance document General Information about Mammalian Virus Vectors into this policy/procedure, delete all BSL information redundant to BS020 - UR Biosafety Level Requirements for BSL1, ABSL1, BSL2, ABSL2, BSL2+ or ABSL2+, harmonize with Lab Safety Training, update references, update appendix table
09/18/2018	2	Update AAV BSL per 8/22/2018 IBC meeting
12/29/2021	3	Triennial review, update references

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Vectors/Biosafety Levels currently approved by the IBC

Agent	In vitro	In vivo (mice, rats)				Notes
		Administration/ sample collection		Housing/ Husbandry		
		ABSL2	ABSL1	ABSL2	ABSL1	
Adeno-associated virus (AAV) vectors	BSL1		X		X	<ul style="list-style-type: none"> <li>- 'Most adults (85-90% in the USA) are seropositive...not an etiological agent for disease.' (Tenenbaum, L. et al.)</li> <li>- BSL2 if adenoviruses used in production</li> <li>- BSL2 if express oncogenes or silence tumor suppressors</li> </ul>
Adenovirus vectors	BSL2	X		X (mucosal routes)	X (non-mucosal routes)	<ul style="list-style-type: none"> <li>- Generally E1-deleted (E1a and partial E1b) or E1-E3-deleted</li> <li>- Replication-competent virus is commonly present</li> <li>- For human serotypes, mice are not permissive for infection</li> </ul>
Baculovirus vectors	BSL1					<ul style="list-style-type: none"> <li>- Not a human pathogen</li> <li>- No VV form required</li> </ul>
Feline immunodeficiency virus (FIV) vectors	BSL2	X			X	<ul style="list-style-type: none"> <li>- See Lentivirus vectors</li> <li>- VSV-G pseudotyped allows entry into non-feline cells</li> </ul>
Herpes simplex virus (HSV) vectors	BSL2	X		X		Vectors developed with helper viruses can be cytopathic on transduced cells (Wang et al.)
Lentivirus vectors	BSL2	X		X (mucosal routes)	X (non-mucosal routes)	<ul style="list-style-type: none"> <li>- BSL2+ if express oncogenes or silence tumor suppressors</li> <li>- Lentiviruses insert themselves into the host's genome, risk of insertional mutagenesis</li> <li>- Newer 'self-inactivating vectors ...no serious adverse events since first use in (human) clinical trials in 2006'. (ref: Cavazzana M et al.) (SIN = truncated 3'LTR)</li> </ul>

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Agent	In vitro	In vivo (mice, rats)				Notes
		Administration/ sample collection		Housing/ Husbandry		
		ABSL2	ABSL1	ABSL2	ABSL1	
Rabies virus vectors	BSL2	X		X		<ul style="list-style-type: none"> <li>- G-deleted and/or pseudotyped with an avian virus envelope</li> <li>- Rabies vaccine offered</li> </ul>
Retrovirus vectors	BSL2 (amphotropic or VSV-G pseudotyped)  BSL1 (ecotropic)					<ul style="list-style-type: none"> <li>- MMLV- or MSCV-based</li> <li>- BSL2+ if pseudotyped and express oncogenes or silence tumor suppressors</li> <li>- Retroviruses insert themselves into the host's genome, risk of insertional mutagenesis</li> <li>- Insertional mutagenesis due to retroviral vectors has occurred in human gene therapy (Kaiser J.)</li> </ul>
Sindbis virus vectors	BSL2	X		X		<ul style="list-style-type: none"> <li>- Infect a wide range of cells and species</li> <li>- Ability to cause cell death and tropism for tumor cells attractive for cancer therapy (Quetglas et al.)</li> </ul>
Vaccinia virus vectors	BSL2	X		X		<ul style="list-style-type: none"> <li>- Generally replication-competent</li> <li>- Vaccinia vaccine offered</li> </ul>
Vesicular Stomatitis Virus	BSL2					<ul style="list-style-type: none"> <li>- G-deleted</li> </ul>
Transduced human cells/cell lines*	BSL2	X		X		
Transduced mouse cells/cell lines*	BSL1		X		X	

\* Cells transduced with viral vectors must be handled at the vector's BSL until the vector and its genetic material has been fully integrated into the cell's DNA by one of the following methods:

- 1) the cells have been washed with growth media to remove extraneous viral vector or
- 2) the viral vector has been inactivated by treating the transduced cells with trypsin (>0.1%) or human serum.

After the vector has been integrated and free virus removed, the BSL may be lowered to that of the cell pre-transduction.