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CANADIAN BIOSAFETY GUIDELINE

Lentiviral Vectors

Canada

The *Canadian Biosafety Guideline – Lentiviral Vectors* is available on the Internet at the following address: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance.html

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PREFACE

PREFACE

In Canada, facilities where Risk Group 2, 3, and 4 human pathogens or toxins are handled and stored are regulated by the Public Health Agency of Canada (PHAC) under the *Human Pathogens and Toxins Act* (HPTA) and *Human Pathogens and Toxins Regulations* (HPTR). The importation of animal pathogens, infected animals, animal products or by-products (e.g., tissue, serum), or other substances that may carry an animal pathogen or toxin or parts thereof are regulated by the PHAC or the Canadian Food Inspection Agency (CFIA) under the *Health of Animals Act* (HAA) and *Health of Animals Regulations* (HAR).

The following figure depicts the document hierarchy used by the PHAC and the CFIA to oversee biosafety and biosecurity operations. Each tier of the pyramid corresponds to a document type, with documents increasing in order of precedence moving upwards. Acts and regulations are the documents that convey the PHAC's and the CFIA's legal authorities, and, therefore, are found at the top of the pyramid. Guidance material and technical pieces are found at the bottom of the pyramid, as they are intended to summarize recommendations and scientific information only.

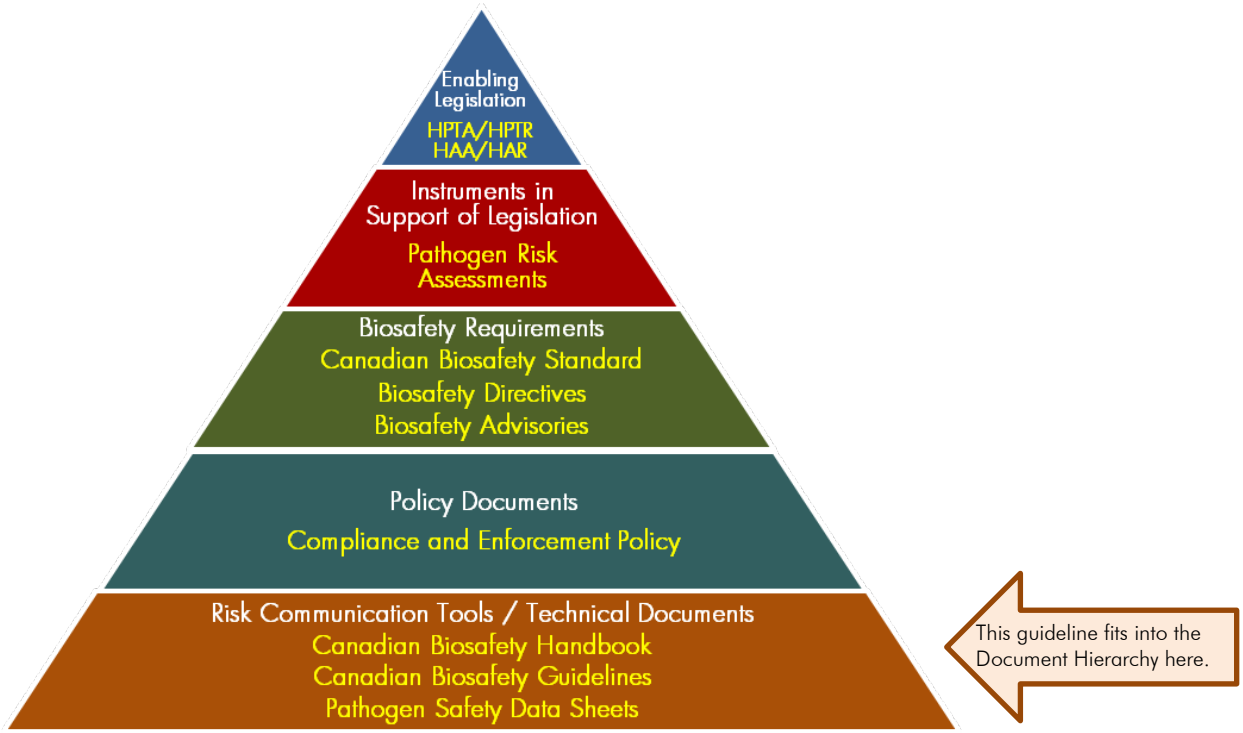

Figure 1: The Government of Canada's Biosafety and Biosecurity Document Hierarchy

The *Lentiviral Vectors* guideline was developed by the PHAC and the CFIA as part of a series of electronic publications that expand upon the biosafety and biosecurity concepts discussed in the current edition of the *Canadian Biosafety Handbook* (CBH), the companion document to the *Canadian Biosafety Standard* (CBS). This guideline describes best practices and recommendations for work involving lentiviral vectors in laboratories and other containment zones.

This guideline is continuously evolving and subject to ongoing improvement. The PHAC and the CFIA welcome comments, clarifications, and suggestions for incorporation into the future versions. Please send this information (with references, where applicable) to:

- PHAC e-mail: PHAC.pathogens-pathogenes.ASPC@canada.ca

ABBREVIATIONS AND ACRONYMS

ABBREVIATIONS AND ACRONYMS

BSC	Biological safety cabinet
CBH	*Canadian Biosafety Handbook*
CBS	*Canadian Biosafety Standard*
CFIA	Canadian Food Inspection Agency
CL	Containment level (i.e., CL1, CL2, CL3, CL4)
CL2-Ag	Containment Level 2 large animal containment zone
DNA	Deoxyribonucleic acid
HIV	Human immunodeficiency virus
LRA	Local risk assessment
LTR	Long terminal repeats
MLV	Murine leukemia virus
PHAC	Public Health Agency of Canada
PPE	Personal protective equipment
RCR	Replication-competent retrovirus
RG	Risk group (i.e., RG1, RG2, RG3, RG4)
RNA	Ribonucleic acid
SOP	Standard operating procedure
VSV-G	Vesicular stomatitis virus glycoproteins

CHAPTER 1

1
2

INTRODUCTION

CHAPTER 1 - INTRODUCTION

The words in **bold type** are defined in the glossary found in Chapter 5.

Lentiviral vectors are retroviruses that are generated *in vitro* by the multi-plasmid transfection of mammalian cells. The retrovirus particles are harvested from the culture medium and used to stably insert a transgene into the genome of a target cell. These vectors have been engineered so that they are able to infect the target cell, but are not able to replicate following the infection. However, as most lentiviral vectors are derived from the human immunodeficiency virus (HIV), concerns relating to the potential reversion of the vector to a replicative state remain, as do risks associated with the transgene and insertional mutagenesis (e.g. resulting in activation or inactivation of genes).

In some cases, applicable **containment** requirements for a laboratory or **containment zone** where **pathogens** are **handled or stored** may need to be modified based upon the procedure being performed, or whether the risk associated with a pathogen has changed (e.g., the pathogen has been modified). Lentiviral vectors are generally classified as **Risk Group** 2 (RG2) human pathogens and RG2 **animal pathogens**. However, some lentiviral vectors and transgenes may possess unique characteristics that increase their risks, leading to additional or modified **biosafety** requirements for their safe handling, or to classification as an RG3 pathogen.

1.1 Scope

The *Lentiviral Vectors* guideline describes biosafety considerations and best practices for conducting a **pathogen risk assessment** or **local risk assessment** (LRA) for a containment zone where lentiviral vectors are handled or stored, so that appropriate mitigation measures may be implemented. The guideline presents risk factors and risk mitigation strategies to be considered when performing risk assessments and when establishing biosafety procedures. This guideline is intended to be used in conjunction with the *Canadian Biosafety Standard* (CBS) and *Canadian Biosafety Handbook* (CBH).[1,2]

> The information provided in this document is meant as guidance only and should not be interpreted as requirements. Regulated parties may choose alternate approaches to meet the requirements specified in the CBS.

1.2 How to Use the *Lentiviral Vectors* Guideline

A detailed list of all abbreviations and acronyms used throughout this guideline is located at the beginning of this document. Each word or term is spelled out upon first use in the guideline, with the abbreviation immediately following in brackets. After its initial definition, the abbreviation is used exclusively throughout the remainder of the document. A comprehensive glossary of definitions for technical terms is located in Chapter 5; words defined in the glossary appear in **bold type** upon first use in the guideline. A list of references and other resources is provided in Chapter 6.

References

1 Government of Canada. (2015). *Canadian Biosafety Standard* (2nd ed.) Ottawa, ON, Canada: Government of Canada. Retrieved 22/10, 2018 from https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines.html

2 Government of Canada. (2016). *Canadian Biosafety Handbook* (2nd ed.) Ottawa, ON, Canada: Government of Canada. Retrieved 22/10, 2018 from https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines.html

CHAPTER 2

DESCRIPTION OF LENTIVIRAL VECTORS

CHAPTER 2 - DESCRIPTION OF LENTIVIRAL VECTORS

Lentiviral vector systems are derived from viruses that belong to the genus of lentivirus from the *Retroviridae* family (Figure 2-1).[1] Lentiviral vectors are most commonly derived from HIV type 1 (HIV-1).[2] However, they may also be derived from HIV-2 and non-human lentiviruses, including simian immunodeficiency virus, feline immunodeficiency virus, equine infectious anemia virus, and bovine immunodeficiency virus.[2,3,4] Lentiviruses are practical vehicles for gene delivery due to their stable integration into the genomes of both dividing and non-dividing cells, as well as their long-term transgene expression.[5] However, the potential pathogenicity of lentiviral vectors is an important consideration when developing biosafety strategies.

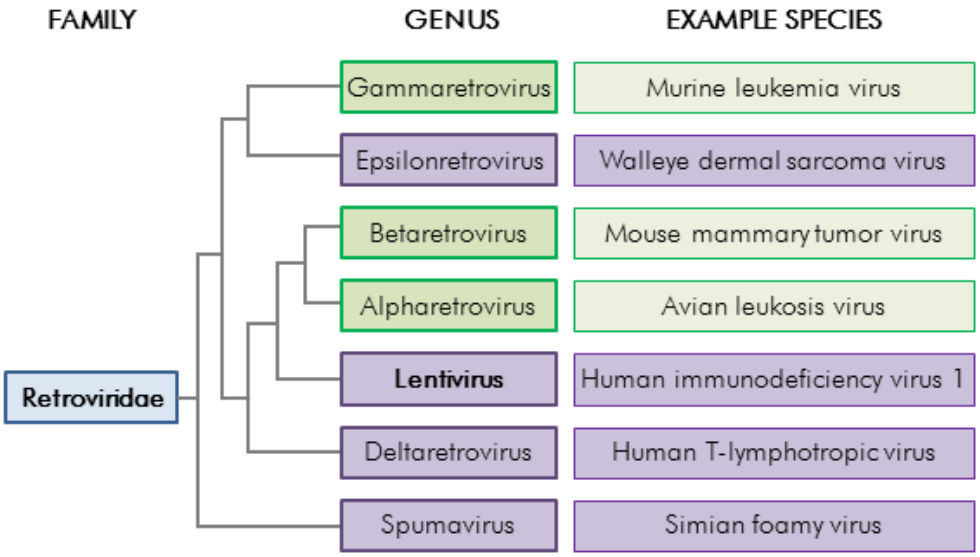

Figure 2-1: *Retroviridae* phylogenetic tree
The seven genera within the *Retroviridae* family are shown; complex retrovirus genera are indicated in purple whereas simple genera are indicated in green. One example species from each of the seven genera is shown. Branch lengths are not meant to represent phylogenetic distance, but are indicated for general reference only.

HIV is the most studied lentivirus, and the predominant primary parent virus from which currently available lentiviral vectors are derived. To overcome the risk of accidental infection, integration into the human genome, and persistent lifelong infection that is associated with HIV pathogenicity, lentiviral vectors are designed to be replication-defective.[2] This is accomplished by segregation or removal of genes that are essential for virion replication and packaging from the rest of the HIV genome. The resulting viral particle is capable of infecting a target cell and delivering its transgene, but is incapable of completing the remainder of the viral life cycle to form a replication-competent retrovirus (RCR), the critical step for propagation.

2.1 Retroviruses

The family of *Retroviridae* is composed of seven genera (Figure 2-1), which consist of single-stranded, enveloped, ribonucleic acid (RNA) retroviruses that are capable of causing disease in humans and animals.[1] They are broadly classed as simple or complex retroviruses, based on RNA splicing patterns and the presence of accessory genes.[6] The interaction of retroviruses and the host cell surface is highly specific, and is the main determinant of the viral host range.[6] After binding and penetrating the host cell, the viral RNA is transcribed into deoxyribonucleic acid (DNA), which is then integrated into the host DNA through processes that use both host- and virally-encoded proteins; following synthesis and assembly, this replication process results in release of infectious virus particles. To perform these tasks, all retroviruses contain the viral genes *gag, pol,* and *env;* the gene products and functions of these are summarized in Table 2-1.

Table 2-1: Roles and functions of retroviral genes and products[1,6,7]

Gene	Products	Function
gag	Capsid	Protects the core
	Matrix	Lines envelope
	Nuclear capsid	Protects the genome and forms the core
pol	Protease	Essential for Gag protein cleavage during maturation
	Reverse transcriptase	Reverse transcribes the RNA genome into double stranded DNA
	Integrase	Required for integration of the provirus
env	Surface glycoprotein	Outer envelope glycoprotein; major virus antigen
	Transmembrane protein	Inner component of the mature envelope glycoprotein

The *gag* gene encodes proteins that make up the internal structure of the virus including the capsid, the membrane associated matrix, and the nuclear capsid. The *pol* gene encodes enzymes that are required for transcription, integration of the virus genome into the DNA of the host cell, and viral maturation. The *env* gene encodes proteins that make up the external portion of the virus including the surface glycoproteins and the transmembrane protein, which form a complex that specifically interacts with target cell receptors.[6] Long terminal repeats (LTR) at the 5' and 3' end of retroviruses contain elements necessary for gene expression, reverse transcription, and integration into host cell genomes; the RNA packaging signal psi (Ψ) is required for the packaging of RNA into virions.[1,7]

In addition to the essential elements found in simple retroviruses, the complex genera of *Retroviridae* (i.e., lentivirus, epsilonretrovirus, deltaretrovirus, and spumavirus) have genes that encode for accessory and regulatory proteins, which provide the retroviruses with some control over gene expression and the virus life cycle.[6] For example, the lentivirus HIV-1 contains the accessory genes *nef, vif, vpu,* and *vpr*, which promote the infectivity and pathogenicity of HIV, and the regulatory genes *tat* and *rev,* which work in coordination with the LTR for virus replication (Table 2-2).[6]

Table 2-2: Roles and functions of HIV-specific genes and products[1,6,7]

Gene	Product	Function
nef	Accessory proteins	Enhances virion infectivity
vif		Affects infectivity of viral particles
vpu		Enhances virion release
vpr		Enhances virion infectivity
tat	Regulatory proteins	Activates transcription
rev		Regulates splicing / RNA transport

2.2 Evolution of Retroviral Vector Systems

The ability of retroviruses to integrate into host genomes rendered them a potential tool for gene therapy; however, the release of RCRs with disease-causing potential was a significant biosafety concern. In an effort to increase the safety of retroviral vector systems, several iterative developments were directed toward reducing the possibility of replicative competency. As detailed below, this was accomplished by replacing or removing the coding regions of genes essential for virion replication and packaging; resultant retroviral particles retain the ability to infect and deliver the transgene to target cells but are unable to complete the remainder of the viral life cycle, including RCR formation. A further level of safety was achieved by separating the viral components needed to produce the infectious virion into multiple plasmids.[8]

Early retroviral vector systems were developed from simple retroviruses; these had limited utility since simple retroviruses can only enter the host's nucleus during the mitotic process and so are only able to integrate into dividing cells. In contrast, complex retroviruses can integrate into both dividing and non-dividing cells due to the additional genome-encoded proteins; as such, lentiviral vector systems are now the predominantly used retroviral vector systems.

2.2.1 Early Retroviral Vector Systems

Initial retroviral vector systems were based on simple retroviruses, such as the murine leukemia virus (MLV; Figure 2-2, panel A), a gammaretrovirus. In these systems, the vector was separated into two plasmids, namely a transfer/vector plasmid and a packaging/helper plasmid (Figure 2-2, panel B). The transfer plasmid included the Ψ and LTR that are required for packaging the transgene into the virion and integrating it into the host genome, respectively. The helper plasmid contained the *gag, pol, and env* genes that encode the proteins that are necessary for virion packaging; the lack of Ψ in the helper vector prevented the transcribed viral DNA from being incorporated into the virion. The end result was a replication-incompetent virus that included the gene of interest, but lacked any of the viral genes required to complete the viral life cycle within the host cell.[9] Despite the separation of the viral genome that was applied in this retroviral systems, a single recombination event could result in the Ψ signal relocating from the transfer plasmid to the helper plasmid, leading to RCR formation.[9] Thus, use of these early retroviral vector systems requires additional biosafety measures.

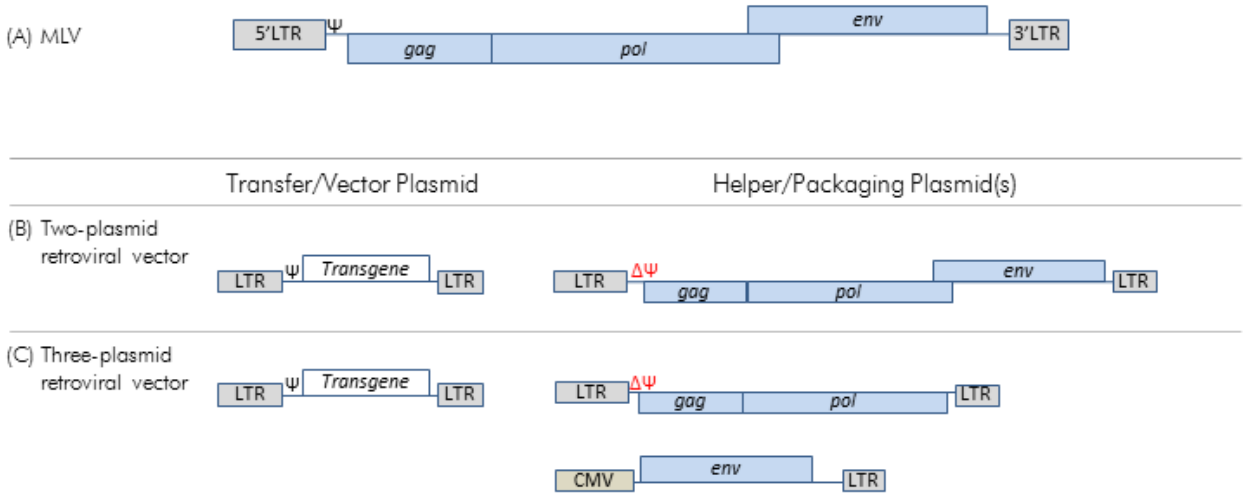

Figure 2-2: Genome of MLV and the evolution to three-plasmid retroviral vector system
(A) The MLV genome with common retroviral genes (blue); note that the figure is not to scale and only meant to show the relative location of genes. (B) A two-plasmid retroviral vector system. (C) A three-plasmid retroviral vector system.

A three-plasmid MLV-based retroviral vector system was developed to address the issue of Ψ signal relocation from the transfer plasmid to the helper plasmid (Figure 2-2, panel C).[10] In this system, the helper plasmid components were divided into two plasmids: one helper (packaging) plasmid contained the *gag* and *pol* genes, while the second (envelope) plasmid contained only the *env* gene. The resulting vector system was as effective at integrating the transgene into dividing cells and at sustaining transgene expression as were earlier vector systems.[9] Furthermore, since at least two recombination events would be required for Ψ, *gag*, *pol*, and *env* to be located together on the same plasmid, RCR formation was less likely, which improved the biosafety of this system. However, the use of MLV demonstrated poor efficiency of gene transfer in human systems, most likely due to these vectors only being able to integrate into dividing cells and inactivation by the human complement system.[11,12]

2.2.2 First Generation Lentiviral Vector Systems

Lentiviral-based vector systems were developed to overcome the deficits of early MLV-based retroviral systems. Unlike simple retroviral vectors, HIV-1 and other lentiviruses are able to infect both dividing and non-dividing cells due to their complex genomes that contain additional regulatory and accessory genes (Figure 2-3, panel A). This broader capacity made lentiviruses promising candidates for gene therapy applications, but their parental origin raised many safety concerns that prevented them from undergoing human testing.

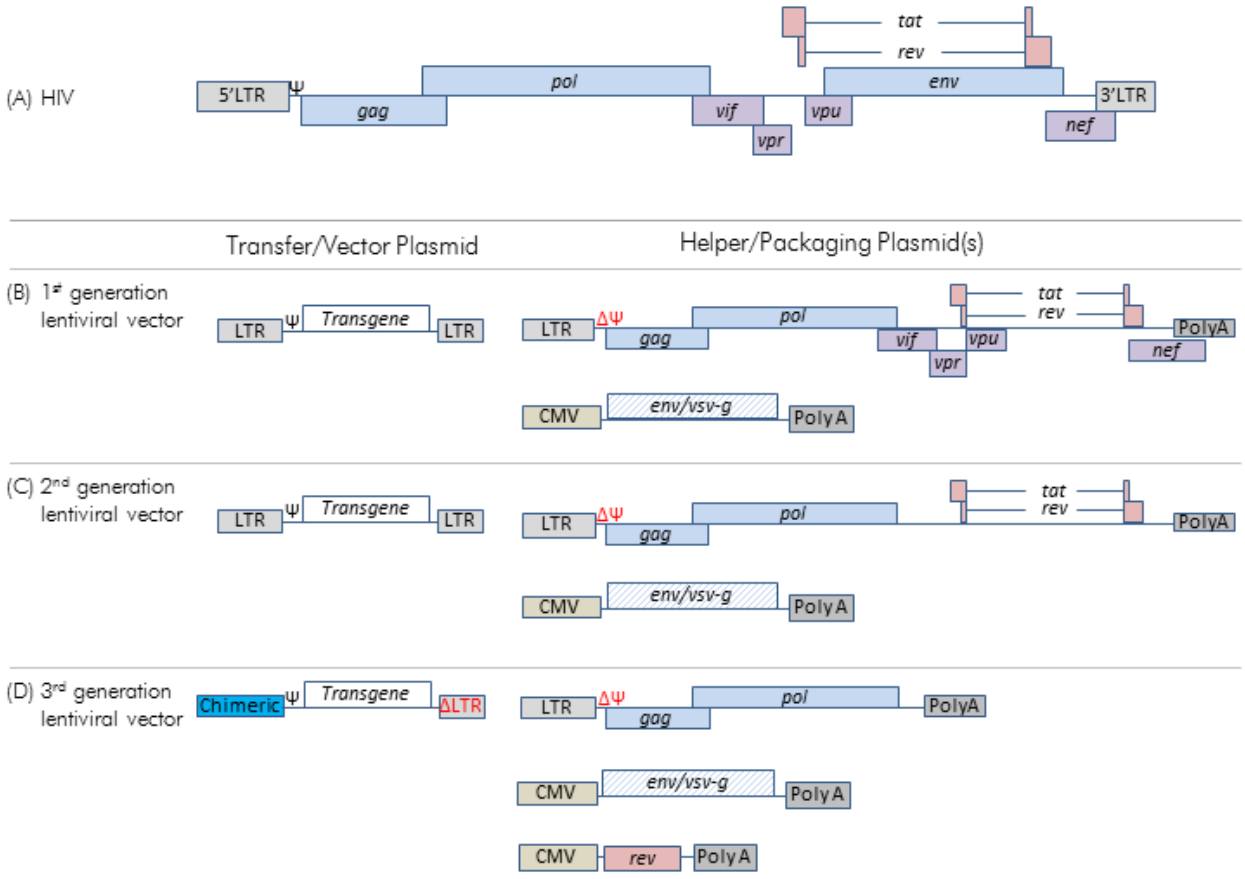

Figure 2-3: Genome of HIV-1 and the evolution to 3rd generation lentiviral vector systems

(A) The HIV genome with common retroviral genes (blue) and HIV-specific genes (purple and pink); note that the figure is not to scale and only meant to show the relative location of genes. (B) A first generation three-plasmid lentiviral vector system. (C) A second generation three-plasmid lentiviral vector system. (D) A third generation four-plasmid lentiviral vector system.

The first generation lentiviral vector systems were three-plasmid systems derived from HIV, and included the standard retroviral genes as well as HIV-specific genes (Figure 2-3 panel B).[13] These first generation lentiviral vector systems were composed of (1) a Ψ–containing transfer plasmid; (2) a packaging plasmid that, in addition to the *gag* and *pol*, contains the HIV accessory genes *nef*, *vif*, *vpu*, and *vpr* and regulatory genes *tat* and *rev*; and (3) an envelope plasmid. To increase the tropism, or cellular targets, of the HIV-based lentiviral vector systems, the HIV *env* gene in the envelope plasmid may be replaced with heterologous glycoprotein genes, a process called pseudotyping; lentiviral vectors are often pseudotyped with genes encoding vesicular stomatitis virus glycoproteins (VSV-G).[1,2] While pseudotyping improves vector stability, altering the tropism of the lentiviral vector to include virtually all mammalian cells increases the inherent risk associated with the lentiviral vector.[2] Also, since the pseudotyped vector is an animal pathogen as well as a human pathogen, it is subject to regulation by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA).

2.2.3 Second Generation Lentiviral Vector Systems

To further improve the level of lentiviral vector system safety, the HIV-specific accessory genes (*nef*, *vif*, *vpu*, and *vpr*) that contribute to pathogenesis were removed from the helper/packaging plasmid in second generation HIV-based vectors (Figure 2-3 panel C). By reducing the number of lentivirus genes from nine to four, the chance of RCR formation was significantly reduced even if multiple recombination events were to occur.

2.2.4 Third Generation and Higher Lentiviral Vector Systems

Third generation lentiviral vector system developments were directed toward safety improvements and reduction in the risk of RCR formation. The 5' LTR was modified into a chimeric 5′ LTR with a heterologous promoter that is no longer dependent on *tat* transactivation. Since *tat* was no longer required, it was removed from the third generation packaging plasmid. In addition, the remaining HIV packaging genes were separated into two packaging plasmids (one with *gag* and *pol*, and a second with *rev*). Along with the transfer and envelope plasmids, the resulting four-plasmid system contains as few as three HIV-specific genes and has significantly reduced RCR formation capability compared with earlier generations (Figure 2-3 panel D). In addition, some third generation systems have a deletion within the 3' LTR in the transfer plasmid, which is transcribed to the 5' LTR during reverse transcription; this results in a self-inactivating vector due to reduced promoter activity. This modification is particularly useful since it addresses biosafety issues that include recombination between the transfer and helper plasmids, insertional oncogenesis due to LTRs, and mobilization of the RCR upon subsequent infection with replication competent HIV-1.[2,14]

Developing fourth generation lentiviral vector systems with improved biosafety has been the aim of multiple scientific studies. In one, a transfer plasmid was reconfigured to prevent transcription of some HIV-1 components into the transduced transgene. This was accomplished

in part by removing the 5' LTR, and placing RNA signals (e.g., Ψ, Rev-response element) downstream of a self-inactivating 3' LTR.[15] The transduction efficiency of this lentiviral vector system is currently lower than standard technology, so it has yet to be implemented commercially. Separately, the packaging plasmids of a lentiviral vector system were divided into five separate plasmids, in part by separating *gag* and *pol* genes, but also by reintroducing the *tat* gene.[16] This latter system, as well as several second and third generation systems, remain in use and are available from commercial sources.

While it is highly unlikely, the risk of an RCR forming when using a second or third generation vector system remains. An RCR that contains HIV genes could demonstrate the viral attributes of HIV if it were introduced into a human or animal host.[17] Monitoring for RCR formation during vector development and manufacturing provides a means to detect its presence before the vector is put to use.

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CHAPTER 3

RISK ASSESSMENT

CHAPTER 3 - RISK ASSESSMENT

Biosafety involves the consistent application of safety measures (i.e., physical containment features and operational practices) that are crucial to prevent harm to personnel, the community, and the environment that could result from exposure to the pathogens handled within a facility, or their release from containment. The appropriate mitigation measures for a given pathogen, containment zone, work area, or procedure are based on risk assessments, which are the foundation for all components of a biosafety program. This chapter describes considerations for conducting a pathogen risk assessment and an LRA, specific to work with lentiviral vectors.

3.1 Pathogen Risk Assessment

A pathogen's risk group is determined through a pathogen risk assessment that evaluates the inherent characteristics that contribute to the pathogen's risk by considering factors such as pathogenicity, availability of effective preventive and therapeutic treatments, and communicability. The pathogen risk assessment will define the risk group, which helps to determine the appropriate containment level, and is taken into account in LRAs.

Routes of infection for lentiviral vectors are similar to those of HIV and include injection and exposure of mucosal surfaces or broken skin with infectious liquids or aerosols. Exposure to intact skin is not considered a significant risk of infection.[1] Effective treatment and preventive measures are available for lentiviruses, and the risk of spread of disease caused by these pathogens is low.

The PHAC has determined that lentiviral vectors meet the definition of RG2 human and RG2 animal pathogens. RG2 pathogens are those that pose a moderate risk to the health of individuals or animals, and a low risk to public health. RG2 pathogens are able to cause serious disease in a human or animal but are unlikely to do so. However, under certain conditions (e.g., use of first generation lentiviral vector systems, a modification of the vector, insertion of an oncogenic or toxic transgene), a resultant increased risk of handling these lentiviral vectors may require additional or modified biosafety procedures, or result in a higher risk group classification (i.e., RG3).

Individuals are encouraged to conduct their own pathogen risk assessments on the lentiviral vector system intended for use, particularly when using first generation systems, or when inserting oncogenic or toxic transgenes; completed risk assessments may be submitted to the PHAC for validation. More information on pathogen risk assessments can be found in the *Canadian Biosafety Guideline - Pathogen Risk Assessment*, and a pathogen risk assessment template is available on the Government of Canada website https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/pathogen-risk-assessment-template.html).[2,3]

The licence holder and BSO must be notified in writing if a pathogen risk assessment results in an increase of the lentiviral vector system to RG3 or if experimental evidence indicates that there is a high likelihood that the lentiviral vector will recombine to produce replication competent HIV (HPTR 5a). In such a case, an RG3 pathogen and toxin licence (hereafter referred to as "licence") issued by the PHAC is required. The licence will specify that all activities with the lentiviral vector system are to be performed in accordance with the *Biosafety Directive for Human Immunodeficiency Virus (HIV), Human T-cell Lymphotropic Virus Type 1 (HTLV-1), and Related Simian Retroviruses* (i.e., may be performed at Containment Level 2 (CL2) with additional precautions).[4]

3.1.1 Pathogen Risk Assessment Considerations for Lentiviral Vectors

The pathogen risk assessment starts by identifying the characteristics that are intrinsic to the pathogen itself and that contribute to its risk. Though not an exhaustive list, the following are considerations for lentiviral vectors:[5,6,7,8,9,10]

- The nature of the transgene insert. Known oncogenes or genes with high oncogenic or toxic potential may require additional biosafety practices.
- The possibility of alternate routes of infection, depending on the lentiviral vector. Pseudotyping with *vsv-g* can enable infection by inhalation under some conditions.
- The possibility of recombination during manufacture, which may lead to RCR. Despite the low probability, monitoring vector preparations for the presence of RCR will assess this. Use of third generation systems (i.e., low RCR formation potential) is recommended.
- Following exposure, there is a potential for oncogenesis due to insertional mutagenesis or transactivation of adjacent gene sequences resulting from the integration of viral DNA into the host genome.
- The possible mobilization (i.e., rescue) by wild type virus (e.g., HIV) and spread of mobilized vector particles to non-target cells or tissues (i.e., particularly with vectors that retain the complete LTR).
- The possibility of recombination with wild type virus in exposed HIV-positive individuals or lentivirus-positive animal hosts, whose native virus could recombine with or complement the vector.
- The possibility of productive infection in experimental animals injected with or exposed to RCR-contaminated vectors that could result in vector/virus shedding.
- The potential for lentiviral vectors to become capable of infecting a broader range of human and mammalian cells as a result of pseudotyping of the viral particles or alteration of the viral promoter sequences.
- Despite being replication incompetent, the infectivity of lentiviral vectors remains. Lentiviral vectors are capable of infecting target cells, which can then express the transgene.

The risk group of the vector-transgene combination will determine the appropriate physical containment requirements and operational practice requirements for safely handling the lentiviral vector. Even in cases where the lentiviral vector remains an RG2 pathogen, the risk assessment may identify additional mitigation measures needed for safe handling.

3.2 Local Risk Assessment

LRAs are site-specific assessments of risks related to activities involving pathogens and toxins. They are used to:

- identify and characterize hazards associated with the activities (i.e., tasks and procedures) being performed with infectious material or toxins;
- assess the risks for each hazard based on the likelihood of incidents occurring (e.g., exposure, release, or loss of pathogens and toxins), and the consequences of those incidents; and,
- develop and implement mitigation measures (e.g., standard operating procedures [SOPs] for safe work practices).

3.2.1 Local Risk Assessment Considerations for Lentiviral Vectors

Since the most likely routes of laboratory exposure to lentiviral vectors are injection and exposure of mucous membranes, key hazards to consider when performing LRAs for work involving lentiviral vectors include:[1]

- Use of sharps: The primary route of infection of lentiviral vectors is inoculation. The accidental injection of a lentiviral vector could result in HIV-positive serological status, transgene expression (the same is true for other lentiviral vectors and retroviruses), or insertional mutagenesis.
- Handling cell cultures transduced with lentiviral vectors: The integration of lentiviral vectors into the cell genome can have harmful effects on the transduced cells including an increased risk of tumour formation, insertional mutagenesis, and transactivation of neighbouring genes.
- Handling lentiviral vector suspensions: Handling high titre stocks of lentiviral vector suspensions increases the possibility of personnel exposure.
- Aerosol-generating procedures: Indirect contact with droplets produced from aerosol-generating procedures (e.g., centrifugation, pipetting) can result in droplet transmission (i.e., the depositing of infectious agents onto surfaces and subsequent transfer to exposed mucosal surfaces of the recipient).
- Work with animals: Evidence indicates that vectors may be shed from infected animals for over 24 hours post-infection, leading to the possibility of transmission to personnel through direct contact of mucous membranes with infected body fluids.

Table 3-1 outlines some of the factors to consider when performing risk assessments related to the use of lentiviral vectors, and how each factor contributes to increasing or decreasing the overall risk.

Table 3-1: Considerations for pathogen risk assessments and LRAs[1]

Pathogen Risk Assessment		
	Lower Risk	Higher risk
Transgene	• Non-oncogene • Non-toxic	• Oncogene • Toxic
Vector design	• Viral genes on four or more plasmids • Deletion of viral accessory genes • Deletion of *tat* regulatory gene • Self-inactivating vector • Weak promoter • Non-human tropism	• Viral genes on two plasmids • Expression of viral accessory genes • Expression of *tat* regulatory gene • Intact 3' LTR • Strong promoters • Pseudotropism with expanded host range
Local Risk Assessment		
	Lower Risk	Higher risk
Animals	• Non-permissive hosts (unlikely to shed virions)	• Permissive hosts or hosts engrafted with human cells (more likely to shed virions)
Production	• Small scale (<10 L)	• Large scale (>10 L)
Manipulations	• No use of sharps • Unlikely to produce aerosols	• Use of sharps • May produce aerosols

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CHAPTER 4

CONSIDERATIONS FOR CONTAINMENT

CHAPTER 4 - CONSIDERATIONS FOR CONTAINMENT

4.1 Containment Level Requirements

Based on the pathogen risk assessments completed by the PHAC, it has been determined that all activities with lentiviral vector systems, including propagative *in vitro* and *in vivo* activities, can be safely conducted at CL2 or in a CL2 large animal containment zone (i.e., CL2-agriculture [CL2-Ag]). CL2 is also appropriate in large-scale production areas (>10 L). Applicability of large-scale production area requirements can be determined in consultation with the PHAC and the CFIA on a case-by-case basis. However, under certain conditions (e.g., use of first or second generation lentiviral vector systems) additional operational requirements at CL2 or CL2-Ag may be required to prevent exposure of personnel and the spread of contamination outside the containment zone (e.g., in accordance with the *Biosafety Directive for Human Immunodeficiency Virus (HIV), Human T-cell Lymphotropic Virus Type 1 (HTLV-1), and Related Simian Retroviruses*).[1]

4.2 Work Practices

Operational practices refer to the administrative and procedural controls, including SOPs, in place to prevent the inadvertent exposure of personnel to potentially infectious material, and the release of pathogens and toxins from containment. Personnel are more likely to follow operational practices when they are documented in SOPs and included in personnel training. Operational practice requirements for CL2 and CL2-Ag are specified in Chapter 4 of the CBS.

In areas where lentiviral vectors are handled or stored, safe work practices include the proper use, and maintenance of, laboratory and biosafety equipment (e.g., centrifuges, biological safety cabinets [BSCs]), and the use of good microbiological laboratory practices.

The use of a BSC or other primary containment device will protect personnel against exposure (e.g., via mucous membranes contact) by containing aerosols during procedures that may result in the production of infectious aerosols, or involve high concentrations or large volumes of lentiviral vectors. The use of sealed safety cups or rotors that are unloaded in a BSC will contain aerosols that may be generated when centrifuging lentiviral vectors.

The main risk of transmission of lentiviral vectors to personnel is through accidental inoculation, cuts or punctures with contaminated instruments, and contact with open wounds. Avoiding the use of needles and other sharps will reduce this risk. In the event that the use of sharps is unavoidable, appropriate SOPs can be developed that limit, or offer proper handling instructions regarding the use of sharps so that the instruments are used in a manner that prevents accidental inoculations.[2] Safety-engineered sharps and needles that reduce the risk may also be applicable.

When used appropriately, personal protective equipment (PPE), such as gloves, lab coats, and safety glasses, protect workers from infectious material with which they may come into contact in the course of performing their work. In addition to the PPE required for CL2 and CL2-Ag, as indicated in Matrix 4.4 of the CBS, an LRA may indicate the need for additional PPE. For example, an LRA may determine that a respirator, eye protection, or face shield be worn during procedures likely to generate aerosols.[2]

4.3 Animal Work Considerations

Pathogen and toxin work performed *in vivo* (i.e., in living animals) is carried out in an animal containment zone. An animal containment zone refers to a series of co-located animal rooms or animal cubicles, as well as associated corridors and support rooms (e.g., storage and preparation areas) of equal containment level. More detailed information on animal containment zones can be found in the CBS and CBH.[3,4]

The delivery of lentiviral vectors or lentiviral vector-transduced cells into an animal must be performed at the appropriate containment level. Animal hosts that are permissive for HIV replication, or those that have been engrafted with human cells, can support replication of infectious HIV, and hence additional precautions may need to be in place. Given the increased hazard posed by animal inoculation, it is important that laboratory personnel handle these materials in a manner that minimizes the risk of incidents, as determined by an LRA. For example, to minimize the risk of self-inoculation, required safety procedures (e.g., animal restraint) may be enhanced with additional precautions and PPE (e.g., puncture-resistant gloves). Where it is not feasible to perform injections in a BSC, additional PPE (e.g., goggles, respirators) may be needed to reduce the possibility of mucosal exposure to aerosols. Following inoculation, disinfecting or cleaning the animal or the site of inoculation will eliminate any lentiviral vector that may remain on the surface of the animal.[2,5]

4.3.1 Testing for replication-competent retrovirus

The PHAC's pathogen risk assessments for lentiviral vector systems clearly indicate that lentiviral vector systems have the potential to form RCR and the potential for oncogenesis following integration. This risk is higher in earlier generation systems; to date, there have been no reports of RCR formation using a third-generation lentiviral vector system. RCR testing during vector manufacture will aid in confirming the presence or absence of RCR; positive detection may negate the vectors subsequent use.[6,7] Contact Health Canada's Biologics and Genetic Therapies Directorate or the CFIA's Canadian Centre for Veterinary Biologics for more information on requirements for RCR testing of lentiviral vectors systems for use in humans or animals clinical applications (respectively).[8,9]

It was also found that, following inoculation and until viral clearance, the vector could be shed from infected animals and transmitted to humans through direct contact of mucous membranes with infected body fluids.[10] Laboratory personnel could also be exposed through accidental

injection of the vector preparation. In both cases, the nature of the inserted gene (e.g., oncogene) will be an important consideration to determine the effective physical containment and operational practices that will minimize the risk of exposure. Certain animals cannot support the replication of infectious HIV and are therefore considered to have negligible probability of RCR formation following injection with a lentiviral vector. In such cases, the probability for shedding of RCR is very low.

A risk assessment that considers RCR formation and shedding as well as other factors (e.g., potential exposure of the animal to higher risk pathogens or non-indigenous animal pathogens) may determine that it is permissible to decrease the containment level following vector delivery. For example, if an LRA determines that the likelihood of infection is extremely low or nonexistent and the site of inoculation has been cleaned and the bedding changed, it may be acceptable to move the animal to a lower containment level within a few days following inoculation (the timing of this will be determined by an LRA, and may range from one to seven days).[5] Similarly, animals that have tested negative for RCR may be moved out of containment a few days following inoculation, provided that the risk assessment determines that there is little to no risk of infection or replication of an infectious virus, and that higher risk pathogens (including non-indigenous animal pathogens) are not handled within the same containment zone.[2,5]

Currently, the most common approach of testing for RCR involves a cell culture based method followed by endpoint detection of RCR components, which can be achieved by polymerase chain reaction (PCR), assays for detection of reverse transcriptase activity, or antigen assays.[7] Notably, some RCR detection assays use an RG3 positive control, thus increasing the risk of incidents to personnel, and requiring a valid RG3 licence from the PHAC.

4.4 Post-Exposure Prophylaxis

Prior to working with lentiviral vectors, a post-exposure response plan that addresses the specifics of the planned lentiviral vector system and activities should be in place as a component of the medical surveillance plan.[11] In case of needlestick or sharps injury with first or second generation lentiviral vectors, prompt administration of post-exposure prophylaxis (e.g., within one hour) will minimize the already low risk of HIV infection.[11] The risks of transduction with potentially oncogenic or toxic genes, or insertional mutagenesis have not been clinically proven to be prevented by HIV prophylaxis.[11] However, antiretroviral drugs (e.g., azidothymidine [AZT]) are capable of blocking transduction completely in cell culture.[12]

4.5 Decontamination and Waste Management

The effective decontamination of waste, materials, equipment, and surfaces that have come in contact with potentially infectious material or toxins is fundamental in limiting the spread of contamination beyond the work area and facility. Decontamination technologies may be provided on site, or contaminated waste can be appropriately packaged and transported to a designated facility for decontamination.

4.5.1 Decontamination of Lentiviral Vectors

Lentiviral vectors are labile, so surfaces can be easily decontaminated by a variety of chemicals, including 5,000 ppm sodium hypochlorite (about 1/10 dilution of commercial bleach) or 70% ethanol, with a sufficient contact time. If bleach is used, fresh dilutions should be prepared and the residue must be rinsed off with water to prevent corrosion of surfaces (e.g., stainless steel).[13] Liquid waste can be decontaminated by autoclaving or mixing with bleach to a final concentration of 5,000 ppm sodium hypochlorite and leaving for at least 30 minutes. Solid waste can be autoclaved or incinerated.[14]

Decontaminating (and labelling as decontaminated) all materials that may have been in contact with lentiviral vectors prior to removal from the containment zone will prevent contaminated material from leaving the containment zone. If the material is to be decontaminated off-site, it can be placed in a closed, labelled, leak-proof container that has been surface decontaminated.

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CHAPTER 5

GLOSSARY

CHAPTER 5 - GLOSSARY

Most of the following list is derived from the CBS and the CBH. It is important to note that while some of the definitions provided in the glossary are universally accepted, many of them were developed specifically for the CBS or the CBH; therefore, some definitions may not be applicable to facilities that fall outside of the scope of the CBS and the CBH. A comprehensive list of terms and their definitions can be found in the Glossary in Chapter 24 of the CBH.

Animal cubicle	A room or space designed to house an animal (or animals) where the room itself serves as primary containment. These spaces are used to house large-sized animals (e.g., livestock, deer), or small-sized animals that are housed in open caging (i.e., not primary containment caging).
Animal pathogen	Any pathogen that causes disease in animals, including those derived from biotechnology. In the context of the CBS, "animal pathogen" refers only to pathogens that cause disease in terrestrial animals; including those that infect avian and amphibian animals, but excluding those that cause disease in aquatic animals and invertebrates.
Animal room	A room designed to house animals in primary containment caging. These spaces are used to house only small-sized animals (e.g., mice, rats, rabbits).
Biological safety cabinet (BSC)	A primary containment device that provides protection for personnel, the environment and the product (depending on BSC class), when working with biological material.
Biosafety	Containment principles, technologies, and practices that are implemented to prevent unintentional exposure to infectious material and toxins, or their accidental release.
Containment	The combination of physical design parameters and operational practices that protect personnel, the immediate work environment, and the community from exposure to biological material. The term "biocontainment" is also used in this context.
Containment level (CL)	Minimum physical containment and operational practice requirements for handling infectious material or toxins safely in laboratory, large scale production, and animal work environments. There are four containment levels ranging from a basic laboratory (CL1) to the highest level of containment (CL4).

Containment zone	A physical area that meets the requirements for a specified containment level. A containment zone can be a single room (e.g., CL2 laboratory), a series of co-located rooms (e.g., several non-adjoining but lockable CL2 laboratory work areas), or it can be comprised of several adjoining rooms (e.g., CL3 suite with dedicated laboratory areas and separate animal rooms, or animal cubicles). Dedicated support areas, including anterooms (with showers and "clean" and "dirty" change areas, where required), are considered to be part of the containment zone.
Good microbiological laboratory practices	A basic laboratory code of practice applicable to all types of activities with biological material. These practices serve to protect workers and prevent contamination of the environment, and the samples in use.
Handled or stored	"Handling or storing" pathogens, toxins, or infectious material includes possessing, handling, using, producing, storing, permitting access to, transferring, importing, exporting, releasing, disposing of, or abandoning such material. This includes all controlled activities involving human pathogens and toxins specified in Section 7(1) of the HPTA.
Incident	An event or occurrence with the potential of causing injury, harm, infection, intoxication, disease, or damage. Incidents can involve infectious material, infected animals, or toxins, including a spill, exposure, release of infectious material or toxins, animal escape, personnel injury or illness, missing infectious material or toxins, unauthorized entry into the containment zone, power failure, fire, explosion, flood, or other crisis situations (e.g., earthquake, hurricane). Incidents include accidents and near misses.
In vitro	Latin for "within glass"; describes experimentation involving components of a living organism within an artificial environment (e.g., manipulation of cells in petri dish), including activities involving cell lines or eggs.
In vivo	Latin for "within the living"; describes experimentation conducted within the whole living organism (e.g., studying the effect of antibiotic treatment in animal models).
Large animal containment zone (LA zone)	Animal containment zone comprised of two or more co-located or adjoining rooms of equal containment level where animals are housed in animal cubicles (i.e., the room itself provides the primary containment). An LA zone may include, for example, large-sized animals, such as livestock or deer, housed in cubicles or, cubicles where small-sized animals, such as mice or raccoons, are housed in open caging (i.e., not primary containment caging). Post mortem rooms, where present, are considered to be part of an LA zone.

Local risk assessment (LRA)	Site-specific risk assessment used to identify hazards based on the infectious material or toxins in use and the activities being performed. This analysis provides risk mitigation and risk management strategies to be incorporated into the physical containment design and operational practices of the facility.
Non-indigenous animal pathogens	A pathogen that causes an animal disease listed in the World Organization for Animal Health's OIE-Listed diseases, infections and infestations (as amended from time to time) and that is exotic to Canada (i.e., foreign animal disease agents that are not present in Canada), or any other animal disease agent that is not indigenous to Canada, as determined by the CFIA. These pathogens may have serious negative health effects to the Canadian animal population.
Oncogene	A mutated form of a gene involved in normal cell growth whose activation is associated with the conversion of normal cells into cancer cells.
Operational practice requirements	Administrative controls and procedures followed in a containment zone to protect personnel, the environment, and ultimately the community, from infectious material or toxins, as outlined in Chapter 4 of the CBS.
Pathogen	A microorganism, nucleic acid, or protein capable of causing disease or infection in humans or animals. Examples of human pathogens are listed in Schedules 2 to 4 and in Part 2 of Schedule 5 of the HPTA, but these are not exhaustive lists.
Pathogenicity	The ability of a pathogen to cause disease in a human or animal host.
Pathogen risk assessment	The determination of the risk group and appropriate physical containment requirements and operational practice requirements needed to safely handle the infectious material or toxins in question.
Personal protective equipment (PPE)	Equipment and/or clothing worn by personnel to provide a barrier against infectious material or toxins, thereby minimizing the risk of exposure. PPE may include, but is not limited to, lab coats, gowns, full-body suits, gloves, protective footwear, safety glasses, safety goggles, masks, and respirators.
Physical containment requirements	Physical barriers in the form of engineering controls and facility design used to protect personnel, the environment, and ultimately the community, from pathogens or toxins, as outlined in Chapter 3 of the CBS.

Primary containment device	Apparatus or equipment that is designed to prevent the release of infectious material or toxins and to provide primary containment (i.e., provide a physical barrier between the individual and/or the work environment and the biological material). Examples of primary containment devices include biological safety cabinets, isolators, centrifuges with sealable cups, process equipment, fermenters, microisolator cages, and ventilated cage racks.
Risk group (RG)	The classification of biological material based on its inherent characteristics, including pathogenicity, virulence, risk of spread, and availability of effective prophylactic or therapeutic treatments, that describes the risk to the health of individuals and the public as well as the health of animals and the animal population.

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CHAPTER 6 - REFERENCES AND RESOURCES

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