Last revised 12/17/2010

COMS Policy on Recommended Containment Levels for use of Retroviral Vectors in Laboratory Rats, Laboratory Mice and Laboratory Rabbits

I. Purpose

Provide containment requirements for use of retroviral vectors in laboratory rats, laboratory mice and laboratory rabbits.

II. Applicability

All COMS projects involving the use of retroviral vectors in Laboratory Rats, Laboratory Mice and Laboratory Rabbits must comply with the requirements of this policy.

III. Definitions

A. *BL2-N(72hr)*

Animals are housed in BL2-N containment for the first 72 hours following inoculation with viral vector according to the guidelines of the specific institution. Animal care during this time period is handled by either the laboratory personnel (best practice) or animal care workers, depending on the institution. Waste materials such as bedding, feces and urine should be disposed as of biohazardous waste. After a minimum of 72 hours, animals must be placed in a clean cage before animals can be housed at BL1-N for the remainder of experiment. Please consult with your Biosafety Officer, IACUC and/or Animal Facility Manager on approved procedures at your Institutions animal facility.

B. Implementation procedures

1. Inoculation

a. Inoculations of retroviral vectors that can infect human cells (e.g., vectors pseudotyped with VSV-G Env protein) into animals are to be performed within a biological safety cabinet under biosafety level 2 (BL2) conditions.

b. Inoculations with ecotropic viral vectors (i.e., vectors that cannot infect human cells) can be performed under biosafety level 1 (BL1) conditions

and inoculated animals housed in animal biosafety level 1 (BL1-N) conditions.

c. Safer, engineered needles or needle less systems should be used, when possible. Inoculations should be conducted by trained personnel only.

d. The site of inoculation should be thoroughly cleansed to prevent contamination of bedding materials.

C. Housing

1. The level of housing containment for animals inoculated with viral vectors that can infect human cells is dependent on the characteristics of the viral vector, the animal host, inoculation method, and the transgene.
2. For most experiments where common, replication incompetent second or third generation retroviral vectors (and packaging systems) are inoculated into animals, the required housing containment is dependent on the expressed transgene (see table below).
3. A partial list of common, well described viral vector systems are located in Appendix A and common packaging systems in Appendix B. Vectors not on this list may be approved with at a higher containment level.

Transgene Type	**Housing**
Reporter genes (e.g., green fluorescent protein)	BL1-N
Genes with biological activity	BL2-N for first 72 hours post inoculation followed by BL1-N housing (denoted as BL2-N(72hr))
Oncogene or toxin gene (or transgenes with high oncogenic or toxic potential)	BL2-N housing for the life of the animal

D. BL2-N(72hr)

1. Rationale: Studies suggest that the potential for shedding of replication competent virus (RCV) is low but not unfeasible in non-permissive hosts

(even if RCV were present in the original vector inoculum)[i]. Therefore, based on guidance from the NIH[ii], a reduction in containment to BL1-N after 72 hours reduces the risk of exposure to shed virus and allows for a sensible safety factor.

E. Exceptions

1. In light of their potential to support replication of infectious HIV-1, animals engrafted or injected with human cells or animal hosts that are permissive for retrovirus replication (e.g., SCID mice with humanized immune systems), may be approved at a higher containment level.
2. Depending on the specific project attributes, COMS may require BL2-N housing for the life of the animal regardless of the expressed transgene.
3. This policy is subject to change as new information on viral shedding becomes available.
4. This policy is specific to lab rats, lab mice, not other rodent species. Other animal species may be examined on a case by case basis by COMS.

IV. Policy Authority

The Office of Biological Safety (OBS) of the Harvard Medical School is responsible for supporting the Committee on Microbiological Safety. This includes preparation and revising of the COMS Policy Manual for committee review. The Committee on Microbiological Safety (COMS) authorizes this policy.

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[i] Karlen S and Zufferey R. (2007). Declassification of rodents exposed to third generation HIV-based vectors into class 1 animals. Applied Biosafety 12(2) pp. 93-99.

[ii] National Institutes of Health Recombinant DNA Advisory Committee (RAC). *Biosafety Considerations for Research with Lentiviral Vectors*.

Appendix A: Top 10 Most Commonly Used Retroviral Vectors in Animals

Vector	Source
pLenti (various versions)	Invitrogen
pRRL	Salk Institute
MSCV	Many
pLKO	Open BioSystems, AddGene, Others
pBabe	Many
MLV	Many
pSICO	AddGene, others
Lenti-Lox	Many
pSMPUW	Cell Biolabs
MoMuLV	Many
pHAGE	R. Mulligan
pMMP	R. Mulligan

Appendix B: Common Packaging Systems

1.	Invitrogen ViraPower
2.	Phoenix Ampho Packaging System (Obigen)
3.	Trans-Lentiviral Packaging System (Open Biosystems)
4.	pPack Packaging Systems (System Biosciences)
5.	Lenti-X Packaging Systems (Takara)
6.	pCMV-R8.74 and pMD2.G (Addgene)
7.	psPAX2—Originally from Trono (deposited at AddGene), second generation packaging vector