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Public Health Agency of Canada
Centre for Biosecurity

Biosafety Directive
for Human Immunodeficiency Virus (HIV), Human T-lymphotropic Virus (HTLV), and Related Simian Retroviruses

April 14, 2019

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Abbreviations

AIDS	Acquired immunodeficiency syndrome
ATL	Adult T-cell leukemia
BSC	Biological safety cabinet
CBS	*Canadian Biosafety Standard*
CBH	*Canadian Biosafety Handbook*
CFIA	Canadian Food Inspection Agency
CL	Containment Level (i.e., CL1, CL2, CL3, CL4)
CL2-Ag	CL2 large animal containment zone
HAA	*Health of Animals Act*
HAR	*Health of Animals Regulations*
HAM/TSP	HTLV-1 associated myelopathy/tropical spastic paraparesis
HIV	Human immunodeficiency virus
HPTA	*Human Pathogens and Toxins Act*
HPTR	*Human Pathogens and Toxins Regulations*
HTLV	Human T-lymphotropic virus
LA zone	Large animal containment zone
LAI	Laboratory acquired infection
LRA	Local risk assessment
NHP	Nonhuman primate
PHAC	Public Health Agency of Canada
RG	Risk Group (i.e., RG1, RG2, RG3, RG4)
RNA	Ribonucleic acid
SA zone	Small animal containment zone
SHIV	Simian-Human immunodeficiency virus
SIV	Simian immunodeficiency virus
STLV	Simian T-lymphotropic virus

1.0 Background

The handling or storing of pathogens, toxins, and other regulated infectious material necessitates an awareness and application of biosafety and biosecurity practices. The release of human and animal pathogens and toxins from laboratories or other containment zones may pose a risk to public health, animal health, or both. Personnel can minimize the risks associated with pathogens or toxins through the application of appropriate biosafety and biocontainment principles and practices.

In Canada, facilities that handle or store human pathogens, or that import animal pathogens, must meet the requirements specified in the current version of the *Canadian Biosafety Standard* (CBS), as well as the requirements of the *Human Pathogens and Toxins Act* (HPTA), *Human Pathogens and Toxins Regulations* (HPTR), *Health of Animals Act* (HAA), and *Health of Animals Regulation*s (HAR).[1,2,3,4,5]

The CBS describes the physical containment requirements, operational practice requirements, and performance and verification testing requirements for handling and storing infectious material according to containment level. Pathogens that have had pathogen risk and containment level assessments completed have been assigned an appropriate risk group and containment level. For the majority of pathogens, the containment level and risk group of the pathogen are the same (e.g., Risk Group 2 [RG2] pathogens are handled at containment level 2 [CL2]), but there are some exceptions. As part of the pathogen risk assessments and containment level assessments conducted by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA), the containment requirements may be reduced or modified for certain activities, which would be communicated in the form of biosafety advisories or biosafety directives. In general, many of the physical containment requirements and operational practice requirements at CL3 are aimed at reducing the risks associated with airborne or aerosol-transmitted pathogens. As such, certain activities involving RG3 pathogens not known to be transmissible by inhalation can sometimes be performed at a lower containment level. As stated in the CBS, the reduced or specific requirements will be determined based on the work involved and the pathogen in question, and would be stipulated on the pathogen and toxin licence or the animal pathogen import permit. It may also be otherwise communicated by the PHAC or the CFIA using another mechanism and, in this case, a biosafety directive.

The human immunodeficiency virus (HIV) and the human T-lymphotropic virus (HTLV) are examples of RG3 human pathogens where the pathogen risk and containment level assessments have been revisited by the PHAC in conjunction with HIV/HTLV specialists. It has been determined that HIV and HTLV can be safely handled at CL2/CL2 large animal containment zone (CL2-Ag) with specific additional operational requirements (see Section 4.0 of this directive). The simian viruses related to HIV and HTLV are RG2 human pathogens, and as such, must also be handled at CL2. This biosafety directive is intended to provide a comprehensive overview of the risk assessment outcomes, subsequent containment level decisions, and considerations that have been made for those working with HIV or HTLV. The *Biosafety Directive for HIV, HTLV, and Related Simian Retroviruses* is to be used in conjunction with the CBS.

2.0 Pathogen descriptions and risk groups

The pathogens within the scope of this directive are retroviruses that are known to infect humans, and that are presented in the following sections. These include the HIV, simian immunodeficiency virus (SIV), HTLV, simian T-lymphotropic virus (STLV), and any strains or constructs engineered from any of the viruses indicated in Table 1. This includes, for example, simian-human immunodeficiency virus (SHIV) which is a chimeric virus engineered from SIV and HIV. In the case of SIV, approximately 40 strains have been identified, often associated with a specific species of nonhuman primate (NHP). Aside from the two SIV strains listed, their ability to infect humans remains unknown. If handling an SIV strain that has not been characterized, contact the PHAC for more information regarding risk group classification and appropriate containment measures.

Table 1: Virus strains covered by this directive

Virus[1]	Strain
HIV	HIV-1 HIV-2
SIV	SIVsm SIVcpz
HTLV	HTLV-1 HTLV-2 HTLV-3 HTLV-4
STLV	STLV-1 STLV-2 STLV-3 STLV-4

[1] This also includes any strains or constructs engineered from any of the strains listed in this table.

2.1 Human Immunodeficiency Virus (HIV) Description and Risk Factors

HIV is classified as an RG3 human pathogen. It is an enveloped, spherical virus that is around 80-100 nm in size, and has a linear, single-stranded positive-sense ribonucleic acid (RNA) genome.[6] The retrovirus exists as two types, HIV-1 and HIV-2.[7] HIV-1 is the more common type, and it is divided into four groups, M, N, O, and P, based on homology of nucleotide sequences and the originating cross-species transmission. HIV-1 group M accounts for an estimated 98% of infections worldwide while groups N, O, and P are considered rare.[8]

HIV-2 is mainly concentrated in West Africa, but its prevalence is increasing in other parts of the world as well, as a result of travel and immigration. The virus is related more closely to SIV (75% similarity) than to HIV-1 (42% similarity).[9] HIV-2 infection does not appear to be as easily transmitted and immunodeficiency takes longer to develop. Unlike HIV-1 which is highly infectious in the acute stage, HIV-2 infectiousness increases in the later stages of acquired immunodeficiency syndrome (AIDS) and the highly infectious stage is shorter when compared with HIV-1.

High rates of HIV infection are observed in Africa, Asia, and Europe.[10] As of 2016, the PHAC estimates 63,110 individuals are living with HIV in Canada, a 5% increase from 2014.[11]

The initial, acute stage of HIV-1 infection results in flu-like symptoms that can last for 3-5 weeks and typically includes lymphadenopathy, malaise, high plasma viremia, and an initial decrease in CD4+ T cells.[12,13] As infection progresses to the clinical latency stage that can last for years, immune responses lead to a decrease in circulating virus and restoration of CD4+ T cell numbers. In the absence of treatment, viral replication continues, leading to the gradual loss of CD4+ T cells. When the number of CD4+ T cells becomes insufficient to maintain effective immunity, the likelihood of opportunistic infections and neoplasia increases. As CD4+ T cell numbers fall to very low levels, the disease progresses to AIDS, with symptoms of lymphadenopathy, weight loss, chronic diarrhea, and nervous system diseases (including dementia, myopathy and pain). Progression to disease depends on viral factors such as the strain or isolate, infectious dose, and viral load. Without treatment, AIDS develops in the majority of people within 10 years after infection.[14] Globally, tuberculosis (TB) is the leading cause of death in patients with AIDS, as it causes mortality in one third of HIV-infected individuals worldwide, and patients with HIV are also about 30% more likely to develop TB disease than those without infection.[15]

The natural host of HIV is humans, and experimental infection with HIV-1 is possible in the chimpanzee, gibbon ape, and rabbit.[16] HIV cannot replicate outside of the host, and is extremely susceptible to drying.[17] The infectious dose of HIV remains unknown. Since the primary route of infection is through sexual and parenteral transmission, it is unlikely that a major HIV epidemic would occur upon the release of the virus from the laboratory environment.

Vaccine development has not yet been successful due to the ever-changing genetic and antigenic properties of HIV-1. There are now more than 30 effective antiviral drugs and combination formulations available for use; however, the emergence of drug-resistant HIV strains threatens to limit the effects of antiviral treatments.[18,19,20] Additionally, in cases of exposure or high likelihood of exposure, there are effective pre- and post-exposure prophylaxis regimes and guidelines to follow.[21,22]

2.2 Simian Immunodeficiency Virus (SIV) Description and Risk Factors

SIV is a species of retrovirus that is able to infect at least 45 species of African primates. HIV-1 and HIV-2 are believed to have resulted from cross-species transmission of primate strains SIVcpz and SIVsmm, respectively. SIVcpz is found in chimpanzees and SIVsmm is found in sooty mangabeys (*Cercocebus atys*).[8]

The main host for SIV is NHPs, although SIV has the potential to be a zoonotic pathogen. Laboratory acquired infections (LAIs) of laboratory workers have been reported in the literature with no associated disease.[23,24,25]

The primary mode of transmission is by injection and thus the use of needles should be limited. However, transmission could also occur through exposure of mucous membranes or breaks in the skin to infected blood or bodily fluids.

In rhesus monkeys and other susceptible NHP species (e.g. pig-tailed macaque, crab-eating macaque), SIV infection will lead to a chronic wasting disease syndrome with depletion of CD4+ T lymphocytes

and lymphadenopathy. The clinical course of this infection in monkeys, like that of AIDS in humans, is complicated by various opportunistic infections.[26] SIV also causes a primary encephalopathy in monkeys with many of the features of HIV-associated encephalopathy in humans.[27]

2.2.1 Simian-Human Immunodeficiency Virus (SHIV)

A SHIV is a chimeric construct engineered from SIV and HIV. Typically, these constructs are created by the addition of genes encoding HIV envelope and accessory proteins (i.e., env, tat, rev, vpu, and nef) onto an SIV genetic backbone, resulting in a replication-competent recombinant virus that exhibits properties of both lineages with the same antigenicity as HIV. Several pathogenic SHIV strains are used as HIV models for research on transmission, pathogenesis, drug potency, and vaccine development.[28,29,30]

The chimeric virus is capable of infecting NHPs not naturally susceptible to SIV infections, including rhesus, pig-tailed, and cynomolgus macaques. SHIV infection in these NHP hosts produces HIV-like equivalents of human disease.[31] There are no reported incidents of exposure, and thus no known infections of humans. However, specific SHIV strains are capable of infecting and replicating in human cells, including peripheral blood mononuclear cells, astrocytes, and T-cell lines. Serial passage and in vivo adaptation can yield variants with increased pathogenicity.[30]

Characteristics of SHIVs are strain-specific and dependent in part upon the founder HIV and SIV isolates. Infectivity is demonstrated by both isolated virus (i.e., cell-free) and cell-associated SHIV. Depending on the specific SHIV, infection can be the result of intravenous or mucosal transmission, and can lead to varying levels of transmissibility, pathogenicity, and host specificity.[30]

SHIVs are classified as RG3 human pathogens and RG3 animal pathogens, but as is the case of other laboratory-engineered organisms, organizations can perform their own pathogen risk assessment to evaluate the unique properties of their pathogen.[32]

2.3 Primate T-lymphotropic Viruses (PTLV) Description and Risk Factors

HTLVs and their STLV counterparts are globally referred to as primate T-lymphotropic viruses (PTLV). To date, there are four distinct groups of PTLV that have been discovered: PTLV-1, -2, and -3 include both human (HTLV-1, 2 and 3) and simian (STLV-1, 2 and 3) viruses. The fourth type (PTLV-4) had only been described in humans until recently (HTLV-4); however, new STLVs, including STLV-4, have been discovered and are under investigation.[8,33]

2.3.1 Human T-lymphotropic Virus (HTLV) Description and Risk Factors

HTLV is an RG3 pathogen, also known as human T-cell leukemia virus. It is a complex human C-type retrovirus that is spherical in structure with a diameter of approximately 100 nm.[34] The core of HTLV is electron-dense and contains two positive-sense single-stranded RNA genomes that upon infection are converted to deoxyribonucleic acid (DNA) by reverse transcription and inserted into the host genome to cause persistent infection.[35] Unlike other retroviruses such as HIV, which increases its progeny by replication of the virus itself, HTLV increases its copy number by proliferation of infected cells.[36,37]

Four distinct HTLV types have been identified, all of which resulted from the zoonotic transmission of simian retroviruses from simian to human hosts.[30] HTLV-1 and HTLV-2 have been linked to human disease. HLTV-1 primarily causes adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HTLV-2 is a less pathogenic strain and has been associated with milder neurological disorders and chronic pulmonary infections. HTLV-3 and HTLV-4 have not been associated with human disease.[38]

It is estimated that 10-20 million individuals worldwide are infected with HTLV-1.[29,30,32,39] HTLV-1 infection is endemic in southern Japan, the Caribbean, parts of Africa (Gabon and Nigeria), the Middle East, South America (Panama, Colombia, Venezuela, Brazil and Bolivia), the Pacific Melanesian islands, and Papua New Guinea.[34] The number of HTLV-1 cases in Canada is unknown; however, it is reported as rare. HTLV-1 infections have been described in indigenous peoples from coastal regions of British Colombia, and in Nunavut.[40,41] A survey of United States first-time blood donors from 2000-2009 found 5.1 cases/10^5 (95% confidence interval [CI], 4.1–6.1) were positive for HTLV-1 and 15.7 cases/10^5 (95% CI, 13.0-16.3) were positive for HTLV-2.[42]

The main hosts of HTLV-1 are humans; however, HTLV-1 is also able to experimentally infect rabbits, rats and monkeys.[43] While the infectious dose of HTLV-1 and -2 remain unknown, the primary mode of transmission is through direct contact with contaminated body fluids with mucous membranes or breaks in the skin (e.g., abrasions, lacerations, etc.). Even though the transmission of HTLV from animals to humans would be rare, it could occur from animal bites or scratches, or contact of mucous membranes with infected body fluids.[44] There is documentation of HTLV infection of a laboratory worker following accidental needle stick puncture.[45]

ATL is a serious disorder involving malignancy of T-lymphocytes. There are four types that are based on clinical presentation. The acute type involves increased numbers of T-cells, skin lesions, systemic lymphadenopathy and hepatosplenomegaly. The lymphoma type is characterized by prominent systemic lymphadenopathy with few abnormal cells in the blood. Both the acute and lymphoma types have poor outcomes with a median survival time of about one year. In chronic ATL, the white cell count is mildly elevated and skin lesions, lymphadenopathy, and hepatosplenomegaly are sometimes present. Smoldering ATL is characterized by a few ATL cells and the absence of other clinical features commonly associated with ATL. Generally, the chronic types of ATL progress to an acute form within a few years.[31,35,38] Development of ATL is strongly linked to childhood infection and has an estimated incubation period anywhere between 20-60 years, with about 2-6% of infected individuals developing the disease.[29,31]

HAM/TSP is a chronic demyelinating disorder characterized by muscle weakness in the extremities, sensory disturbances, urinary incontinence, impotence, and lower back pain.[21,22,29,35,38] This is a progressively disabling condition and with time, the patient may become wheelchair bound, and death may result from secondary complications.[31] The median incubation period of HAM/TSP is 3.3 years, with less than 1% of HTLV-1 infected patients having the condition.[40] Although the majority of HTLV-1 infected patients remain asymptomatic, they are still capable of transmitting the virus.[38] There is no cure for HTLV infection, and effective and long-term therapeutic protocols are lacking for HTLV associated diseases.

2.3.2 Simian T-lymphotropic Virus (STLV) Description and Risk Factors

Four distinct STLV types (STLV-1, STLV-2, STLV-3, and STLV-4) have been identified that correspond to the four HTLV types. STLV-1 and STLV-3 exhibit high genetic diversity, can infect many different NHP species, and have numerous subtypes. In contrast, two strains of STLV-2 have only been found in bonobos. STLV-4 was recently discovered in gorillas in Central Africa with corresponding evidence of zoonotic transmission to hunters severely bitten by gorillas.[28,46] STLV is endemic in Africa and Asia with STLV strains clustering geographically and infecting many different NHP species.[8]

2.4 Laboratory Acquired Infections

From 1981 to 2002, 106 cases of occupationally acquired HIV infections were documented among healthcare workers: 57 in the United States, 35 in Europe, and 14 elsewhere (including 3 cases in Canada). Two hundred and thirty eight (238) cases of possible occupational acquired infections have also been reported.[47] The United States Centers for Disease Control and Prevention latest available data (December 2010) found no new documented infections since 1999, but several cases were still under investigation.[48] Of the 200 documented and possible occupationally acquired HIV infections, 36 (18%) were laboratory workers or technicians.

The risks of acquiring HIV infection in the laboratory environment include transmission via exposure to blood and infected material, or from infected animals. The most common exposure is needle stick injury, which causes about 80% of all LAIs; however, it is reported that the risk of acquiring an HIV infection following this type of exposure is 0.3-0.5%.[49]

The routes of occupational exposure to HTLV are the same as for HIV, but the risk of transmission is much lower as indicated by the few documented occupational infections.[40] In addition, a study of occupational exposures in Australia found no transmissions of HTLV-1 among 53 exposed healthcare workers despite only three having received post-exposure prophylaxis.[50]

2.5 Risk Group Determination

The risk group is based on the outcome of a pathogen risk assessment which assesses the inherent risk of a pathogen considering risk factors such as pathogenicity, availability of effective preventive and therapeutic treatments, and communicability. The categories range from RG1 (low individual and low community risk) to RG4 (high individual and high community risk). A full list of pathogen risk factors and the definitions of the risk group levels can be found in the *Canadian Biosafety Handbook* (CBH).[51]

In collaboration with experts, the PHAC performed pathogen risk assessments on HIV and HTLV and determined that the viruses meet the definition of a RG3 human pathogen. RG3 pathogens are those that pose a high risk to the health of individuals and/or animals and a low risk to public health. RG3 pathogens are likely to cause serious disease in a human or animal. Effective treatment and preventive measures are usually available and the risk of spread of disease caused by these pathogens is low. The risk group determination for HIV and HTLV is in line with assessments that have been completed by international counterparts as shown in Table 2.

SIV and STLV meet the definition of a RG2 human pathogen. RG2 pathogens are those that pose a moderate risk to the health of individuals and/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. Effective treatment and preventive measures are available and the risk of spread of disease caused by these pathogens is low.

Table 2: International Risk Group Classification of HIV, SIV, and HTLV

Country	HIV Risk Group	SIV Risk Group	HTLV Risk Group
Australia/New Zealand 2010	2	--	2
Belgium 2008	3	3	3
Germany 2013	3	2	3
United Kingdom 2013	3	3	3
European Community 2000	3	3	3
National Institute of Health 2016	3	3	3
Singapore 2004	Schedule 1	Schedule 1	Schedule 1
Switzerland	3	3	3

From https://my.absa.org/Riskgroupsl

3.0 Containment Level Requirements

3.1 CBS Containment Zone Overview

The CBS specifies the physical containment requirements, operational practice requirements, and performance and verification testing requirements for handling human and terrestrial animal pathogens and toxins. This section describes the distinction between the animal containment zones. More detailed information on the types of containment zones can be found in the CBS.

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, serviced by a single entry/exit. In a small animal containment zone (SA zone), animals are housed in primary containment caging. The room encompassing these cages is referred to as an "animal room". SA zones must generally meet the same requirements as those for laboratory work areas that appear under the CL2 or CL3 columns of the CBS.

In a large animal containment zone (LA zone), the room itself provides the primary containment. The room or space housing the animals is referred to as an "animal cubicle". Where small-sized animals are housed in open caging only intended for the confinement of animals to an area (i.e., it does not include filtration to prevent the release of infectious material and toxins), it is considered to be an animal cubicle inside an LA zone, despite the actual size of the animal. The CBS provides distinct columns for LA zones at CL2 and CL3, labelled CL2-Ag and CL3-Ag, respectively.

3.2 Containment Level Requirements

The containment level provides the end-user with a description of the minimum physical containment requirements, operational practice requirements, and performance and verification testing requirements for handling pathogens safely in a laboratory setting. Containment levels range from a basic laboratory (i.e., CL1) to CL4, the highest level of containment. Refer to the CBS for full descriptions of the containment levels.

The risk assessments for HIV and HTLV clearly indicate that these pathogens have the ability to produce serious disease in humans. However, the assessments also report that transmission is largely dependent on parenteral or mucous membrane exposure; survival of the virus outside the host is limited and occurs only under ideal conditions; airborne transmission is not possible; and the risk for laboratory users as well as the environment is limited. It has also been determined that the culturing (propagation) or *in vivo* work involving these pathogens does not increase the risk for the laboratory user and that proper handling and operational practices can mitigate the risk.

Based on the risk assessments completed by the PHAC in conjunction with HIV/HTLV specialists, it has been determined that HIV, HTLV, and related simian retroviruses (including SHIVs) can be safely handled at CL2/CL2-Ag with specific additional operational requirements. See Section 4 for the additional requirements that are to be followed in order to work at CL2/CL2-Ag and Section 5 for additional biosafety considerations for working with HIV, HTLV, and related simian retroviruses. For a complete listing of the physical containment requirements, operational practice requirements, and performance and verification testing requirements, refer to Chapters 3, 4, and 5 of the CBS.

3.3 Specimen and Culture Type

The different types of specimens and cultures containing HIV or HTLV may result in different risks. For the purpose of this directive, specimens and activities have each been classified into the three broad categories described below.

3.3.1 Inactivated biological material: These include products (e.g., pellets, concentrated virus) concentrated from primary specimens (e.g., blood, plasma, and semen), as well as cultures (propagated material), that have been inactivated using a validated and routinely verified method. The inactivation must be performed at the containment level required for the pathogen and type of specimen (e.g., if a culture of HIV must be handled at CL2, the inactivation must take place at CL2 or higher). Inactivation methods can include heat or chemicals. The ability of a nucleic acid extraction method to inactivate a pathogen must be validated and verified in house.

Specimens and cultures that have been inactivated using a validated method (e.g., autoclaved waste, heat-treated proteins) are not expected to be pathogenic, and are therefore not regulated by the PHAC.

3.3.2 Primary specimens: These include specimens collected directly from patients or animals for the purpose of detecting or monitoring an infection (e.g., blood, plasma, semen and tissues containing blood). Diagnostic specimens from naturally exposed animals (i.e., **not** resulting from in vivo studies) are also included in this category. Primary specimens will generally contain much

lower concentrations of pathogen than found in cultures. Primary specimens containing a human pathogen are excluded from the HPTA and do not require a pathogen and toxin licence issued by the PHAC unless the pathogen has been cultivated or intentionally collected or extracted; however, the importation of primary specimens containing an animal or zoonotic pathogen does require an animal pathogen import permit issued by the CFIA.

3.3.3 Propagated or concentrated pathogen: These include any sample where the pathogen has been propagated by culturing, including stock cultures of clinical isolates or pathogen reference strains, as well as cultures for diagnostic purposes, all of which will result in an increase in the amount of pathogen. It also includes pathogen that is concentrated in any way (e.g., by centrifugation, filtration, chromatography). Propagated and concentrated pathogens are under the authority of the HPTA, and require a pathogen and toxin licence issued by the PHAC. Also, the importation of cultures of animal pathogens is under the authority of the HAA and HAR, and regulated by the PHAC or the CFIA.

3.4 Activities

In addition to specimen and culture type, specific laboratory procedures can also influence the risks associated with laboratory activities. The following definitions and examples of laboratory activities are also intended to help classify new activities.

3.4.1 Activities with inactivated biological material: The killing or inactivation process renders the sample essentially free of the pathogen such that it is unlikely to be infectious. Inactivation using an effective, validated and routinely verified method must be performed either at the containment level of the specimen or culture (as indicated in Table 3), or before opening the culture tube (i.e., if the culture was performed in a sealed vessel at CL2 with additional biosafety requirements). Examples of these activities include, but are not limited to: antigen assay, reverse transcriptase assay, and nucleic acid extraction, provided that the method has been validated to inactivate the pathogen.

3.4.2 Non-propagative clinical/diagnostic activities with primary specimens: These include activities with primary, patient-derived specimens that have not been cultured (propagated), and that are performed for the purpose of diagnosing or monitoring an infection. Only activities that aim to propagate, concentrate, or purify pathogens (i.e., in this case HIV, HTLV, or other retroviruses covered by this directive) from primary specimens are regulated by the PHAC; therefore, diagnostic activities that do not increase the number or concentration of the pathogen are excluded from this directive. Nonetheless, it is strongly recommended that, at minimum, routine practices are followed in work areas, especially in health care environments, where primary specimens are handled to protect against the potential of exposure to any pathogen that may be present in the specimen.[52]

Examples of diagnostic activities include, but are not limited to: enzyme linked immunosorbent assay (ELISA); centrifugation of primary specimens (e.g., to separate plasma, not to pellet virus), inoculation of sensitive cells for culture, and nucleic acid extraction or nucleic acid amplification test (NAAT).

3.4.3 Propagative in vitro activities: Propagating pathogens increases the concentration and number of organisms, thereby greatly increasing the infectiousness of the sample. As described in Section 3.3.3, these activities are regulated by the PHAC.

Examples of these include, but are not limited to: culture, cultivation or propagation of specimens likely to contain a particular pathogen, processing of positive cultures for packaging and distribution to laboratories, and research activities involving culture of the pathogen.

The following table describes the containment level required for containment zones working with HIV, HTLV or related simian retroviruses based on sample type and activity. HIV and HTLV are categorized as RG3 human pathogens but work with these pathogens can be carried out safely at CL2 with additional biosafety requirements. See Section 4 of this directive for the additional biosafety requirements that are to be followed when working at CL2, and Section 5 of this directive for the additional biosafety considerations for working with HIV, HTLV, and related simian retroviruses. For a complete listing of the physical containment requirements, operational practice requirements, and performance and verification testing requirements, refer to Chapters 3, 4, and 5 of the CBS.

Table 3: Containment Levels for Activities with HIV, HTLV, and Related Simian Retroviruses

Sample Type and Activity	Minimum Containment Level Required
Non-propagative clinical/diagnostic activities Examples of these activities include, but are not limited to: • preparing diagnostic specimens with the goal of concentrating or isolating HIV, HTLV, or a related simian retrovirus (e.g., concentration or centrifugation of sample).	CL2[a]
Propagative *in vitro* activities Examples of these activities include, but are not limited to: • culturing of specimens that contain or are likely to contain the pathogen; • *in vitro* research with the pathogen; • preparatory work for *in vivo* activities; and • processing positive cultures for packaging and distribution to other facilities.	CL2[a]
***In vivo* work activities** Examples of these activities include, but are not limited to: • preparing inoculum; • inoculating animals; and • collecting specimens from experimentally-infected animals (e.g., blood, cerebrospinal fluid).	CL2[a,b]

a With additional biosafety requirements as described in Section 4.0.

b Work in SA zones must meet the requirements in the CL2 column of the CBS and work in LA zones must meet the requirements in the CL2-Ag column of the CBS.

4.0 Additional Biosafety Requirements

In addition to the requirements listed for CL2 specified in Chapters 3, 4, and 5 of the CBS, the requirements below (designated 'CBS R' followed by the requirement number) are to be followed for activities listed in Table 3 as requiring CL2 with additional biosafety requirements (CL2[a]). The following requirements apply to all personnel entering the containment zone.

4.1 Additional Operational Requirement for all Activities (CL2[a,b])

- Centrifugation of infectious material to be carried out in sealed safety cups (or rotors) that are unloaded in a biological safety cabinet (BSC) (CBS R4.6.29). Sealed safety cups and rotors that are unloaded in a BSC prevent the release of infectious aerosols and protect individuals from exposure.

4.2 Additional Operational Requirements for Propagative *in vitro* Activities (CL2[a])

- All activities involving open vessels of infectious material to be performed in a certified BSC or other appropriate primary containment device (CBS R4.6.25). BSCs provide effective primary containment while simultaneously providing personnel and environmental protection from infectious aerosols; and

- An additional layer of protective clothing to be donned in accordance with entry procedures prior to work with infectious material (CBS R4.4.7). An additional layer of protective clothing (e.g., solid-front gowns with tight-fitting wrists, second pair of gloves, waterproof aprons or head covers) protects personnel from exposure by providing an additional layer of protection in the event that the outer layer of protective clothing is compromised or contaminated.

4.3 Additional Operational Requirement for *in vivo* Activities (CL2[a,b])

- An additional layer of protective clothing to be donned in accordance with entry procedures prior to work with animals infected with HIV, HTLV, or related simian retrovirus (CBS R4.4.7).

5.0 Biosafety Considerations

The importance of local risk assessments (LRAs) in the implementation of requirements is discussed in the CBS. Many of the requirements in the CBS are risk- and performance-based and as such, are dependent on the LRA performed. Based on the risks associated with work involving HIV, HTLV, and related simian retroviruses, the following list of CBS requirements are being highlighted to assist with the development of LRAs and standard operating procedures (SOPs). Although these requirements or parts thereof are listed as CL2/CL2-Ag operational requirements in Chapter 4 of the CBS, they are critical for the safe handling of HIV, HTLV, and related simian retroviruses.

- Good microbiological laboratory practices to be employed (CBS R4.6.18). Good microbiological laboratory practices (e.g., including the use of personal protective equipment [PPE], handwashing, disinfecting work areas, use of procedures that minimize the creation of aerosols, and proper decontamination and disposal of materials) protect containment zone personnel from exposure by reducing the risk of cross-contamination and the spread of contamination;

- Use of needles, syringes, and other sharp objects to be strictly limited and avoided when suitable alternatives are available (CBS R4.6.9);

- A certified BSC to be used for procedures involving open vessels of infectious material that may produce infectious aerosols when aerosol generation cannot be contained through other methods, or that involve high concentrations or large volumes of infectious material (CBS R4.6.24);

- Face protection to be used where there is a risk of exposure to splashes or flying objects (CBS R4.4.2);

- Open wounds, cuts, scratches, and grazes to be covered with waterproof dressings (CBS R4.6.6);

- Procedures, as determined by an LRA, to be in place to prevent a leak, drop, spill, or similar event, during the movement of infectious material within the containment zone, or between containment zones within a building (CBS R4.6.31);

- A medical surveillance program, based on an overarching risk assessment and LRAs, to be developed, implemented, and kept up to date (CBS R4.1.12). The surveillance plan should include a protocol for the evaluation of potentially exposed personnel to receive post exposure prophylactic treatment; and

- Disinfectants effective against the pathogens in use to be available and used in the containment zone (CBS R4.8.2).

6.0 Contact and additional information

This directive is based on currently available scientific evidence and is subject to review and change as new information becomes available. If the directive is amended, the PHAC will communicate the updated information to the impacted regulated parties and distribute the amended directive. For more information on this directive, please contact:

Public Health Agency of Canada
Centre for Biosecurity
Biosafety Standards and Guidelines Program
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Canadian Biosafety Standards and Guidelines website: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines.html

PHAC Pathogen Safety Data Sheets (PSDS): https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html

7.0 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.

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 comprised of dedicated laboratory areas and separate animal rooms/ 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 practice	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.
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 organisms (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.
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 or 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 and/or animal host
Pathogen risk assessment	The determination of the risk group and appropriate physical containment 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 infectious material or toxins, as outlined in Chapter 3 of the CBS.
Primary containment	The first level of physical barriers designed to contain pathogens and toxins and prevent their release. This is accomplished by the provision of a device, equipment, or other physical structure situated between the infectious material or toxins and the individual, the work environment, or other areas within the containment zone. Examples include biological safety cabinets, glove boxes, and animal microisolators. In animal cubicles, the room itself provides primary containment, and personal protective equipment serves as primary protection against exposure.

Primary Containment caging	Animal caging serving as a primary containment device to prevent the release of infectious material and toxins. Examples include ventilated filter-top cages and ventilated micro-isolator cage rack system, with or without high efficiency particulate air (HEPA) filters.
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.
Propagation	The act of multiplying pathogens under controlled laboratory conditions.
Risk group (RG)	The classification of biological material based on its inherent characteristics, including pathogenicity, 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. The definitions of RG2-4 human pathogens are specified in Section 3 of the HPTA.
Small animal containment zone (SA zone)	Animal containment zone comprised of one or several co-located or adjoining rooms of equal containment level where animals are housed in animal rooms inside primary containment caging (e.g., microisolators). An SA zone may contain, for example, mice, rats, rabbits, ferrets or nonhuman primates, provided that they are housed in primary containment caging.
Virulence	The degree or severity of a disease caused by a pathogen.

8.0 References and resources

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