Biosafety Directive
for
New and Emerging Influenza A Viruses

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Abbreviations and acronyms

BSC	Biological safety cabinet
CBS	*Canadian Biosafety Standard*
CBH	*Canadian Biosafety Handbook*
CFIA	Canadian Food Inspection Agency
CL	Containment Level (i.e., CL2, CL3, CL4)
CL2-Ag	CL2 large animal containment zone
HAA	*Health of Animals Act*
HAR	*Health of Animals Regulations*
HPAI	Highly pathogenic avian influenza
HPTA	*Human Pathogens and Toxins Act*
HPTR	*Human Pathogens and Toxins Regulations*
LPAI	Low pathogenic avian influenza
LRA	Local risk assessment
PHAC	Public Health Agency of Canada
RG	Risk Group (i.e., RG1, RG2, RG3, RG4)
SSBA	Security sensitive biological agents
WHO	World Health Organization

1.0 Background

The words in **bold type** are defined in the glossary found in Section 6.

In Canada, facilities that **handle or store Risk Group** 2 (RG2), RG3, and RG4 human **pathogens** or toxins are regulated by the Public Health Agency of Canada (PHAC) under the Human Pathogens and Toxins Act (HPTA) and the 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 a part of one (e.g., toxin) 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).

An Influenza A virus that is a **new or emerging pathogen** is often classified as an RG3 human pathogen by the PHAC and RG3 animal pathogen by the CFIA. This Biosafety Directive has been developed by the PHAC and the CFIA to assist facilities in determining the appropriate **containment level** and additional **biosafety** requirements for the safe handling of samples that are suspected of or confirmed to contain a new or emerging strain of influenza A virus. Facilities such as diagnostic and research laboratories may use this Directive to implement the appropriate physical requirements and operational practices when handling samples containing new or emerging subtypes or strains of influenza A virus. General information on influenza A virus, including **circulating strains of influenza virus** (i.e., not new or emerging), is also included. The Biosafety Directive for New and Emerging Influenza A Viruses is to be used in conjunction with the Canadian Biosafety Standard (CBS).[1]

2.0 Pathogen description and risk group

Influenza viruses are part of the *Orthomyxoviridae* family, with a genome comprised of segmented, negative sense, single-stranded RNA.[2] The four influenza virus types are A, B, C and D. Type C is endemic worldwide and usually associated with mild infections.[3,4,5] Type D was recently identified in animal populations, with no known transmission to humans.[6] Variants of influenza virus types A and B circulate among the Canadian population and cause seasonal influenza, which is among the leading causes of acute respiratory infections in humans.[7] Annually, there are an estimated 12,200 flu-related hospitalizations and 3,500 deaths in Canada.[8,9,10] While influenza virus type B has been the cause of epidemics, it has not caused pandemics.[11] Type A is considered to be the influenza virus type with the greatest impact on public health as it has caused more than a dozen documented pandemics since the 1700s, with the latest occurring in 2009.[5]

2.1 Description of influenza A viruses

Influenza A viruses are classified into subtypes based upon the combination of surface glycoproteins haemagglutinin (H1 to H16) and neuraminidase (N1 to N9).[12] The haemagglutinin protein binds to receptors that are on the surface of epithelial cells in the upper respiratory tract and on the surface of erythrocytes, and is one of the factors that determines whether a host is susceptible to infection or not. Neuraminidase is an enzyme that cleaves bound virus from the host cells to permit further distribution.

Influenza A viruses are endemic throughout the world, and can infect and cause mild to severe disease to humans and a wide range of animals, including swine and birds.[2,13] Swine influenza A viruses regularly cause

respiratory disease outbreaks in pigs and, while severe illness may occur, death is infrequent.[14] Avian influenza A viruses often exists as harmless (i.e., non-pathogenic) residents in the gut of their natural hosts, which are waterfowl and shorebirds, but can infect poultry and other bird species.[2]

Infection of poultry with avian influenza A viruses can result in mild to severe disease, depending upon the genetic make-up of the virus. Therefore, in addition to haemagglutinin and neuraminidase classification, avian influenza A viruses are broadly divided according to their **pathogenicity** in poultry. Transmission of avian influenza A viruses to poultry generally results in a mild disease, in which case the strain is classified as low pathogenic avian influenza (LPAI). LPAI can evolve or mutate into a highly pathogenic avian influenza (HPAI) form that typically causes severe disease with high mortality rates in poultry.[15] An example of this is H5N1, of which there are both LPAI and HPAI forms. To date, HPAI have only been identified within subtypes H5 and H7, which have been the focus of influenza A virus surveillance in poultry worldwide (e.g., H5N1, H5N2, H5N8, and H7N9,).[16,17,18,19,20]

Transmission of influenza A viruses is predominantly through inhalation of airborne particles into the respiratory tract, while contact transmission through mucous membranes can also lead to infection.[21] Human-to-human transmission of human influenza A virus strains can occur by aerosols or droplets, as well as by direct or indirect contact with contaminated surfaces.[2] Zoonotic transmission of influenza A virus is infrequent but can occur through contact of mucous membranes with infectious secretions, excretions, and tissues when handling infected animals or ingesting undercooked infected poultry.[22] There is limited evidence to support human-to-human transmission of influenza A viruses following direct animal-to-human transmission.

2.1.1 New and emerging iInfluenza A viruses

A strain of influenza A virus is deemed to be new or emerging if it meets one of the following criteria:[23,24]

- It is a novel pathogen for human or animal hosts, from natural or engineered origins.
- It is an existing pathogen that has been introduced (or reintroduced) into a new host population with no or low immunity.
- It is an existing pathogen whose incidence is increasing in the host population as a result of uncharacterized changes to the pathogen.

In Canada and around the world, new and emerging influenza A viruses that can infect both humans and animals are frequently identified. Adaptation and the emergence of new strains of influenza A virus are caused by antigenic drift and antigenic shift, which frequently affect the haemagglutinin and neuraminidase surface antigens. Antigenic drift occurs when small changes in the virus's ribonucleic acid (RNA) genome happen over time, eventually resulting in new strains that are antigenically different from existing strains.[2,26Error! Bookmark not defined.] This is common in seasonal circulating strains, which may undergo some gene variation that does not significantly alter the pathogenicity in humans. Antigenic shift is a rarer type of change that can produce a completely new strain as a result of genetic reassortment or novel zoonotic transmission to humans from an animal source.[26Error! Bookmark not defined.] A new strain, especially one created through antigenic shift, has the potential of introducing a glycoprotein combination that has not been previously observed in existing strains. This can lead to sudden changes in pathogenicity and unexpected outbreaks in the community.

Genetic manipulation in the laboratory can also alter the host range, pathogenicity, and antigenic profile of the virus. Strains can be artificially created through reverse genetics, a method by which plasmids containing

all of the required influenza genes (from circulating, pandemic, or vaccine strains) are used to transfect a permissive cell and generate a biologically active influenza virus, including reassortant viruses.[25] Influenza A viruses generated by reverse genetics are important tools for understanding the biology of the virus and factors that determine host range, inter-species transmission, and mechanisms for infection.[26] This method is especially common for developing live attenuated vaccines. Although reverse genetic methods generally reduce **virulence** by creating mutations in the viral genes, activities must be performed with caution as mutations occurring in reassortant strains can potentially revert or increase virulence.[25]

New and emerging influenza A viruses have an increased potential of causing pandemics as there is often little or no population immunity against a novel viral structure. An example of this was the 2009 pandemic outbreak of H1N1 (influenza A(H1N1)pdm2009), a virus of swine origin that was transmissible to the human population.[2,25]

Due to the unpredictable emergence of new strains, there is generally a delay between the detection of a new or emerging influenza A virus and the communication of appropriate biosafety guidance. Often, guidance is required for facilities that handle these pathogens before they have been well-characterized and their specific risks identified.

2.2 Risk group for influenza A viruses

A **pathogen risk assessment** evaluates the inherent characteristics of a pathogen in order to assign a risk group. The categories range from RG1 (low individual and low community risk) to RG4 (high individual and high community risk). The risk factors evaluated include:

- pathogenicity;
- communicability;
- availability of effective pre- or post-exposure measures (e.g., vaccines, antivirals), including estimates of immunity in the current population (based on time elapsed since an antigenically similar strain last circulated in the community); and
- impact on the animal population, including host range and endemicity.

Circulating strains of influenza A virus (excluding the 1918 H1N1 strain, subtype H2N2, and HPAI subtypes) have been classified by the PHAC as RG2 human pathogens.

The risk group assignment for over 40 strains of influenza A viruses can be found in the PHAC's ePATHogen Risk Group Database.[27]

New or emerging strains may have increased pathogenicity and virulence in humans and animals. They may also evade existing population immunity and current vaccines or treatments. This has been observed in strains implicated in recent human and avian outbreaks and pandemics, such as HPAI subtypes H7N9, H5N1, H5N2, and H5N8.[16,17,19,28] **Such new or emerging strains are often classified as RG3 human pathogens or RG3 animal pathogens, or both.**

HPAI strains (notably, from subtypes H5 and H7) that have not undergone evaluations for virulence or attenuation are considered by the PHAC to be **security sensitive biological agents (SSBAs)** as there is a greater biosecurity risk associated with these strains due to their potential for use as biological weapons. As such, they

are subject to increased biosecurity requirements, as specified in the CBS and HPTR. Contact the PHAC directly for requirements and guidance prior to conducting activities involving SSBAs.

H5 and H7 LPAI subtypes, and all HPAI subtypes are considered **non-indigenous animal pathogens**. Under the Health of Animals Act and the Reportable Diseases Regulations, non-indigenous animal pathogens are reportable to the CFIA. Contact the CFIA directly for requirements and guidance prior to conducting activities involving non-indigenous animal pathogens.

2.2.1 Biotechnology and vaccine development

Genetic manipulation of a pathogen can alter the host range, pathogenicity, and antigenic profile.[29] A pathogen risk assessment takes into consideration whether the virus has been genetically modified (e.g., insertions, deletions, or point mutations), and, if the goal is to create a reassortant virus, the source strains of the contributing elements. The World Health Organization's (WHO's) Technical Report Series No. 941, Annex 5, WHO biosafety risk assessment and guidelines for the production and quality control of human influenza pandemic vaccines, outlines the potential hazards of working with reassortant viruses, as well as a risk assessment approach to genetic modification or creation of influenza virus, and vaccine production activities.[30] These guidelines also specify a number of tests necessary for evaluating the safety of potential influenza candidate vaccine viruses prior to release for vaccine production, which include:[31]

- sequencing to confirm identity and verify the presence of attenuating and other phenotypic markers;
- pathogenicity in chickens;
- testing for attenuation in ferrets; and
- genetic stability testing.

2.3 Factors to consider in a local risk assessment

Based on the specific activity being conducted, there are potential hazards that may exist for work involving influenza A viruses. When performing a **local risk assessment** (LRA), consideration should be given to the factors influencing the risks associated with pathogen handling and storage, including:

- Potential for aerosol generation:

  Some influenza A viruses remain viable in aerosols for up to 24 hours, presenting a risk for infection following aerosol-generating activities.[32] Similarly some influenza viruses can survive on surfaces for several hours.[32,33,34] When airborne particles generated during work activities settle on a surface, and inadequate decontamination is performed, infection may result from contact with the contaminated surface.

- Concentration and quantity of pathogen handled:

  The risk of infection may be increased during work involving **in vitro** or **in vivo propagation** of the virus, wherein the concentration or quantity of pathogen is increased.

- In vivo work:

  Working with animals can increase the risk of laboratory acquired infections (LAIs) from exposure of mucous membranes with contaminated material when handling tissues or secretions from infected animals.

- Selected species for in vivo work:

  Some animal models have increased susceptibility to reassortment, which can increase the possibility of creating a new strain with pandemic potential. The receptors expressed on epithelial cells in swine respiratory tracts include the preferred receptors for both human and avian influenza A viruses.[35] As such, swine are susceptible to influenza A viruses that are not only endemic in swine, but also those from human and avian origin, and so may act as a mixing vessel with increased reassortment potential. In fact, reassortant swine influenza A viruses carrying swine, human, and avian influenza genes have been identified.[36] Similarly, transgenic animals may have altered susceptibility to influenza A viruses originating from different species, thereby increasing the chance of strain reassortment.

- Other strains of Influenza A virus handled and stored within the facility:

  The presence of multiple strains and sources of influenza A viruses increases the risk of accidental reassortment if they are handled in temporal and physical proximity.

- Immune status of laboratory personnel:

  Personnel may be partially or fully protected from infection if they have been infected by or vaccinated against seasonal influenza. Antibody titres will provide an indication of immune status; however, immunity against seasonal influenza may not confer effective protection against a new or emerging strain.[37]

- Handling engineered reassortant influenza viruses:[32]

  Verifying clonal purity and phenotypic stability will confirm that only the strain of interest is present and that it is unlikely to recombine or revert to a more pathogenic strain during experimental procedures.

3.0 Containment level overview

The CBS specifies the minimum **physical containment requirements**, **operational practice requirements**, and performance and verification testing requirements for the handling and storing of human and terrestrial animal pathogens and toxins. The CBS is used by the PHAC and the CFIA to verify the ongoing compliance of facilities regulated under the HPTA, HPTR, HAA, and HAR. Containment levels range from a basic laboratory (Containment Level 1 [CL1]) to the highest level of **containment** (CL4). Full descriptions of containment levels can be found in Chapter 2 of the CBS.

3.1 Containment level assessment for influenza A viruses

Circulating strains of influenza A viruses (excluding the 1918 H1N1 strain, subtype H2N2, and HPAI subtypes) have been classified by the PHAC as RG2 human and animal pathogens and can be handled at CL2. Diagnostic activities for influenza will generally be performed at CL2 unless there is reason to believe a new or emerging strain may be present (e.g., reports, prior diagnosis). **Once evidence supports the presence of a new or emerging strain (e.g., such a virus has been identified within the facility or elsewhere), this Directive is to be followed.**

Inadequately characterized new or emerging strains of influenza A virus, including any HPAI subtype, are often initially classified as RG3 human pathogens or RG3 animal pathogens. Due to the potential increased risk of these strains, they must be handled at CL3 for most activities until the available information indicates that they can be safely handled at a lower containment level. Section 4.0 describes the circumstances where CL2 may be acceptable, particularly for diagnostic activities. The containment level recommendations determined by the PHAC are aligned with those of various national and international agencies (Appendix A). [38,39,40,41,42,43]

3.2 Sample types

The different types of specimens and cultures in facilities where influenza A viruses are handled and stored may result in different risks and are therefore taken into account during containment assessment. For the purpose of this Directive, specimens have each been classified into the three broad categories described below:

3.2.1 Inactivated biological material:

Any material that has been inactivated using a validated and routinely verified method, including inactivated cultures and products concentrated from primary specimens (e.g., pellets, concentrated virus). The inactivation must be performed at the containment level required for the pathogen or type of specimen (e.g., if a culture must be handled at CL3, the inactivation must take place at CL3). Inactivation methods can include heat, chemicals, and irradiation. The ability of a nucleic acid extraction method to inactivate a pathogen must also be validated and verified in house.

Specimens and cultures that have been inactivated using a validated and routinely verified method (e.g., autoclaved waste, lysed cultures, heat treated proteins) are not regulated by the PHAC or the CFIA.

3.2.2 Primary specimens:

These are specimens collected directly from individuals or animals, generally for diagnostic, monitoring, or research purposes (e.g., nasopharyngeal swab, nasal swab, nasal wash and aspirate, endotracheal aspirate, bronchoalveolar lavage fluid). Quality assurance and proficiency panel specimens that mimic primary specimens are also included.

Primary specimens generally contain much lower concentrations of pathogen than cultures. Primary specimens containing a human pathogen (i.e., a pathogen in its natural environment) are excluded from the HPTA, and work with these specimens does not require a Pathogen and Toxin Licence issued by the PHAC, though the CBS and CBH can be consulted to determine best practices. However, the importation of primary specimens

containing an animal pathogen or a zoonotic pathogen does require an animal pathogen import permit issued by the CFIA.

3.2.3 Propagated or concentrated pathogens:

These include any sample where a pathogen has been propagated by culturing, including stock cultures of clinical isolates or pathogen reference strains, as well as diagnostic cultures that are likely increasing the amount of pathogen (e.g., culture in embryonated eggs). It also includes pathogens concentrated by various procedures (e.g., by centrifugation, filtration, chromatography). Propagated and concentrated RG2, RG3 and RG4 human pathogens fall under the HPTA, and work with these pathogens requires a Pathogen and Toxin Licence issued by the PHAC, unless otherwise exempt. The importation of cultures of animal pathogens falls under the HAA and HAR, and is regulated by the PHAC or the CFIA.

3.3 Activity types

In addition to sample type, specific laboratory procedures can influence risk and are also taken into account during containment assessments. The following definitions provide examples of laboratory activities and may help classify new activities as they become available.

3.3.1 Handling inactivated biological material:

These include activities with material where pathogens were previously inactivated by a validated method. Examples include antigen assays, reverse transcriptase assays, and nucleic acid extraction

3.3.2 Non-propagative clinical/diagnostic activities with primary specimens:

These include activities with primary, patient-derived specimens and specimens that mimic primary specimens (e.g., quality assurance specimens) performed for the purpose of diagnosing or monitoring an infection. Examples of diagnostic activities include antibody and antigen testing, nucleic acid testing, and rapid molecular assays that do not involve pelleting bacteria or live virus.[44] Other activities may include centrifugation of primary specimens (e.g., to separate plasma, not to pellet a pathogen), and nucleic acid extraction or nucleic acid amplification.

Diagnostic activities with primary specimens that propagate, concentrate, or purify pathogens are regulated by the PHAC under the HPTA, as they result in the pathogen no longer being in its natural environment (i.e., it is no longer a primary specimen), whereas diagnostic activities with primary specimens that do not increase the number or concentration of the pathogen are excluded from this Directive and the HPTA. Nonetheless, it is strongly recommended that diagnostic specimens are handled in a facility that meets the requirements for CL2 specified in the CBS to prevent exposure to any pathogens that may be present in the specimen.[1]

3.3.3 Propagative in vitro activities:

Propagating pathogens increases the concentration and number of organisms, thereby greatly increasing the infectivity of the sample. Propagative in vitro activities with RG2, RG3 and RG4 pathogens and toxins are regulated by the PHAC unless exempted [HPTR 27(1)]. Examples include the culture of influenza A virus in

embryonated chicken eggs or in tissue culture (e.g., mammalian cell culture), rapid cell culture, and processing of positive cultures for packaging and distribution to other laboratories.

3.4 Genetically engineered strains

Activities with engineered strains that have not undergone safety and evaluation tests are to be conducted at the highest containment level required for either of the donor strains. For example, if the engineered virus is a result of portions from donors classified as RG2, the resultant virus can be handled at CL2. If, however, one portion is from an RG3 donor strain and another from an RG2 donor strain, the resultant strain is to be handled at CL3. Activities with untested recombinant strains with HPAI virus as a donor strain must be conducted at CL3.

4.0 Biosafety requirements for new and emerging influenza A viruses

4.1 Requirements for all sample types and activities

To prevent the unintended generation or release of a new or emerging influenza A virus, laboratories must comply with the following requirements:

- To prevent accidental reassortment, manipulations involving propagation (i.e., culture) of new or emerging influenza viruses are not to be performed simultaneously in a laboratory space where material that may contain other influenza strains (human or animal) is occurring. Separation of such work can be spatial (e.g., different **biological safety cabinet** [BSC] or room) or temporal (e.g., scheduling).

- In the event that a human diagnostic laboratory detects a non-negative sample (i.e., tests positive for the new or emerging influenza A virus or an unknown strain), all work with the sample is to be stopped. This sample is to be transferred to a **containment zone** of the appropriate containment level (e.g., CL3 or the National Microbiology Laboratory [NML]) for confirmatory testing and any further manipulations. Contact information for the NML Viral Diseases section can be found at https://cnphi.canada.ca/gts/laboratory/1013.

- In the event that a veterinary diagnostic laboratory detects a non-negative sample, all work with the sample is to be stopped and the sample transferred to the National Centre for Foreign Animal Disease (NCFAD).[45]

4.2 Containment requirements for new and emerging influenza A viruses

New and emerging influenza A viruses that have not been fully characterized are usually classified as RG3 human and animal pathogens in Canada. They must be handled in a CL3 facility that meets the minimum requirements specified in Chapters 3, 4, and 5 of the CBS until updated information supports that sufficient safety can be provided at a lower containment level. However, based on the sample type and the activity being performed, new or emerging influenza A viruses may be handled in a lower containment level if additional biosafety requirements are respected. As described in Table 1, diagnostic activities with the intent

to concentrate or isolate the virus can be safely conducted at CL2 with additional biosafety practices; these practices are aligned with international approaches (Appendix A).[38,39,40,41,42,43] The additional biosafety requirements to be followed when working at CL2 are described in Section 4.3 of this Directive.

Table 1 – Canadian containment level requirements for RG3 new and emerging influenza A viruses

Sample type and activity	Minimum containment level	
	CL2	CL3
Non-propagative clinical/diagnostic activities with primary samples containing wild type strains Examples of these activities include: • preparing human or animal diagnostic specimens with the goal of concentrating or isolating influenza A virus (e.g., concentration of virus by filtration or centrifugation of sample).	■[a]	
Propagative *in vitro* activities with wild type strains Examples of these activities could occur in diagnostic or research facilities, and include: • intentional propagation of the virus (e.g., virus tissue culture); • culture of specimens likely to contain the virus (e.g., egg culture, cell/tissue culture); • preparatory work for *in vivo* activities; and, • processing positive cultures for packaging and distribution to other laboratories or facilities.		■ SSBA
***In vivo* work activities with wild type occurring strains** Examples of these activities include: • preparing inoculum; • inoculating animals; and • collecting specimens from experimentally infected animals (e.g., nasal/throat swab, blood, bronchial lavage).		■[c], SSBA
Genetically engineered strains Propagative *in vitro* and *in vivo* work activities with genetically engineered strains that have undergone testing according to the WHO's guidelines for safety testing.[31]	■[a,b]	
Genetically engineered strains, untested for safety Propagative *in vitro* and *in vivo* work activities with genetically engineered strains that have not undergone testing according to the WHO's guidelines for safety testing (including HPAI strains).[31]		■[c], SSBA

[a] With additional operational requirements as described in Section 4.3.

[b] Work in **small animal containment zones** (SA zones) must meet the requirements in the "CL2" column of the CBS matrices and work in **large animal containment zones** (LA zones) must meet the requirements in the "CL2-Ag" column of the CBS matrices.

[c] Work in SA zones must meet the requirements in the "CL3" column of the CBS matrices and work in LA zones must meet the requirements in the "CL3-Ag" column of the CBS matrices.

SSBA All HPAI strains are considered SSBAs, and the handling or storing of these strains must meet the SSBA requirements specified in the CBS, unless confirmed otherwise by the PHAC.

N/A Not applicable

4.3 Additional operational requirements

Certain activities with new and emerging influenza A viruses can be safely performed at CL2 with additional biosafety requirements. These requirements are to be followed in addition to the applicable minimum physical containment requirements, operational practice requirements, and performance and verification testing requirements for CL2 specified in Chapters 3, 4, and 5 of the CBS, respectively. The requirements below (designated as 'CBS R' followed by the requirement number) are to be followed for activities listed in Table 1 as requiring CL2 with additional biosafety requirements (i.e., activities denoted with the letter a). The following requirements apply to all personnel entering the containment zone.

- CBS R4.6.25 All activities involving open vessels of infectious material to be performed in a certified BSC or other appropriate **primary containment device** [Not required when collecting samples from or inoculating animals housed in an **animal cubicle**]. This operational practice is required as manipulations of the infectious material have the potential to generate infectious aerosols. BSCs provide effective **primary containment** while simultaneously providing personnel and environmental protection from infectious aerosols.

- CBS R4.4.9 Respirators to be worn where there is a risk of exposure to infectious aerosols that can be transmitted through the inhalation route, as determined by an LRA. Respirators protect personnel from airborne pathogens or infectious aerosols that are not contained in a primary containment device (e.g., a certified BSC or high efficiency particulate air [HEPA]-filtered cage).

- CBS R4.6.29 Centrifugation of infectious material to be carried out in sealed safety cups (or rotors) that are unloaded in a BSC. Sealed safety cups (or rotors) for centrifugation prevents the release of infectious aerosols. The sealed safety cups (or rotors) are unloaded in a BSC to protect individuals from exposure to any aerosolized material and prevent the spread of contamination.

- CBS R4.4.7 An additional layer of protective clothing to be donned in accordance with entry procedures prior to work with infectious material or animals infected with zoonotic pathogens. An additional layer of protective clothing (e.g., solid-front gowns with tight-fitting wrists, waterproof aprons, 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.

- CBS R4.5.12 Personal belongings not required for work to be left outside the containment zone or in change areas outside the containment zone. This operational practice protects individuals from exposure by preventing the contamination of these items and the spread of contamination outside of the containment barrier.

- Vaccination against seasonal influenza, or administration of an approved commercial vaccine for a new or emerging strain (if available), based on risk assessment and medical surveillance program, to be included in organization policy. The National Advisory Committee on Immunization (NACI)'s annual Statement on Seasonal Influenza Vaccine recommends vaccination unless there is a medical contraindication.[37]

5.0 Contact and additional information

Please note that 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 post the amended Directive on the Government of Canada Website. For more information on this Directive or for further biosafety information, please contact:

Public Health Agency of Canada
Centre for Biosecurity
Email: PHAC.pathogens-pathogenes.ASPC@canada.ca
On the web: https://www.canada.ca/en/services/health/biosafety-biosecurity.html

Canadian Food Inspection Agency
Office of Biohazard Containment and Safety
Email: cfia.biocontainment-bioconfinement.acia@canada.ca
On the web: www.inspection.gc.ca/english/sci/bio/bioe.shtml

6.0 Glossary

Most of the following list is derived from the CBS and the Canadian Biosafety Handbook (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. Some definitions may therefore 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., no primary containment caging).
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.
Circulating strains of influenza virus	Influenza viruses that are adapted to humans and cause seasonal outbreaks. Strains are based on international surveillance review by the World Health Organization (WHO) in consultation with directors of the WHO Collaborating Centers to determine optimal vaccine composition.
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. These are four containment levels ranging from a basic laboratory (Containment Level 1 [CL1]) to the highest level of containment (Containment Level 4 [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., Containment Level 2 [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., Containment Level 3 [CL3] suite comprised of 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.

Handle or store	"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 Human Pathogens and Toxins Act.
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.
New or emerging pathogen	A pathogen that is either: a novel pathogen for human or animal hosts; an existing pathogen introduced (or reintroduced) into a new host population with no or low immunity; or an existing pathogen whose incidence is increasing in the host population as a result of changes to the pathogen.
Non-indigenous animal pathogen	A pathogen that causes an animal disease listed in the World Organisation 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). These pathogens may have serious negative health effects to the Canadian animal population.
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 Canadian Biosafety Standard.
Pathogen(s)	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 Human Pathogens and Toxins Act, but these are not exhaustive lists.

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.
Pathogenicity	The ability of a pathogen to cause disease in a human and/or animal host.
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 BSC, glove boxes, and animal microisolators. In animal cubicles, the room itself provides primary containment, and PPE serves as primary protection against exposure.
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 BSC, 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.
Security sensitive biological agents (SSBAs)	The subset of human pathogens and toxins that have been determined to pose an increased biosecurity risk due to their potential for use as a biological weapon. SSBAs are identified as prescribed human pathogens and toxins by Section 10 of the Human Pathogens and Toxins Regulations. This means all Risk Group 3 and Risk Group 4 human pathogens that are in the List of Human and Animal Pathogens for Export Control, published by the Australia Group, as amended from time to time, with the exception of Duvenhage virus, Rabies virus and all other members of the Lyssavirus genus, Vesicular stomatitis virus, and Lymphocytic choriomeningitis virus; as well as all toxins listed in Schedule 1 of the Human Pathogens and Toxins Act that are listed on the List of Human and Animal Pathogens for Export Control when in a quantity greater than that specified in Section 10(2) of the Human Pathogens and Toxins Regulations.

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.

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Appendix A: International containment level requirements

Table A1- International containment level requirements for influenza A viruses[38,39,40,41,42,43]

Influenza strain	Containment Level	Agency
Circulating seasonal influenza A strains	CL2	CDC; ECDC; HSE
LPAI	CL2	CDC; ECDC; WHO
HPAI (i.e., H5, H7)	CL3	CDC; ECDC; HSE; NIH; WHO
LPAI with human transmission	CL3	HSE
Pandemic potential (i.e., H1, H2, H3) Non-circulating strains of H2N2	CL3	CDC; HSE
HPAI H5N1 strains from A/goose/Guangdong/1/96 lineage	CL4 CL3-Ag for large animal (LA) zone	CDC

CDC – United States Centers for Disease Control and Prevention; ECDC – European Centre for Disease Prevention and Control; HSE – United Kingdom Health & Safety Executive; NIH - United States National Institutes of Health; WHO – World Health Organization

Table A2 – International containment level requirements for activities with characterized RG3 influenza A viruses[38,39,40,41,42,43]

Type of activity	Containment level	Agency
Diagnostic testing (i.e., initial processing and serological identification), excluding testing of respiratory secretions	CL2	CDC; HSE
Non-propagative work	CL2[a]	CDC
Aliquoting/diluting specimens Preparing smears using heat or chemical fixation Performing nucleic acid extractions	CL2[a]	WHO
Diagnostic work with respiratory secretions or strains known to be resistant to antivirals	CL3	HSE
Propagative *in vitro* (including cell and egg culture) and *in vivo* activities	CL3	CDC; HSE; WHO

CDC – United States Centers for Disease Control and Prevention; HSE – United Kingdom Health & Safety Executive; WHO – World Health Organization

[a] with additional biosafety considerations