Genetic Modification Safety Committee (GMSC) Guidance

Classifying GM activity with lentiviral vectors

Introduction

General guidance on risk assessment and classification of GM research projects is provided on the UCL Safety Services website. 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. 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)
- Insertional mutagenesis
- Oncogenic properties of vectors
- Biological activity or toxicity of the inserted gene sequence (i.e. a harmful insert)

A 'harmful insert' can be defined as a sequence that may have harmful biological activity such as 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.

Classification

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 Activity	✓ Use of third generation* (or safer) replication incompetent, self-inactivating vectors ✓ Use of non-harmful inserts ✓ Lower viral titres, e.g. less than $5x10^9$ pfu/ml
Class 2 Activity if **any** of the criteria apply	➢ Use of first or second generation vectors* ➢ Use of inserted sequences with harmful properties (as described above) ➢ Viral vector containing the X protein expressing forms of WPRE ➢ Higher viral titres, e.g. above $5x10^9$ pfu/ml **plus** 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.

An authoritative summary of the nature of the biohazard and risk associated with lentiviral vectors is given in the SACGM Compendium of Guidance published by the Health & Safety Executive (HSE). Refer to pages 116-126. Notwithstanding the table above, the degree of control needed and therefore classification should be determined by risk assessment on a case-by-case basis.

Extracts from the HSE guidance are given below, covering use of sharps, large volumes/titres and aerosol generation.

Use of sharps

The risk of insertional mutagenesis, an inherent hazard associated with lentiviral vectors, is difficult to quantify given the current available data. However, the potential likelihood of this hazard along with others conferred by the transgene being realised is increased where work involves the use of sharps to deliver the viral vector.

Whilst 'control of sharps' is not one of the specified control measures in the containment tables, other containment level 2 (CL2) measures are necessary to facilitate their control, e.g. access restricted to authorised and trained personnel only; written training records; and the use of gloves. This necessitates a classification of Class 2.

High titres and aerosol generating procedures

In certain circumstances, where large volumes/titres of virus and/or aerosol generating procedures are used, it may also be necessary to use additional CL2 measures to control exposure of the operator. These measures include microbiological safety cabinets, contained equipment, restricted access or other specific measures to minimise aerosols. Aerosol generating procedures include the use of FACS and other flow cytometry methods. As above, the use of CL2 measures means activity must be classified as Class 2.

After consultation with the HSE, the GM Safety Committee recommends that UCL should take the approach outlined in the sections above, in respect of the use of sharps, large volumes/titres and aerosol generating procedures.

Note: If CL2 measures such as use of gloves and microbiological safety cabinets are used only to protect the sterility of the product, rather than the person, the work can be categorised as Class 1.

Projects involving *in vitro* and *in vivo* work

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 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 for the reasons quoted in the extract above (control of sharps). However, the inoculation stage may be the only part of a project that should be classified as activity class 2. The vector preparation stages may be determined as containment level 1, therefore class 1.

Document control

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