

# A General Introduction to and Guidance on Retroviruses and Retrovirus (including Lentivirus) Vectors

## Guidance

### 1. Classification/Nomenclature

Retrovirus nomenclature can be confusing because a variety of terms are used to describe various properties of the family. The International Committee for Taxonomy of Viruses (ICTV) split the Retroviridae family

into two sub-families, Orthoretrovirinae and Spumaretrovirinae. The first sub-family contains six genera, Alpharetrovirus, Betaretrovirus, Gammaretrovirus, Deltaretrovirus, Epsilon-retrovirus and Lentivirus while the second contains the single Spumavirus genus (Table 1).

**Table 1: Retroviridae Family Classification**

Subfamily	Genus	Typical Examples
Orthoretrovirinae	Alpharetrovirus	Avian leukosis virus (ALV), Rous sarcoma virus (RSV) Avian myeloblastoma virus Moloney Murine leukaemia virus MMLV (defective - encodes v-myb oncogene) Moloney Murine sarcoma virus (encodes v-src oncogene)
	Betaretrovirus	Mouse mammary tumor virus (MMTV), Jaasichte sheep retrovirus and Mason-Pfizer monkey virus (MPMV)
	Gammaretrovirus	Murine leukemia virus (MLV), Feline leukemia virus (FELV)
	Deltaretrovirus	Bovine leukemia virus, Human T-cell lymphotropic virus -1 (HTLV-1) Human T-cell lymphotropic virus -2 (HTLV-2)
	Epsilonretrovirus	Walleye dermal sarcoma virus
	Lentivirus	Human immunodeficiency virus 1 and 2 (HIV1, 2) Simian immunodeficiency virus (SIV) Feline immunodeficiency virus (FIV) Equine infectious anaemia virus (EIAV) Visna/maedi virus
Spumaretrovirinae	Spumavirus	Simian foamy virus, Human foamy virus

## 2. Virus Morphology

Historically speaking retroviruses were classified into groups based on their morphology in negatively-stained electron microscope pictures. **A-type** virus possessed a non-enveloped immature intracellular particle believed to result from endogenous retrovirus like genetic elements. **B-type** viruses were extra-cellular with prominent envelope spikes and an electron dense, acentric core, typified by MMTV. The **C-type** group included most mammalian and avian retroviruses and was similar to B-type viruses with a central core but with poorly visible envelope spikes. The **D-type** virus group appeared slightly larger, up to 120nm in size, with less prominent envelope proteins e.g. Mason-Pfizer monkey virus (which is now classified as a Betaretrovirus).

Classification based on these morphological details has now been largely superceded by information derived from sequence data.

## 3. Virion Structure

As implied above by the morphological appearance of virions in the EM there are considerable differences between various types of retrovirus; what follows is a simplified description of the particle. The standard nomenclature for retrovirus proteins along with their basic function is illustrated in Table 2. All of these proteins are essential for replication; some retroviruses, particularly those in the delta, epsilon, lentivirus and spumavirus genera encode additional essential and non-essential proteins.

<b>Protein</b>	<b>Function</b>
Matrix (MA)	A matrix protein (gag gene product) which lines the envelope
Capsid (CA)	A capsid protein (gag gene product); protects the core and is the most abundant protein in the virus particle
Nucleocapsid (NC)	A capsid protein (gag gene product); protects the genome and forms the core of the particle
Protease (PR)	Required for gag protein cleavage during maturation
Reverse transcriptase (RT)	Reverse transcribes the RNA genome; also has RNaseH activity
Integrase (IN)	Encoded by the pol gene; needed for integration of the provirus
Surface glycoprotein (SU)	The outer envelope glycoprotein; binds to the viral receptor on the target cell surface
Transmembrane protein (TM)	The inner component of the mature envelope glycoprotein, mediates cell membrane fusion with virion

## 4. Virus tropism

The commonly used terms, ecotropic, xenotropic, amphotropic and polytropic relate to the type of cells that can be infected by the virus. For retroviruses this host tropism is largely determined by the viral envelope glycoprotein and whether the target cells have an appropriate receptor i.e. cellular entry involves interaction between the surface subunit of the virion envelope glycoprotein and cell-surface determinants. The receptor binding triggers membrane fusion, mediated by transmembrane subunits, resulting in delivery of the virus capsid into the target cell.

An **ecotropic retrovirus** is a retrovirus that will grow in cells of the species from which it was isolated, but to a very limited or undetectable level in cells of other species, for example, Friend murine leukaemia virus (FrMuLV). A **xenotropic retrovirus** is a retrovirus that does not produce disease in its natural host and replicates only in tissue culture cells derived from a different species, for example, the murine leukaemia virus NZB. An **amphotropic retrovirus** will grow in the cells from which it was isolated and also in cells from a wide range of other species, for example, Moloney murine leukaemia virus (MoMuLV). Other terms such

as polytropic have also been used to refer to viruses that are capable of infecting murine and non murine cells.

More recently, with the possibility of pseudotyping viruses with non-retrovirus derived glycoproteins, other terms such as pantropic and dualtropic have been coined to describe host range. For example retroviruses pseudotyped with vesicular stomatitis virus glycoprotein (VSV G) can infect most cultured cell types resulting in these viruses being referred to as pantropic. Entry into cells is not dependant on the presence of a receptor because VSV G binds lipid and induces cell fusion to mediate virus infection. The widely used cell line PT76 expresses 10A1 an envelope glycoprotein from a murine leukaemia virus which recognises two different receptors, the amphotropic retrovirus receptor RAM 1 (Pit2) and the gibbon ape leukaemia virus (GALV) receptor (Pit1). Thus virus produced in PT76 or stable virus producing derivatives of these cells can be thought of as dualtropic with an increased host range relative to amphotropic viruses i.e. two receptors for a virus to interact with means that if one is not expressed at sufficient levels by a given species or cell type, the other may still allow viral entry. Experimental use of pseudotyping with VSV-G has important safety implications that will be discussed in [section 9.6](#).

Target Cell Origin	Viral Envelope			
	Dualtropic e.g. MLV 0A1*	Ecotropic e.g. ga70	Amphotropic e.g. 4070A	Pantropic i.e. pseudotyped with VSV G
Mouse	+	+	+	+
Rat	+	+	+	+
Hamster	+	–	+/-	+
Mink	+	–	+	+

<b>Target Cell Origin</b>	<b>Viral Envelope</b>			
	<b>Dualtropic e.g. MLV 0A1*</b>	<b>Ecotropic e.g. ga70</b>	<b>Amphotropic e.g. 4070A</b>	<b>Pantropic i.e. pseudotyped with VSV G</b>
Cat	+	–	+	+
Dog	+	–	+	+
Monkey	+	–	+	+
Human	+	–	+	+
Avian	–	–	–	+
Fish	–	–	–	+
Insect	–	–	–	+

\* Present on PT76 packaging cell line.

Many other glycoproteins of viral and cellular origin have been used to give novel target cell specificities to retroviral vectors. Of particular note is the RD114 envelope from a feline endogenous virus that has been shown to transduce human haemopoietic stem cells i.e. CD34+ cells and may be useful for a number of gene therapy applications.

## 5. Virus Genome/Replication

Retroviruses have a truly diploid genome consisting of 2 single stranded positive sense RNA molecules which vary from 8-11Kb and possess a 5' cap and 3' poly A. The RNA is hydrogen bonded and requires a specific cellular tRNA (usually trp, pro or lys) for replication which is also packaged into the viral particle. Although the genomic RNA is equivalent to mRNA it does not become translated immediately after infection probably because the virus is only partially uncoated resulting in a core (nucleocapsid) particle in the cytoplasm. Reverse transcription of the viral RNA genome

occurs in conjunction with/within the core particle giving rise to a DNA provirus that contains a repeated U3, R and U5 at either end (i.e. the long terminal repeat – LTR). Proviral DNA migrates into the nucleus and inserts into the host chromosome where it can act as a template for viral mRNA and copies of the viral genome. Viral gene expression is regulated in part by the LTR's that contain a strong viral transcriptional promoter and enhancer regions (the U3 region).

All retroviruses contain the same three gene clusters in the same order i.e. 5' - gag - pol - env - 3'. gag codes for structural proteins, pol codes for reverse transcriptase and integrase, while env codes for the envelope glycoprotein's. The more complex families of retroviruses (delta-retroviruses, spumaviruses and lentiviruses) have additional genes that affect various aspects of the viral life cycle. For example HIV *vif* appears to enhance viral infectivity, *rev* acts as a post-transcriptional activator of HIV gene transcription, while HIV *tat* enhances replication

by transcriptional activation and other HIV genes such as *nef* and *vpr* have diverse biological functions.

## 6. Oncogenesis

In the early years of the 20<sup>th</sup> century Ellerman and Bang and later Peyton Rous reported the identification of transmissible cancer-causing agents. These "filterable" agents later turned out to be avian retroviruses. Subsequently many other retroviruses causing malignant disease have been identified and a number of them have been shown to be tumorigenic as a result of acquiring host oncogenes. This gene acquisition is almost always at the expense of viral genomic sequences (Rous sarcoma virus being a notable exception) and results in a defective virus which is acutely transforming, producing polyclonal tumours rapidly. Such defective strains are dependent on a co-infecting helper virus for replication.

Not all retroviruses induce oncogenesis via captured cellular genes. Many induce tumours as a result of insertional mutagenesis where proviral genomes insert into host DNA and activate genes adjacent to the site of insertion. This is generally a slow process e.g. mouse mammary tumour virus induces mammary tumours by insertion at what turns out to be a restricted number of sites in the murine genome, taking many months to produce tumours.

Other mechanisms of oncogenesis have also been described for the retrovirus family. For example HTLV1 encodes and expresses a pleiotrophic transcriptional activator, Tax, which is thought to deregulate infected T-cell growth. Ultimately those cells that escape immune surveillance may then go on to form adult T-cell leukaemia. In another instance the simple

Jaagsiekte sheep retrovirus, an ovine betaretrovirus, possesses a native envelope (Env) structural protein that is itself the active oncogene and activates the PI3K/Akt and MAPK signaling cascades. In this example, other activation events mediated by the binding of *env* to its receptor hyaluronidase 2 or RON tyrosine kinase may also contribute to oncogenesis.

## 7. Retrovirus Vectors

Retrovirus vectors have become standard tools in the study of many aspects of molecular biology. They can deliver functional gene products as well as molecules that modulate the expression of target genes, e.g. shRNA, *in vitro* and *in vivo*. Indeed more than 17% of clinical gene therapy trials have used retrovirus vectors (see <http://www.abedia.com/wiley/vectors.php>), making them the second most common vector system used after adenovirus. A notable use of the technology involved a series of experiments with co-infection of over twenty different retrovirus vector constructs leading to the delineation of genes that can reprogram differentiated cells into stem cells – the so called induced pluripotent stem (iPS) cells (Takahashi and Yamanaka, 2006, Cell. 126:663-76).

The utility of these vectors is such that large panels of vectors have now been developed and one company claims to have available lentivirus vectors expressing shRNA that targets over 99% of the open reading frames in the human and mouse genomes – a total of over 48,000 constructs!

### 7.1 Basic Oncogenic Virus Vectors

The majority of non lentivirus vectors have been derived from simple oncogenic retroviruses,

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such as Avian leukosis virus (ALV), Moloney murine leukemia virus (MoMLV) or Feline leukemia virus (FeLV). These vectors will infect actively dividing cells and generally integrate into transcriptionally active areas of the chromosome.

For many years it was known that the vast majority of acutely transforming retroviruses were defective and required co-infection with replication competent virus in order for oncogenic viral genomes to be packaged into viral particles. This permissiveness where virus structural components can be provided in trans is the basis of all retrovirus vector systems. Once a region of the genome that was required for viral genomes to be packaged into the particle had been identified the first steps to a functioning vector system were taken. The 'First Generation' of retrovirus vectors were based on a DNA copy of the retrovirus genome which can code for the viral gag, pol and env genes but that has had its packaging ( $\Psi$ ) sequence deleted (known as the psi sequence). This construct is either co-transfected with the transfer vector, or is stably incorporated into the host-cell chromosomes generating a helper cell line. The construct that provides the proteins for forming capsids cannot be packaged because it lacks the Psi site whereas the transfer vector designed to express the gene under study can be packaged.

Such systems are inherently the most hazardous since a single recombination event is all that is required to generate replication competent viruses (RCV). ***First generation vector systems should not be used in HKU without full consultation with the Biosafety Committee.***

'Second Generation' packaging systems have deleted the 3' LTR from the packaging construct. This improves biosafety on two counts:-

- (1) by reducing the likelihood of RCV generation (two recombination events are required to produce RCV in these constructs).
- (2) reducing the possibility that the packaging construct will be mobilised.

***Second generation vector systems should also not be used in HKU without full consultation with the Biosafety Committee.***

'Third Generation' systems, also delete the 5' LTR from the packaging system and split the packaging sequences between two constructs, gag/pol being encoded by one and env by the second. (In helper cell lines this equates to two separate insertion events). This significantly reduces the likelihood of RCV generation by increasing the number of recombination events that are required to reconstitute a competent viral genome. Additional biosafety can also be achieved by using self-inactivating (SIN) transfer vectors. These vectors have been designed so that the viral enhancer and/or promoter sequences are deleted from the U3 region of the 3' LTR. Following reverse transcription in transduced cells, the 3' LTR deletions will be copied to the 5' LTR by template switch rendering the vector transcriptionally inactive, thereby reducing the risk of aberrant activation of cellular oncogenes adjacent to the integrated provirus site and by minimizing the risk of production of replication competent retroviruses (RCRs). When MoMLV, MSC or similar basic retrovirus vectors are used in HKU two component packaging systems of this type must be used.

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## 7.2 Lentivirus Vectors

In contrast to the basic retrovirus vectors described above lentiviruses can infect resting cells. As a consequence there has been a shift towards using lentivirus vectors for various gene transfer protocols. A further perceived advantage is that unlike oncogenic retrovirus vectors, transformation has not been seen when using lentivirus systems. Evidence from a broad range of *in vitro* studies and animal studies using both *in vivo* and *ex vivo* protocols can be cited as well as data from human gene therapy trials (<http://www.abedia.com/wiley/vectors.php>).

Indeed in humans wild type HIV infection *per se* is not associated with cancer formation (i.e. in the absence of other factors and agents HIV is not oncogenic). However, in common with AAV and MLV vectors, liver tumours have been observed following administration of some lentiviral vectors to foetal or neo-natal animals (see Themis *et al*, 2005 in Mol. Ther. 12(4):763-71 – it should be noted that the mechanism of the oncogenesis remains unclear and that some of the constituents of the vector construct may contribute e.g. the woodchuck hepatitis virus post-transcriptional regulatory element).

Basic lentivirus systems are composed of a transfer vector containing all virus components except gag, pol and env which is provided in trans by two helper constructs. ***These systems are less safe than those where accessory proteins are deleted and should only be used in HKU if the Biosafety Committee has been fully consulted.***

In some systems the lentivirus accessory proteins vif, vpr, vpu and nef have been removed primarily because they are not required for *in vitro* replication but also because they encode

proteins that have cytotoxic activities. In most commercially available vectors the tat gene is also deleted and the Tat-responsive promoter present in the 5' LTR has been replaced with heterologous promoters, for example with the Rous sarcoma virus U3 region. Additional biosafety is achieved by deletion of the rev gene from the transfer vector and expressing this from a third packaging construct as well as employing the SIN principle. Minimising overlap between all vectors is also important in reducing the likelihood of recombination. Some vectors also have conditional promoters i.e. ones that require induction, on the packaging genes (e.g. Lenti-X from Clontech) or on the gene of interest (e.g. some ViraPower constructs from Invitrogen) thus also improving safety.

## 8. Vector Choice

From this discussion it is clear that there are a variety of vector systems and that they have a spectrum of safety profiles. It is important to choose a system that not only fulfils the experimental requirements but one that also offers a high level of safety for the user. Third-generation lentivirus systems have a much-improved biosafety profile when compared to first- or second-generation oncogenic retrovirus systems. Safety versus functionality considerations should therefore be carefully weighed and the safest system possible should ultimately be employed. The major commercial suppliers of retrovirus vectors supply detailed user manuals and these can be very helpful for providing information on the available systems.

It might be argued that the safest, readily available, version of retrovirus vector technology not involving a packaging line is a 4

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plasmid system where the plasmids have no overlap, are FIV based, contain SIN deletions in the LTR, where the viral glycoprotein used is ecotropic and where the gene expressed is a marker such as GFP. While it is unlikely that all these criteria will be met BSL2 is generally acceptable for the majority of vector systems. In the future, particularly for use in gene therapy, further safety features may be built into the vectors. For example insulator sequences have been described that isolate the retrovirus transcription unit and decrease the likelihood of read through to adjacent cellular sequences. Split protease packaging systems have also been described further decreasing the opportunity of generating replication competent viruses.

## 9. Safety Issues with Retrovirus Vectors

Many laboratories throughout the world are using retroviral vectors as a standard tool in cell and molecular biology. As mentioned elsewhere these vectors are also being developed as therapeutic agents for human disease with numerous clinical trials of gene therapy being reported over the past 15 years. A consequence of this widespread use is that many researchers think of them as very safe systems with few associated problems. Without wishing to overstate the dangers this view is shortsighted and safety concerns should be assessed for each individual system being used. Current biosafety guidelines and distributors of vectors recommend using most vectors under biosafety level-2 containment (BSL-2) but some experiments may even require BSL-3. Interestingly many of the commercial suppliers of retrovirus vector systems include prominent warnings against incorporating oncogenic sequences into their vectors.

### 9.1 Unintentional Induction of Lymphomas and Leukaemias by Retrovirus Vectors

Several incidents with retrovirus vectors highlight the issues involved and the need to carefully assess the safety of each vector being used. In one case pre-clinical trials of retrovirus vectors were being conducted in bone marrow transplantation. Three of ten monkeys developed fatal lymphomas following transplantation of CD34+ autologous progenitor cells that had been treated with a retrovirus vector. This appears to have been due to the generation of a replication competent retrovirus (RCR) which arose as a result of two recombination events during vector production. This RCR infected monkey T-lymphocytes and induced the lymphomas by insertional mutagenesis (Donahue *et al*, 1992, J Exp Med 176, 1125-35).

A second study in mice showed that murine bone marrow cells transduced with a moloney murine leukaemia virus-based retroviral vector expressing a truncated form of low-affinity nerve growth factor receptor (dLNGFR) caused leukaemia (Li *et al*, 2002, Science 296: 497). The leukaemic cells contained the vector integrated by the *Evil* gene, a potential mutagenic event that by itself, or combined with the effect of dLNGFR on intracellular signaling pathways, appeared necessary for oncogenesis.

A third incident involved the development of leukaemia's in 4 children undergoing a gene therapy trial of a retrovirus designed to correct X-linked severe combined immunodeficiency. Vector treated bone marrow was transplanted into 9 individuals and a number of initiating events, including insertional activation of LMO2 a transcription factor involved in T-cell development led to development of the leukaemias (Hacein-Bey-Abina *et al*, 2008, J Clin Invest 118, 3132-42).

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In short safety concerns over retroviruses revolve around

- (1) The possibility of producing replication competent recombinant retroviruses (RCR),
- (2) The activity of the genes expressed by the vectors,
- (3) The potential for insertional activation of genes and resultant oncogenesis/aberrant pathology – insertional inactivation may also be relevant in some situations e.g. where shRNA is expressed,
- (4) In some circumstances such as in cell culture but more particularly in animal experiments issues arise from the potential for packaging of the construct by super-infecting retroviruses, endogenous retroviruses or retrovirus like elements.

## 9.2 Replication Competent Retrovirus (RCR) Production

The inadvertent production of replication-competent retroviruses (RCR) is the major safety concern in the use of retroviral vectors. Infection with RCR can result in chronic viraemia and subsequent formation of malignant tumors. This hazard has been shown to occur in a variety of settings including in mice, monkeys and in one particular gene therapy trial (see Section 9.1). As described in Section 7 many of the packaging lines used currently have the helper genes, gag-pol and env introduced as separate transcriptional units, so that at least three recombination events would be needed to form a replication competent virus. These "split-function" packaging lines have decreased the likelihood of RCR production dramatically but it

is still possible to produce RCR in these cell lines Chong, Starkey and Vile, 1998 (*J. Virol.*, 72, 2663-70) In this case RCR was generated by recombination events between the vector, one of the packaging constructs and endogenous retroviral sequences. The recombination events were not present in stocks of the packaging cell line or in an initial stock of the vector-producing line, indicating that these events occurred while the vector-producing line was being passaged for harvest of supernatant stocks.

Consequently it is important to ensure that sequence homology between the various components of the vector system is minimized. Thus if a helper cell line is used a third generation system is preferable (see Sections 7.1 and 7.2 above) where the packaging components are encoded by separately integrated genes which lack homology to the vector construct. Where a completely plasmid based system is used 4 plasmids are preferable to three. It is also worth noting that the probability of either homologous or illegitimate recombination after transient transfection with three or four plasmids is higher than the probability of recombination in stable producer cell lines containing single-copy cassettes with integrated elements. However prolonged passage of producer cell lines is not required and ultimately both systems can be used safely.

In terms of replication competence the FIV lentivirus based system has an additional protective factor as even the unmodified vectors will not replicate in murine or human cells. NIH has indicated that working with these replication defective vectors at Class 1 might be acceptable depending on the gene being expressed

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### 9.3 Activity of the Expressed Gene

The expected activities or toxicity of the gene products incorporated into the vector should be assessed. For example, a cytokine or a bacterial toxin would represent greater risk of harm than reporter genes such as Green Fluorescent Protein (GFP), Luciferase and  $\beta$ -galactosidase. Properties of the gene products with respect to individual cell types should also be considered.

The expression characteristics of a particular gene will be dependent on the cell type and the regulatory sequences used to control expression.

Take the down regulation of IL-10 production in mouse joint macrophages with an siRNA vectored by a lentivirus vector as an example. Pertinent questions include, would the siRNA used also down regulate the human counterpart? If it did, what is the consequence of down regulation of the gene? (IL-10 down regulates aspects of inflammation – would its down regulation result in inflammation? If the gene of interest were a tumour suppressor gene e.g. Rb/P53, would its down regulation lead to oncogenesis if human cells were transduced?) In the event of an accident what type of cells are likely to be exposed and what expression can be expected? Is the promoter active in these cell types? For a spill it might only be epithelial cell that are exposed whereas a needle inoculation might mean more cell types might be exposed.

Clearly questions other than those surrounding the likely impact of the gene would also need to be asked in the event of an accident e.g. how much virus is likely to be in an inoculum and what is its half life – in some packaging cell lines (e.g. FLY lines) the natural sensitivity of retroviruses to complement is altered. Basically, highly biologically active molecules will tend to increase the need to adhere to a precautionary approach. These questions would be part of a

risk assessment and answer the basic question what would happen if a worker was accidentally exposed to this virus ?

### 9.4 Insertional Mutagenesis

**9.4.1** The cases of leukemogenic complications referred to in Section 9.1 involve the use of conventional retroviral vectors with long terminal repeats (LTRs) containing strong enhancer/promoters. This configuration is derived from their strongly leukemogenic parental viruses and may trigger distant enhancer interactions and activation of 3' located genes by promoter insertion.

**9.4.2** Extensive experience with HIV infected individuals has led to the belief that lentiviruses are highly unlikely to promote insertional mutagenesis (this is in contrast to some other retrovirus vectors and gene products). However the expression of lentivirus control proteins such as nef is lost in vector constructs and insertional mutagenesis is still a possibility even if a remote one.

**9.4.3** Self-inactivating (SIN) retroviral vectors that contain only one internal enhancer/promoter reduce the incidence of interactions with nearby cellular genes. (Modlich, *et al* 2006; Blood 108(8): 2545–2553). Where possible this type of vector should be used.

### 9.5 Mobilisation/ "Trans-packaging"

In some circumstances e.g. cell culture but more particularly in animal experiments issues may arise from the potential for packaging of the construct by superinfecting retroviruses, endogenous retroviruses or retrovirus like elements.

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## 9.6 Pseudotyping with VSV-G

Frequently it is experimentally desirable to expand the cellular range of lentiviral vectors by use of a helper cell line that expresses VSV-G (see section 4). As long as the pseudotyped viral vectors produced are not themselves infective this is a perfectly reasonable and relatively safe experimental strategy assuming any risks from the expressed gene (section 9.3) are taken into account. However, if there is the possibility that adventitious viral agents are present risks are greatly increased, for example if primary clinical material is involved in the overall experimental strategy.

Two main additional risks are present:

i) naturally occurring retroviruses incidentally present could become pseudotyped by the VSV-G used in the experimental system, expanding their cellular host range. For example if HIV-1 is pseudotyped with VSV-G during viral particle assembly it may acquire the ability to infect other cell types as well as lymphocytes. A laboratory worker was infected with human immunodeficiency virus (HIV) type 1 in a biosafety level 2 containment facility, without any apparent breach. It is thought this occurred because of cross contamination with HIV from an adjacent BSL-3 facility, in combination with her own work with VSV-G expressing cells. It was unlikely that she could have had blood contact with HIV infected material at work. <https://academic.oup.com/cid/article/64/6/810/2747460>

ii) a much more remote but even more significant risk, given the propensity of retroviruses to undergo recombination (see section 9.2), is that replication competent retroviruses such as HIV acquire the ability to express VSV-G by recombination. It is notable that VSV-G is a smaller protein than the endogenous HIV-1 env, and thus this substitution is well within the coding

capacity of the virus. Such a virus, should it ever be produced, could become an enhanced pathogen of pandemic potential (EP3).

Ensuring that viral vector production is always kept separate from any tissues where replication competent viruses are present is a straightforward and highly effective way of reducing these risks to a manageable levels, as long as there is effective safety management in departments where both entities are present. As long as the vectors produced are replication defective they can be safely used to infect and transduce tissue samples where replication competent retroviruses may be present at low frequency eg. primary human material.

## 10. Control Measures

### 10.1 Use of Class II Biological Safety Cabinets

Many retrovirus vectors will be considered low risk GM activity Class 1 or Class 2 and can be handled in standard laboratory conditions. This means that virus preparations could be handled on the open bench. However, retroviruses although fairly labile are transmitted effectively in aerosols and droplets, even if disabled or attenuated. Therefore measures are required to control aerosol generation and airborne dissemination.

Most work with retroviruses will take place within a microbiological safety cabinet for two reasons. Use of a cabinet will keep the materials under study free from bacterial (or adventitious viral) infection and to avoid cross contamination from gloved hands etc.

More importantly, use of a cabinet will protect the worker against airborne virus generated by the manipulations being carried out. Injection of animals in a cabinet is not usually necessary because there is a low risk of generating aerosols during this type of manipulation. However, where

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the risk assessment shows that exposure to *airborne* retrovirus represents a hazard, the use of a cabinet might be required as a control measure.

## 10.2 Guidelines for the Safe Handling of Retrovirus and Retrovirus Vectors

Control measures will also depend on the nature of the risks with particular experiments. The experiments must be risk assessed before work commences to ensure appropriate safety measures are employed. Below is a brief outline of factors to consider:-

### 10.2.1 Laboratory Organisation

Because of the risks arising from the presence of replication-competent retroviruses, it is essential to ensure that production of viral vectors is kept separate from material which may be infected with similar agents. Under most circumstances this should be achieved by separation of the laboratory where vectors are produced from the laboratory where they are used.

### 10.2.2 Transport of retroviruses:

Transport all material in a double-sealed leakproof container. Label the container with a biohazard symbol, the name of the agent, the amount, and the Principal Investigator's name and telephone number.

### 10.2.3 Waste

Decontaminate all cultures, stocks, and other biological wastes before disposal using approved decontamination methods, such as autoclaving. Before decontamination outside of the laboratory the biological materials should be placed in a sealed, durable, leak-proof container for transport.

The most effective decontamination is given (with a minimum of 15 minutes

contact time) by

1% Sodium hypochlorite, 2% Glutaraldehyde or 5% Phenol

OR

Autoclaving for 30 minutes at 121°C or 250°F (15 lbs per square inch of steam pressure)

### 10.2.4 For general laboratory work and tissue culture:

A biological hazard sign indicating the use of retrovirus vectors should be placed outside of the laboratory or tissue culture room and on the biological safety cabinet.

Laboratory coats, gloves, and safety glasses or goggles must be worn.

Where virus is handled laboratory coats etc. used inside the laboratory should not be worn outside of the laboratory.

Materials containing retroviruses should be handled inside biological safety cabinets capable of protecting the product and personnel, whenever possible.

When performing centrifugation procedures, use sealed rotors with primary and secondary containment. Open rotors in a biological safety cabinet.

### 10.2.4 Biological safety cabinets

Materials should not be stored inside the biological safety cabinet (BSC). Take only what is needed to perform the procedure(s) and place it in the BSC upon initiation of the procedure. Upon conclusion of the procedure(s), remove everything from the

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BSC.

Serological pipettes and pipette tips should be decontaminated with an appropriate anti-viral agent such as a 1:10 dilution of household bleach (final concentration 0.525%), for at least 15 minutes prior to discarding in solid biohazard waste. For this purpose, a beaker containing the virucide can be kept inside the BSC while experimental procedures are being performed.

Avoid vacuum lines but if carrying out large numbers of manipulations these can be used. As a minimum protect them with disinfectant traps and filters. Use plastic containers rather than glass for traps, as glass can implode under pressure. If aspirated liquid waste is 2/3 full, aspirate sodium hypochlorite through the suction tube so that the final concentration is appropriate, allow it to soak for at least 15 minutes, and empty entire contents down the drain.

Upon conclusion of procedures in the BSC spray all work surfaces with a virucide and then with 80% ethanol. Allow the surface to air dry.

### 10.2.5 Sharps policy

Adopt a stringent sharps policy particularly when handling needles i.e. use sharps only. When absolutely necessary, no re-sheathing, limiting multiple uses of the syringe, disposal directly to sharps bins, appropriate restraint/anesthesia if injecting animals etc.

### 10.2.6 Emergency procedures

Clear procedures for emergencies must be documented in advance of the work being carried out. Details for different scenarios are appropriate e.g. dropping the stock vial of virus inside or outside of a hood, accidental injection or spilling/dripping virus containing material in a hood or onto the

bench or even contaminating an animal while injecting.

## 10.3 University Administrative Procedures

**10.3.1** Complete risk assessment form [RA3](#). Worked examples of several retrovirus vectors can be found on the University Safety Office website under the Biosafety microsite at <http://www.hku.hk/safety>.

**10.3.2** Return the completed assessment to the University Biological Safety Officer via e-mail [paulhunt@hku.hk](mailto:paulhunt@hku.hk) or [safety@hku.hk](mailto:safety@hku.hk) or by internal post. The proposal, which is treated as confidential information, will be reviewed by the University Biosafety Committee and will be approved if deemed appropriate. It is the intention of the committee to carry out the review within 5 working days but in some circumstances such as holiday periods or if large numbers of proposals are submitted it may take longer.

**10.3.3** It is advisable for departments to develop their own local code of practice (COP), sometimes referred to as a standard operating procedure (SOP), for the areas where they will work with retrovirus or retrovirus vectors. The type of information that might be included in a COP are an introduction, general procedures, the operation of the laboratory, any local rules, procedures for safe handling of waste, any staff health issues, what testing and maintenance is required and emergency procedures. An SOP is likely to contain more detail in terms of the specific experimental protocols to be adopted.

## 10.4 Lentiviruses

The documents referred to in the following URLs are from the NIH office of biotechnology

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affairs (the office that oversees the NIH Recombinant Advisory Committee, RAC) and indicate that NIH will accept virtually anything expressed by lentiviruses as BSL2 i.e. Class 2 (even in large volumes). Their view (by implication) is that these systems may be safer than other retrovirus vectors.

[http://www4.od.nih.gov/oba/RAC/Guidance/Lentivirus\\_Containment/index.htm](http://www4.od.nih.gov/oba/RAC/Guidance/Lentivirus_Containment/index.htm)

[http://www4.od.nih.gov/oba/RAC/Guidance/Lentivirus\\_Containment/pdf/RAC\\_containment\\_Soemia.pdf](http://www4.od.nih.gov/oba/RAC/Guidance/Lentivirus_Containment/pdf/RAC_containment_Soemia.pdf)

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