

Note that the IBC must review each individual project involving viral vectors to determine the appropriate biosafety level prior to initiating work with the vector. The biosafety levels listed below apply to replication incompetent vector systems only for *in vitro* (during production in tissue culture) and *in vivo* (rodents only, where all experiments would be terminal). In all cases, additional biosafety precautions may be recommended.

The IBC always recommends testing for replication competent virus (RCV) in the vector stock. However, prior RCV testing of vector stock may be required in order to house injected animals at Animal Biosafety Level-1 (ABSL-1) as indicated below.

Viral Vector	Risk Group	Biosafety Level		Special Requirements	Acceptable RCV levels
		<i>in vitro</i>	<i>in vivo</i> (rodents)		
Adenovirus (Ad 2 or Ad 5)*	2	BSL-2	ABSL-1	Animals may be housed at ABSL-1 if vector stock is tested for RCV and found to be negative before injection	<1 PFU of RCV/10 <sup>6</sup> PFU recombinant virus
			ABSL-2	Without RCV testing of vector stock, or if stock tests positive, animals must be housed at ABSL-2	
Murine Retrovirus-Ecotropic	1	BSL-1	ABSL-1 w/ special precautions <sup>1</sup>	Animals may be housed at ABSL-1 with special precautions <sup>1</sup>	RCV testing is not required
Murine Retrovirus-Amphotropic (or VSV-G pseudotyped)	2	BSL-2	ABSL-1 w/ special precautions <sup>1</sup>	Animals may be housed at ABSL-1 <sup>1</sup> if vector stock is tested for replication-competent virus (RCV) before injection	0 RCV in 10 <sup>6</sup> infectious units
			ABSL-2	If insert codes for a toxin <sup>2</sup> or oncogene <sup>3</sup> , or if stock is not tested for RCV, animals must be housed at ABSL-2	
Lentivirus-3rd generation (3 or 4 vector system)	3	BSL-2 or BSL 2+	ABSL-1, ABSL-2, or ABSL-2+	See Biosafety assignment for lentiviral vectors for guidance on biosafety level and special requirements.	See Biosafety assignment for lentiviral vectors for RCV requirements
AAV (w/ adenovirus* helper)	2	BSL-2	ABSL-1	Animals may be housed at ABSL-1 if vector stock is tested for RCV (adenovirus) and found to be negative before injection	<1 PFU of RCV/10 <sup>6</sup> PFU recombinant virus
			ABSL-2	Without RCV testing of vector stock, or if stock tests positive, animals must be housed at ABSL-2.	

AAV (helper-free)	1	BSL-1	ABSL-1	Animals may be housed at ABSL-1	Not required
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**Footnotes:**

\* For all adenovirus serotypes other than 2 or 5, the Committee will consider the characteristics of the serotype and the experiments proposed. Special precautions may be required; see footnote 1 following.

<sup>1</sup>ABSL-1 special precautions must be followed including: handling infected animals last, changing gloves after handling, clearly labeling cages and notifying DCM or DAR prior to moving animals into room;

<sup>2</sup>link to biological toxin definition on IBC website: <http://www.ohsu.edu/xd/research/about/integrity/ibc/#agentToxin>

<sup>3</sup>If not known, but can demonstrate that no oncogenic properties exist in cell culture, okay for ABSL-1 housing with testing

**Links to RCV testing protocols and references are available at:** <http://www.ohsu.edu/xd/research/about/integrity/ibc/protocols.cfm>

## Biosafety assignment for lentiviral vectors

The use of lentivirus vector systems is becoming more prevalent in research, especially with the development of later generation systems that have reduced biosafety concerns. This document serves as general guidance on biosafety levels for *in vitro* and *in vivo* work with lentivirus.

Note that the IBC must review each individual project involving viral vectors to determine the appropriate biosafety level prior to initiating work with the vector. In all cases, additional biosafety precautions may be recommended.

System	Parameters of use	Biosafety Level	Special Requirements
<b><i>In vitro</i></b>  <b>Lentivirus- 2<sup>nd</sup> or 3<sup>rd</sup> generation*</b>	Non-oncogenic insert	BSL-2	
	Insert: -has oncogenic potential -expresses biological toxin <sup>1</sup>	BSL-2+	Special practices as outlined in the BSL-2+ template biosafety manual or as determined by a Biosafety Officer risk assessment.
	All inserts: Production scale	BSL-2+	- Any insert with a production scale > 500 ml of unconcentrated virus - Special practices as outlined in the BSL-2+ template biosafety manual or as determined by a Biosafety Officer risk assessment.
<b><i>In vivo</i></b>  <b>Animal models other than non- human primates</b>  <b>Lentivirus- 2<sup>nd</sup> or 3<sup>rd</sup> generation*</b>	All inserts: Direct injection -or- Non-human cells infected <i>ex vivo</i>	ABSL-1	-Injection should take place using BSL-2 containment and practices, and needle protective devices should be used for injection procedures -After injection of animals, the injection site should be cleansed with 70% ethanol and then animals should be placed into a secondary container without bedding. Once injection site is dry, animals can be returned to original cage(s) and returned to the ABSL-1 animal facility. The secondary container should also be cleaned with 70% ethanol.
	All inserts: Human cells infected <i>ex vivo</i> –	ABSL-2	If lentiviral vectors are used to infect human cells which are subsequently injected into animals.
<b><i>In vivo</i></b>  <b>Non-human primates</b>	All inserts: Lentivirus - 2nd generation	ABSL-2+	Must always have RCV <sup>2</sup>
	All inserts: Lentivirus - 3rd generation	ABSL-2	Must always have RCV <sup>2</sup>

\*The IBC always recommends testing for replication competent virus (RCV) in the vector stock.

## Guidance on use of 2<sup>nd</sup> or 3<sup>rd</sup> generation lentivirus vector systems<sup>3</sup>

*The following are typical characteristics of 2<sup>nd</sup> and 3<sup>rd</sup> generation systems. Unique/novel systems will be reviewed by the IBC to determine the appropriate safety precautions.*

### 2nd Generation Systems

- Uses two helper plasmids to separate the packaging and gene transfer functions in addition to the plasmid containing your gene of interest
  - o Plasmid with Gag, Pol, Rev, and Tat and plasmid with envelope protein
- May either express the HIV envelope protein or may be pseudotyped with the vesicular stomatitis virus G (VSV-G) protein
- May or may not be self-inactivating

### 3<sup>rd</sup> Generation Systems

- The IBC recommends the use of 3<sup>rd</sup> Generation Systems for safety purposes
- Uses three or more plasmids to separate the packaging and gene transfer functions in addition to the plasmid containing your gene of interest
  - o Plasmid with Gag and Pol, plasmid with Env (VSV-G), and plasmid with Rev
- Does not include Tat
- Typically pseudotyped to express VSV-G
- Is self-inactivating

### **Footnotes:**

<sup>1</sup>link to biological toxin definition on IBC website: <http://www.ohsu.edu/xd/research/about/integrity/ibc/#agentToxin>

<sup>2</sup> Links to RCV testing protocols and references are available at: <http://www.ohsu.edu/xd/research/about/integrity/ibc/protocols.cfm>

There are labs at OHSU who routinely perform p24 ELISA tests. You may contact one of these labs for assistance:

- Dr. Ashlee Moses, [mosesa@ohsu.edu](mailto:mosesa@ohsu.edu)
- ONPRC Virology Core - CoreyAyne Singleton, [singletonc@ohsu.edu](mailto:singletonc@ohsu.edu) or call 503-690-5568  
or Greg Dissen, [disseng@ohsu.edu](mailto:disseng@ohsu.edu) or call 503-690-5382

<sup>3</sup>For additional information regarding the safety of Lentiviral vectors, please see:

- NIH guidance: [http://oba.od.nih.gov/rdna\\_rac/rac\\_guidance\\_lentivirus.html](http://oba.od.nih.gov/rdna_rac/rac_guidance_lentivirus.html)
- State-of-the-Art Lentiviral Vectors for Research Use: Risk Assessment and Biosafety Recommendations. 2009. [Current Gene Therapy Vol 9\(6\): 459-474](#)