Information Sheet

Classifying Genetically Modified Activity with Lentiviral Vectors

1.0 Introduction

Lentiviruses are a subset of retroviruses, specifically based on the Human Immunodeficiency virus (HIV). They have an RNA genome which is reverse transcribed into DNA in the Host cell using Long Terminal Repeats (LTR). Because they stably integrate, into the host DNA they can be used for long term studies. However, the location the virus can integrate into is mostly random, meaning it can inadvertently activate or disrupt gene expression. Although highly unlikely, this could include oncogenes.

The three main genes involved in survival and function are *gag* (structural proteins), *pol* (reverse transcriptase) and *env* (viral envelope protein). First generation lentiviral vectors included *vpr, vit, vpu, net, tat* and *rev. Tat* and *rev* are involved in viral replication, the others in viral survival *in vivo* but not *in vitro.* Second generation vectors removed *vpr, vit, vpu, and net.* Which made the virus safer to work with.

Third-generation lentiviral vectors further improved safety by splitting the viral genome into four separate plasmids, making recombinant virus generation even more unlikely. the *gag* and *pol* genes were encoded on a different plasmid from that of the *rev* or *env* genes, resulting in a vector made from four separate plasmids containing the necessary viral sequences for packaging. The *tat* gene was removed in third-generation lentiviral. The introduction of deletions into the 3' Long Terminal Repeat (LTR) of the viral genome to create self-inactivating (SIN) lentiviral vectors disrupted the promoter/enhancer activity of the LTR, further improving safety. Fourth-generation vectors separate components into more plasmids and further improve recombination risk safety – but importantly these changes have not addressed other hazards.

In most cases, determining the containment level required for the work (1, 2 or 3) and therefore activity classification (class 1, 2 or 3) is straightforward. In the case of integrating viral vector systems, this is not always the case, as plasmids and/or live virus can be used, additionally the tropism of virus (i.e. can it infect humans) and integration method needs to be considered.

This guidance document is intended to help you determine a classification that addresses the real risk associated with your work.

The hazards associated with lentiviral vectors can be summarised as:

- Potential for generation of a replication competent lentivirus (RCL).
- Adverse inflammatory event.
- Oncogenesis.

2.0 Replication Competent Lentivirus generation

When using third or fourth generation lentiviruses, the risk of recombination (i.e. a virus stealing the components of other plasmids) is negligible – but not absent, and therefore we need to carefully monitor volumes of virus produced, production techniques, and disposal methods.

3.0 Adverse inflammatory event

A virus is recognised by the body as a pathogen and will mount an immune response. As a result of exposure. The integration of the virus will permanently infect human cells, and these are likely to be removed by the immune system. This would cause a local inflammation at the site of infection and would be a minor severity.

4.0 Oncogenesis

The risk of oncogenesis means how likely the virus is to cause cancer. Though this includes 'harmful inserts,' however, with integrating lentiviruses it is important to remember that LTR integration is 'random' and can affect cells in an unknown variety of ways – including inactivating a tumour suppressor gene or turning on an oncogene.

(Note: "Harmful insert' - defined as a sequence that may have harmful **biological** activity such as coding for oncogenes, toxins, cytokines, growth factors and immunomodulatory proteins. The nature of regulatory elements involved in the control of expression of such inserts should also be considered).

5.0 Classification

Genetic modification (GM) activities involving lentiviral vectors can be either Class 1 (non-notifiable) or Class 2 (notifiable to the Health & Safety Executive with an associated fee) depending on how well the hazards of the vector and inserts are reduced or controlled. The table below explains how to select the appropriate classification when completing a risk assessment:

Class 1	• Use of third generation (or safer) replication incompetent, self-inactivating vectors • No live virus used (only plasmid) or viral tropism restricted (e.g. ecotropic [will only replicate in host species]) • Use of non-harmful inserts • Lower viral titres, e.g. less than $5x10^9$ pfu/ml when using ecotropic viruses
Class 2 **if ANY** apply	• Use of first- or second-generation vectors* • Use of inserted sequences with harmful properties (as described above) • Use of live virus particles that have pantropism or likely to infect humans (e.g. VSV-G)

	• Viral vector containing the X protein expressing forms of WPRE (The woodchuck hepatitis virus post-transcriptional regulatory element) truncated X protein has been linked to liver tumours. • Higher viral titres, e.g. above 5x109 pfu/ml when using ecotropic viruses. • The use of sharps**

*Second and third generation vectors separate transfer, envelope, and packaging components of the virus onto different vectors. Third generation systems are considered safer than second generation because the packaging vector has been divided into two separate plasmids, resulting in a four-plasmid system in total. The HIV *tat* gene has also been removed from third generation vectors.
**Care must be taken to consider sharps such as cover slips, glass pasteur pipettes and sharp-pointed forceps, as well as needles and scalpels.

Notwithstanding the table above, the degree of control needed and therefore classification should be determined by risk assessment on a case-by-case basis. These are then reviewed by the Bio/ GM scientific sub-committee so that multiple specialists will be involved in the decision-making process.

Further information is available in the Scientific Advisory Committee on GM compendium of guidance - Part 2 Risk assessment of genetically modified microorganisms (other than those associated with plants) page 116 https://www.hse.gov.uk/biosafety/assets/docs/part2.pdf.

6.0 Common hazards and mitigations in virus use

Lentiviruses cannot readily pass the human skin barrier; however, they can enter through cuts and cracked skin. It is recommended not to work on lentiviruses if such wounds are present on hands. PPE such as gloves would be required even if no visible wounds are present, wounds can be dressed (e.g. plaster) prior to gloves in emergencies.

Lentiviruses can also pass mucosal barriers, such as in the mouth and lungs. This is an important risk to identify as lentiviruses can become aerosolised with techniques such as centrifugation and pipetting. If centrifuging lentiviral solutions, you must use centrifuge bucket lids because in the case of a split tube, virus could be aerosolised in the laboratory. Centrifuge buckets would also need to be opened in a Class II Microbiological safety cabinet (MSC) to ensure no tubes opened or split during the run.

Pipetting or pouring is almost always required and therefore MSCs are recommended for all steps in which lentiviruses are used (particularly pantropic viruses containing VSV-G Glycoprotein shown to improve DNA transfection). Double gloving is also recommended to allow quick glove changing to avoid contaminating surfaces after manipulating lentiviral solutions.

7.0 Downstream applications

If the downstream application involves a 'lysis' step, then lentivirus can be considered inactive **after** this step. For example, RLT buffer for RNA extraction, cell, or protein lysis steps for Western blotting – though be sure to assess and evidence whether a lysis solution destroys lentiviral particles.

However, some techniques require use of live infected cells e.g. microscopy and flow cytometry. There is no method by which we can guarantee all virus is removed from a sample and therefore specific risk assessment is required. For example, when using VSV-G enveloped lentivirus the half-life is 35h (https://www.nature.com/articles/s41434-020-00193-y#Sec17), this means that after 3 days in culture there will still be 25% the level of infectious virus. This can be drastically reduced with washing the cells (e.g. 50-fold reduction per wash), but virus is never completely removed and though risk of aerosolization and infection is reduced, it is never gone. It is reasonable to assume the likelihood of a risk is drastically reduced if the viral titre is reduced by many thousand-fold (multiple washes after a week in culture).

8.0 Projects involving multiple steps

It is usual to set a classification level for a whole project, the highest classification applied to any of its component procedures. When preparing a viral vector in a laboratory for administration to animals, this may not be necessary. Unless using a harmful insert, the point of risk is the preparation of the syringe, then its use and disposal. This part of the project should be classified as class 2. However, the inoculation stage may be the only part of a project that should be classified as activity class 2. The vector preparation stages (e.g. plasmid propagation) may be determined as containment level 1, therefore class 1.