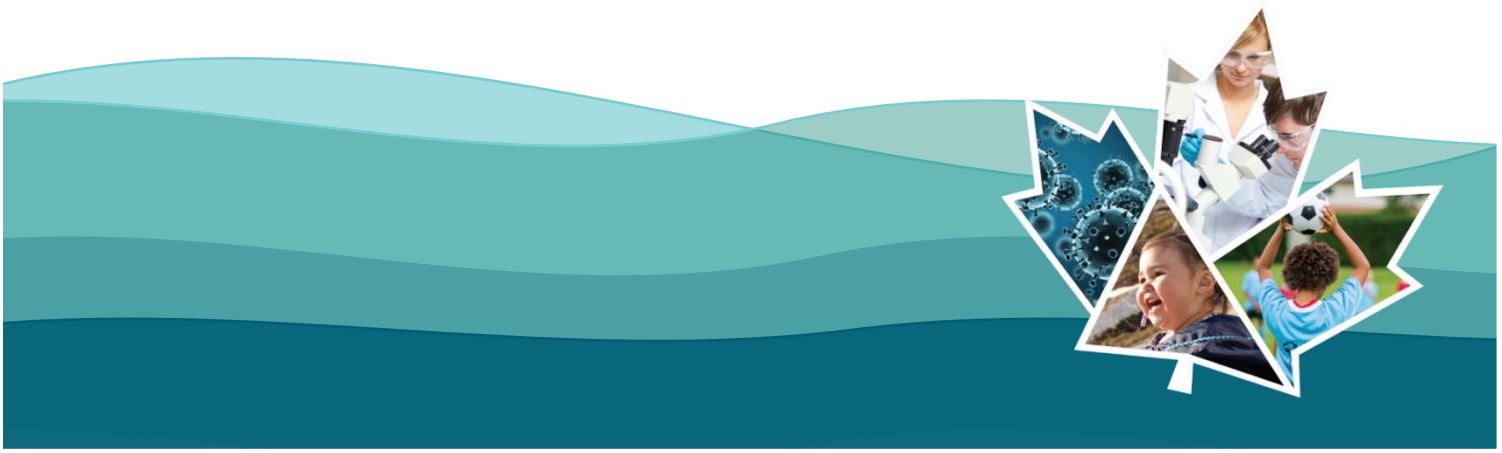
Government of Canada Gouvernement du Canada

Canada

Biosafety Directive for New and Emerging Influenza A Viruses (Alphainfluenzavirus influenzae)

February 2026



TO PROMOTE AND PROTECT THE HEALTH OF CANADIANS THROUGH LEADERSHIP, PARTNERSHIP, INNOVATION AND ACTION IN PUBLIC HEALTH.

—Public Health Agency of Canada

Également disponible en français sous le titre :

Directive en matière de biosécurité portant sur les virus de la grippe A (*Alphainfluenzavirus influenzae*) nouveaux et émergents

To obtain additional information, please contact:

Public Health Agency of Canada
130 Colonnade Rd
A.L 6501H
Ottawa, ON K1A 0K9
Toll free: 1-844-280-5020
Fax: 613-941-5366
TTY: 1-800-465-7735
E-mail: publications-publications@hc-sc.gc.ca

Publication date: November 2024
Minor Update: February 2026

Cat.: HP45-39/2024E-PDF
ISBN: 978-0-660-73007-3
Pub.: 240415

Table of Contents

Abbreviations and Acronyms

BSC	Biological safety cabinet
CBS	*Canadian Biosafety Standard*
CD-TAP	Canadian Food Inspection Agency Designated Terrestrial Animal Pathogen
CFIA	Canadian Food Inspection Agency
CL	Containment Level (i.e., CL1, CL2, CL3, CL4)
CLA	Containment level assessment
CL2-Ag	CL2-Agriculture (i.e., CL2 large animal containment zone)
CL3-Ag	CL3-Agriculture (i.e., CL3 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*
LA zone	Large animal containment zone
LAI	Laboratory-acquired infection
LPAI	Low pathogenic avian influenza
LRA	Local risk assessment
PAO	Plan for Administrative Oversight
PRA	Pathogen risk assessment
PHAC	Public Health Agency of Canada
RG	Risk Group (i.e., RG1, RG2, RG3, RG4)
SA zone	Small animal containment zone
SOP	Standard operating procedure
SSBA	Security sensitive biological agent
WHO	World Health Organization

Executive Summary

- New or emerging strains of influenza A that have not been assigned a risk group are considered to be Risk Group (RG3) human and animal pathogens, until a pathogen risk assessment is completed. These strains must be handled in a containment level 3 (CL3) facility and stored according to applicable *Canadian Biosafety Standard* (CBS) requirements, unless otherwise specified by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA).
- Once identified as such, all highly pathogenic avian influenza (HPAI) strains are considered security sensitive biological agents (SSBAs) and CFIA Designated Terrestrial Animal Pathogens (CD-TAPs). Thus, they must be handled in a facility that meets the minimum applicable requirements for SSBAs and non-indigenous terrestrial animal pathogens specified in Chapters 3, 4, and 5 of the CBS. Refer to Section 2.2.1.1 of this Directive for additional considerations.
- In the case of influenza A candidate vaccine viruses, tests to evaluate their safety and proposed containment levels for their handling are described in the World Health Organization's (WHO's) Technical Report Series No. 1016, Annex 3, *Guidelines for the safe development and production of vaccines to human pandemic influenza viruses and influenza viruses with pandemic potential* (as amended from time to time).
- It is strongly recommended that CL2 laboratories in which influenza A identification activities are performed (e.g., in diagnostic settings), which are exempt from the licensing requirements of the *Human Pathogens and Toxins Act* (HPTA) and *Human Pathogens and Toxins Regulations* or to which exclusions apply under the HPTA, adopt the additional operational practices detailed in Section 4.3.
- This Directive does not apply to seasonal strains of influenza A circulating in humans, which are RG2 human and animal pathogens and can be handled at CL2. These strains include influenza A viruses that are adapted to humans and cause seasonal outbreaks. These strains are based on international surveillance review by the WHO in consultation with WHO Collaborating Centers to determine optimal vaccine composition.
- Once biological material has been completely inactivated (i.e., rendered completely non-infectious), subsequent laboratory procedures with the inactivated material are not regulated by PHAC or CFIA.

1.0 Background

The words in **bold type** are defined in the glossary found in Section 6.0.

In Canada, the **handling or storing** of **Risk Group** 2 (RG2), RG3, and RG4 human **pathogens** or regulated **toxins** is regulated under the *Human Pathogens and Toxins Act* (HPTA) and the *Human Pathogens and Toxins Regulations* (HPTR).[1,2] The handling and storing of imported RG2, RG3, and RG4 animal pathogens or parts thereof are regulated under the *Health of Animals Act* (HAA) and the *Health of Animals Regulations* (HAR).[3,4]

Under the authority of the HPTA and the HPTR, the Public Health Agency of Canada (PHAC) regulates the handling and storing of human pathogens and toxins. Furthermore, under the authority of the HAA and the HAR, PHAC regulates the importation and subsequent transfer or receipt of indigenous **terrestrial animal pathogens** or parts thereof in pure **culture** or non-animal matrix (e.g., human, plant, food or environmental sample), with the exception of bee pathogens or parts thereof.

The Canadian Food Inspection Agency (CFIA) regulates the importation and subsequent transfer or receipt of the remaining animal pathogens or parts thereof (e.g. toxin) under the HAA and HAR. This includes CFIA Designated Terrestrial Animal Pathogens (CD-TAPs), any terrestrial animal pathogen when in animals, animal products, and animal by-products (e.g., tissue, allantoic fluid, serum), bee pathogens, and aquatic animal pathogens. CD-TAP is a term used to capture **foreign animal diseases** (FADs)**, emerging animal diseases** (EADs), and **non-indigenous terrestrial animal pathogens** under CFIA's authority.

Pathogens are assigned a risk group and **containment level** based on a **pathogen risk assessment** (PRA) and **containment level assessment** (CLA) conducted by PHAC and CFIA. A PRA evaluates the inherent characteristics of a biological agent (i.e., microorganism, protein, nucleic acid), including **pathogenicity**, **virulence**, communicability, host range, and the availability of effective prophylactic or therapeutic treatments. Risk group categories range from Risk Group 1 (RG1) (low individual and low community risk) to RG4 (high individual and high community risk). The definitions of the risk groups and a list of pathogen risk factors can be found in the *Canadian Biosafety Standard, Third Edition* (CBS) and in the *Canadian Biosafety Guideline – Pathogen Risk Assessment*, respectively.[5,6] The CLA examines the risks associated with a pathogen as detailed in the PRA and evaluates whether the minimum **containment** requirements corresponding to the pathogen's risk group classification mitigate those risks. It also takes into consideration whether additional precautions may be warranted in certain situations, or whether

exemptions to specific **physical containment** or **operational practice requirements** may be considered.

The CBS sets out the minimum physical containment, operational practice, and **performance and verification testing requirements** for facilities where RG2, RG3, and RG4 human or terrestrial animal pathogens or toxins are handled or stored.[5] In general, the containment level and risk group of a pathogen are the same (e.g., RG2 pathogens are typically handled at containment level 2 [CL2]); however, there are exceptions. Many of the physical containment 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 that are of lower risk for aerosol transmission (e.g., select diagnostic activities) can sometimes be performed at a lower containment level provided that the necessary operational practices are in place to mitigate the risks associated with that activity.

Biosafety directives, such as this one, describe the containment requirements for certain types of activities with a specific pathogen or group of pathogens when the containment level does not align directly with the risk group. This Biosafety Directive has been developed by PHAC and CFIA to assist facilities in determining the appropriate containment level and additional operational practices for the safe handling of samples that are suspected or confirmed to contain a new or emerging strain of influenza A virus. This Biosafety Directive provides a comprehensive overview of the risk assessment outcomes, subsequent CLA decisions, and considerations for those working with new or emerging influenza A viruses.

This *Biosafety Directive for New and Emerging Influenza A Viruses (Alphainfluenzavirus influenzae)* is to be used in conjunction with the CBS. More comprehensive information on the regulatory oversight of pathogens and toxins in Canada can be found in the *Canadian Biosafety Guidelines* series.[7] Regulated parties that are issued a **Pathogen and Toxin Licence** by PHAC, and/or a **Terrestrial Animal Pathogen Import** and/or **Transfer Permit** by CFIA, may be required to adhere to this Biosafety Directive as a condition of licence/permit.

Certain facilities where new and emerging influenza A viruses are handled or stored may be excluded or exempt from licensing requirements under the HPTA or HPTR. These are referred to in two PHAC Statements of Administrative Intent and in Sections 1.1.1 and 1.1.2 of the CBS.[5,8,9] In situations where activities carried out with new and emerging influenza A viruses are excluded from the HPTA, or where facilities are exempt from requiring a Pathogen and Toxin Licence under the HPTA and/or HPTR, PHAC strongly recommends

following the content outlined in this Directive. **It is important to note that while there are exclusions and exemptions under the HPTA, these do not impact the regulatory requirements in place under the HAR that must still be adhered to.**

Exemptions and exclusions do not apply to any activities with imported materials or their derivatives that may contain, are suspected to contain, or contain a new or emerging strain of influenza A. This includes cultures, human **primary specimens**, animal primary specimens, environmental samples, and animal products and by-products as these are regulated under paragraph 51 of the HAR. Requirements for diagnostic laboratories and veterinarian clinics are in Section 4.1 of this Directive.

2.0 Pathogen Description and Risk Group Classification

Influenza viruses are part of the *Orthomyxoviridae* family, with a genome comprised of segmented, negative sense, single-stranded ribonucleic acid (RNA).[10] This family consists of influenza A, B, C, and D. Influenza C is endemic worldwide and usually associated with mild infections.[11,12,13] Influenza D was recently identified in animal populations, with no known transmission to humans.[14] Variants of influenza virus A and B circulate throughout Canada and cause seasonal influenza, which is among the leading causes of acute respiratory infections in humans.[15] In Canada, there are an estimated 12,200 flu-related hospitalizations and 3,500 deaths annually.[16,17,18] While influenza virus B has been the cause of epidemics, it has not caused pandemics.[19] Influenza A is considered to be the influenza virus with the greatest impact on human and animal health, and has caused more than a dozen documented pandemics since the 1700s.[13] Since 2020, an influenza A(H5N1) epizootic has led to outbreaks in wild birds, mammals, and farmed poultry, and has caused a considerable number of avian deaths in Asia, Europe, Africa, South America, and North America.[20]

2.1 Influenza A Viruses

Influenza A viruses are currently assigned to the *Alphainfluenzavirus influenzae* species and are classified into subtypes based on the characterization of surface glycoproteins haemagglutinin (H1 to H18) and neuraminidase (N1 to N11).[11] The haemagglutinin protein binds to receptors that are expressed on the surface of epithelial cells in the upper or lower respiratory tract.[21] Neuraminidase is an enzyme that cleaves a bound virus from the host cells to permit further distribution.

Influenza A viruses are endemic throughout the world. They can infect and cause mild to severe disease in humans and a wide range of animals, including swine and birds.[22,23] Swine influenza A viruses regularly cause respiratory disease outbreaks in pigs and, while severe illness may occur, death is infrequent.[24] Avian influenza A viruses often exist 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 and cause disease.[22]

Infection of poultry with avian influenza A viruses can result in mild to severe disease, depending on 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. Avian influenza A virus infection in poultry generally results in 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.[25] An example of this is influenza A(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, H7N9).[26,27,28,29,30]

Human-to-human transmission of human influenza A viruses can occur by inhalation of infectious aerosols or droplets, as well as by direct or indirect contact of mucous membranes with contaminated surfaces.[22] Zoonotic transmission of influenza A virus can occur through contact of mucous membranes with infectious secretions, excretions, and tissues when handling infected animals or ingesting undercooked infected poultry.[31,32]

2.1.1 New and Emerging Influenza A Viruses

A strain of influenza A virus is deemed to be new or emerging by PHAC and CFIA if it meets one of the following criteria:[33,34]

- It is a novel pathogen for human or animal hosts, from natural or engineered origins.
- It is a new pathogen resulting from the evolution or change of an existing pathogen.
- It is an existing pathogen that has been introduced (or reintroduced) into a new host population with no or low immunity or is spreading to a new geographic area.
- It is an existing pathogen with an increasing incidence 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 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, as well as other viral genes. Antigenic drift occurs when small changes in the virus's genome happen over time, eventually resulting in new strains that are antigenically different from existing strains.[22,35] This is common in seasonal **circulating strains of influenza virus**, which may undergo some genetic variation that does not significantly alter the pathogenicity in humans. Antigenic shift is a change that can produce a completely new strain as a result of genetic reassortment or novel zoonotic transmission to humans from an animal source.[35] 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 circulating strains. This can lead to sudden changes in pathogenicity and unexpected outbreaks in the community.

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 where these pathogens are handled before they have been well characterized and their specific risks identified. As such, new or emerging influenza A viruses that have not been assigned a risk group are to be handled at CL3 and stored according to applicable CBS requirements.

2.1.1.1 Genetic Modification

Genetic reassortment can occur when two distinct influenza viruses infect a single host simultaneously and swap genetic information.[11] The natural evolutionary process can be linked to the generation of influenza viruses with pandemic potential as the novel gene combinations of reassortant viruses are often not recognized by the host's immune system.[36,37] 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.[22,38]

Some animals have increased susceptibility to genetic 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.[39] 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.[40] Similarly, transgenic animals may also be susceptible to influenza A viruses originating from different species, thereby increasing the chance of strain reassortment.

Genetic reassortment is also a mechanism that can be used for the production of influenza virus vaccines through the intentional coinfection of a host with an attenuated virus and a pandemic strain of influenza to obtain an attenuated reassortant influenza virus. Due to the unpredictable nature of viruses, genetic reassortment used for the production of influenza virus vaccines must be performed with caution since the influenza virus produced may also be a virus with increased **virulence**.[38]

Genetic manipulation of the virus in the laboratory can also alter its host range, pathogenicity, and antigenic profile. Strains can be artificially created through reverse genetics, a method by which plasmids containing all of the required influenza genes are used to transfect permissive cells and generate a biologically active influenza virus, including reassortant viruses.[38] These generated influenza A strains are important tools for understanding the biology of the virus, mechanisms for infection, and developing vaccines.[35] 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.[35]

Serial passage of a recombinant influenza A virus through different animal models, such as swine, ferrets, and guinea pigs, must also be performed with caution as this can result in increased pathogenicity and transmissibility in influenza A viruses.[41]

2.2 Risk Group Classification and Containment Level Assessment

2.2.1 Risk Group Classification

The human and animal risk group classification for numerous strains of influenza A viruses can be found in PHAC's ePATHogen Risk Group Database.[42]

It has been determined by PHAC and CFIA that seasonal strains of influenza A circulating in humans are RG2 human and animal pathogens and can be handled at CL2. These include influenza viruses that are adapted to humans and cause seasonal outbreaks. These do not include 1918 H1N1, subtype H2N2, and HPAI subtypes. Strains are based on international surveillance review by the WHO in consultation with WHO Collaborating Centers to determine optimal vaccine composition.

New or emerging strains of influenza A 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 involved in human and avian outbreaks and pandemics, such as HPAI subtypes H5N1, H5N2, H5N8, and H7N9.[26,27,28,29] **Once assigned a risk group, such new or emerging strains are often classified as RG3 human pathogens, RG3 animal pathogens, or both. As noted elsewhere in this Directive, these strains must be handled at CL3 and stored according to applicable CBS requirements, unless otherwise specified by PHAC and CFIA.**

2.2.1.1 Additional Requirements for HPAI Strains

All HPAI strains (i.e., H5 and H7 subtypes) are considered by PHAC to be **security sensitive biological agents** (SSBAs) as they pose a greater biosecurity risk. As such, they are subject to increased biosecurity requirements, as specified in the CBS, HPTA, and HPTR. Contact PHAC directly for requirements and guidance prior to conducting activities involving SSBAs. Refer to Section 5.0 for contact details.

All HPAI subtypes and H5 and H7 LPAI subtypes are considered CD-TAPs. As such, they are subject to increased biosafety and biocontainment requirements, as specified in the CBS, as well as regulatory oversight. Under the HAA and the *Reportable Diseases Regulations*, LPAI and HPAI are reportable to CFIA. Contact CFIA's Office of Biohazard Containment and Safety directly for requirements and guidance prior to conducting activities involving CD-TAPs. Refer to Section 5.0 for contact details.

2.2.2 Containment Level Assessment

New or emerging strains of influenza A virus that have not been assigned a risk group, including any HPAI subtype, are to be handled at CL3 and stored according to applicable CBS requirements until a PRA and CLA have been conducted and PHAC and CFIA have indicated that they can be safely handled at a lower containment level. In the case of candidate vaccine viruses, tests to evaluate their safety and proposed containment levels for their handling are described in the World Health Organization's (WHO's) Technical Report Series No. 1016, Annex 3, *Guidelines for the safe development and production of vaccines to human pandemic influenza viruses and influenza viruses with pandemic potential* (as amended from time to time).[43]

Once evidence supports the potential presence of a new or emerging strain (e.g., a virus has been identified within the facility or elsewhere), this Directive is:

- **to be followed in Pathogen and Toxin licensed facilities when work is conducted at CL2 (with additional operational practices listed in Section 4.3 of this Directive)**
- **strongly recommended to be followed in facilities excluded from the application of the HPTA or exempt from the requirement of a Pathogen and Toxin Licence (e.g., diagnostic laboratories) for implementation of proper biosafety practices (see Section 4.3) to mitigate the risks associated with handling new or emerging influenza A viruses**
- **to be followed in all facilities where avian influenza or any product that may contain avian influenza (e.g., human, animal, environmental samples) is imported**
- **to be followed in all facilities when handling or storing HPAI or LPAI of H5 or H7 sub-types that have been domestically acquired (i.e., non-imported)**
- **strongly recommended to be followed in facilities when work is conducted at CL2 (with additional operational practices listed in Section 4.3 of this Directive) with animal primary samples and environmental primary samples (associated with an animal) that have been domestically acquired (i.e. non-imported).**

In addition to the operational practices listed in Section 4.3 of this Directive, a site-specific **local risk assessment** (LRA) is critical to evaluate and determine which work-specific operational practices and mitigation strategies are needed to achieve the appropriate level of protection for the various activities involving new or emerging strains of influenza A (see *Canadian Biosafety Guideline – Local Risk Assessment*).[44]

2.2.3 Vaccine Development

The WHO's Technical Report Series No. 1016, Annex 3, *Guidelines for the safe development and production of vaccines to human pandemic influenza viruses and influenza viruses with pandemic potential* outlines the potential hazards of working with reassortant candidate vaccine viruses, as well as a risk assessment approach to genetic modification or creation of candidate vaccine viruses and vaccine production activities.[43] These guidelines also specify a number of tests necessary for evaluating the safety of influenza candidate vaccine viruses prior to use for vaccine production. The tests required to determine the containment level at which these strains are to be handled are dependent on the parental

virus being modified, their genetic similarity to a previously tested strain, and the activities being performed. Potential tests include:[43]

- sequencing to confirm identity and verify the presence of attenuating and other phenotypic markers
- testing for pathogenicity in chickens
- testing for ability to plaque in the absence or presence of added trypsin
- testing for attenuation in ferrets
- testing for genetic stability

New influenza A candidate vaccine viruses that have not been tested for safety must be handled at CL3 and stored according to applicable CBS requirements.

3.0 Specimen, Culture, and Activity Types

The type of influenza A specimen and culture, as well as the specific procedures conducted with these specimens and cultures, influence risk. Therefore, the following sections provide various examples of activities with different specimen and culture types and are intended to help classify new activities.

3.1 Non-propagative Diagnostic Activities

These include activities with primary specimens taken directly from patients or animals that have not been cultured and are being performed for the purpose of diagnosing or monitoring an infection. Biosafety recommendations for human diagnostic activities can be found in the *Canadian Biosafety Guideline - Human Diagnostic Activities*.[45]

Examples of non-propagative diagnostic activities include, but are not limited to:

- enzyme-linked immunosorbent assay (ELISA)
- centrifugation of primary specimens (e.g., respiratory secretions) without the intent to pellet the virus
- nucleic acid extraction or nucleic acid amplification (e.g., reverse transcription polymerase chain reaction) from primary specimens

Additionally, this includes activities with primary specimens that remain in the same medium from which they were collected, even if the sample has been modified by other ingredients,

such as stabilizers. Primary specimens that have been processed, as long as the pathogen has not been extracted, are also included in this category. Primary specimens will generally contain a much lower viral load than cultures.

Due to the aerosol transmissibility of influenza A, there is an inherent risk associated with these activities. Adherence to this Directive is strongly recommended when solely performing non-propagative diagnostic activities with domestically-acquired primary specimens in diagnostic facilities (see Table 1 in Section 4.2). Additionally, the development of best practices to reduce the risk of exposure to new or emerging influenza A viruses and **laboratory-acquired infections** (LAIs) is strongly encouraged. However, primary specimens being imported that contain or are suspected to contain new or emerging influenza A viruses are regulated under the HAA and, therefore, adherence to this Directive is required when importing or transferring such specimens.

3.2 Non-propagative and Propagative *In Vitro* Activities

This includes activities with propagated influenza A viruses, whether in cell culture or eggs, including stock cultures of clinical isolates or pathogen reference strains, as well as diagnostic cultures from primary specimens once the pathogen has been identified. It also includes influenza A viruses that are knowingly concentrated in any way (e.g., by ultracentrifugation).

Examples of these activities include, but are not limited to:

- culture of new or emerging influenza A virus for research purposes (e.g., embryonated chicken eggs or in tissue culture, rapid cell culture)
- cultivation or **propagation** of primary specimens that are confirmed to contain new or emerging strains of influenza A
- preparatory work for ***in vivo*** activities
- processing of influenza A positive cultures for packaging and distribution to other laboratories
- other research activities involving cultured or concentrated new or emerging influenza A

When conducting diagnostic activities that aim to knowingly propagate, concentrate, or purify influenza A from primary specimens in an open container, these activities are no longer exempt from the HPTA licensing requirement and therefore, adherence to this

Directive is required (as per Table 1 in Section 4.2). There are no exemptions or exclusions under the HAA for any activity involving the handling of imported new or emerging influenza A specimens.

3.3 *In Vivo* Activities

Under the HPTA and HAR, *in vivo* activities include experimentally exposing an animal to influenza A and the subsequent handling of the animal (and specimens obtained from it) in a research setting. Under the HAA, *in vivo* activities also include any naturally infected animals with an animal pathogen.

Examples of these activities include, but are not limited to:

- inoculating animals
- monitoring infected animals
- collecting specimens from infected animals (e.g., nasal or throat swab, blood, bronchial lavage)

These activities are regulated under the HPTA and HAA; therefore following this Directive is required (as per Table 1 in Section 4.2).

3.4 Activities with Inactivated Biological Material

Inactivated biological material is any primary specimen (e.g., nasopharyngeal specimens), product (e.g., pellets, concentrated virus), or culture (e.g., propagated influenza) that has been rendered completely non-infectious by a **validated inactivation** method. Inactivation methods can include heat or chemicals as well as nucleic acid extraction. The ability of any method to inactivate a pathogen must be validated and routinely **verified**.

Inactivation must be performed at the containment level required for the pathogen and type of specimen or sample (see Table 1 in Section 4.2 for containment level requirements and recommendations).

Examples of activities with inactivated biological material include antigen assays, reverse transcription polymerase chain reaction, and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry.

Once influenza A infectious material has been completely inactivated by a validated and routinely verified method, subsequent activities with the inactivated material are not regulated under the HPTA and the HAA.

3.5 Activities with Genetically Engineered Strains

Modifying a pathogen or toxin to increase pathogenicity and/or infectivity, avoid diagnostic techniques or detection, and/or resist treatment is considered gain-of-function research with **dual-use potential**. A PRA and CLA on the modified virus is used to determine its appropriate risk group and the containment requirements for its handling, respectively. Additionally, it is required that those holding authorizations from PHAC and CFIA conduct a biosecurity risk assessment and develop a biosecurity plan to determine and implement appropriate mitigation strategies to reduce the risk of unauthorized **release**, loss, theft, misuse, or diversion of regulated materials and other related assets.[46] Further, a Plan for Administrative Oversight (PAO) is required under the HPTR. The PAO provides an overview of mechanisms in place to manage and control biosafety and biosecurity risks. For more information on dual-use potential, see the *Plan for Administrative Oversight for Pathogens and Toxins in a Research Setting*.[47]

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

In order to prevent further reassortment, it is strongly recommended to avoid working with various strains of influenza A at the same time, particularly in *in vivo* models. Separation of such work can be spatial (e.g., different **biological safety cabinet** [BSC]) or temporal (e.g., scheduling). This applies to RG2 and RG3 influenza A strains as well as influenza A strains that have not yet been assigned a risk group.

4.0 Containment Level Requirements

Containment levels describe the minimum physical containment, and operational practices that a **containment zone** requires for the safe handling or storing of **regulated materials**. Containment levels range from a basic laboratory (i.e., CL1) to the highest level of containment (i.e., CL4). Detailed information on the different containment levels and types of work areas (e.g., laboratory work areas, **small animal containment zones** [SA zones], **large animal containment zones** [LA zones]) can be found in the CBS.

4.1 Requirements for All Sample Types and Activities

To prevent the unintended generation or release of a new or emerging influenza A virus, all laboratories where activities with influenza A are conducted, regardless of risk group, must comply with the following requirements:

- In the event that a human diagnostic laboratory detects a positive sample for a 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 handling. Contact information for the NML Influenza, Respiratory Viruses and Coronaviruses Section can be found in the NML Guide to services.[48]
- In the event that a veterinary diagnostic laboratory detects a non-negative or positive sample for influenza A virus (H5 or H7 subtypes), all work with the sample is to be stopped. This sample is to be transferred to the National Centre for Foreign Animal Disease (NCFAD) in Winnipeg for confirmatory testing.[49] Additionally, under the HAA and the *Reportable Diseases Regulations*, LPAI and HPAI H5 and H7 subtypes are reportable to the CFIA. Refer to Section 5.0 for contact details.

4.2 Containment Level Requirements for the Safe Handling of New and Emerging Influenza A Viruses

In Canada, new and emerging influenza A viruses that have not been assigned a risk group are presumed to be RG3 human and animal pathogens, until a risk group is assigned. These viruses are to be handled under the normal procedures for RG3 pathogens at CL3 (see Table 1 in Section 4.2).

HPAI strains are to be handled as SSBAs and CD-TAPs. As such, they must be handled in a facility that meets the minimum applicable requirements specified in Chapters 3, 4, and 5 of the CBS. These chapters provide a complete list of the physical containment, operational practice, and performance and verification testing requirements for these containment levels, respectively. If a containment zone cannot meet the containment level requirements for the specimen or culture types or the activities outlined in Sections 3 and 4, or if the handling of SSBAs or CD-TAPs in the facility is not authorized, the specimens or cultures are to be transferred without delay to a containment zone that meets the requirements.

It is strongly recommended that those conducting activities of type 3 in Table 1 below follow the containment level outlined in Table 1. However, if the sample is imported, following the containment level is a requirement. Those performing activities of types 1, 2, 4, 5, 6 and 7 must adhere to the containment level requirements outlined in Table 1 below.

Table 1. Containment levels for activities with influenza A. [a]

Activity Types		Containment Level [a]
1. Propagative *in vitro* activities with influenza A candidate vaccine viruses tested for safety [c]		CL2 with additional operational practices [b]
2. *In vivo* activities with influenza A candidate vaccine viruses tested for safety [c] • CL3 is required for some challenge studies, see activity type 7	Small animal containment zone	CL2 with additional operational practices [b]
	Large animal containment zone	CL2-Ag with additional operational practices [b]
3. Non-propagative diagnostic activities with primary specimens • See Section 3.1 for a description of non-propagative diagnostic activities		CL2 with additional operational practices [b]
4. Propagative *in vitro* activities with influenza A candidate vaccine viruses untested for safety [c]		CL3
5. *In vivo* activities with influenza A candidate vaccine viruses untested for safety [c]	Small animal containment zone	CL3
	Large animal containment zone	CL3-Ag
6. Non-propagative and propagative *in vitro* activities with new and emerging strains influenza A that are RG3 or have not been assigned a risk group [d]		CL3
7. *In vivo* activities with new and emerging influenza A strains that are RG3 or have not been assigned a risk group [d]	Small animal containment zone	CL3
	Large animal containment zone	CL3-Ag

[a] All HPAI strains are considered SSBAs and CD-TAPs, and the handling or storing of these strains must meet the SSBA and non-indigenous terrestrial animal pathogen requirements specified in the CBS, unless confirmed otherwise by PHAC and/or CFIA.

[b] Additional operational practices are described in Section 4.3. These practices are required for activity types 1 and 2, and strongly recommended for activity type 3.

[c] "Tested for safety" refers to testing according to the WHO's guidelines for candidate vaccine viruses (including HPAI strains).[43] Testing may indicate that the candidate vaccine strain needs to be handled at CL3 or higher. It is only acceptable to perform activity types 1 and 2 with candidate vaccine viruses at CL2 with additional operational practices listed in Section 4.3 when safety testing indicates that it is safe to do so.

[d] New and emerging strains are defined in Section 2.1.1 of this Directive.

4.3 Operational Practices for Activities with Influenza A at CL2 and CL2-Ag

In addition to the requirements listed for CL2 specified in Chapters 3, 4, and 5 of the CBS, the operational practices (i.e., CL3 operational practices) below are required for activity types 1 and 2, and strongly recommended for activity type 3 in Table 1. That is unless the specimen or sample is imported, in which case following the additional operational practices detailed below is a requirement. These apply to all personnel who are authorized to enter the containment zone.

In the context of this Directive, all operational practices listed below that may already be required for CL2 and/or CL2-Ag are to be implemented with the rigor of a CL3 operational practice to mitigate the risks associated with new and emerging influenza A viruses.

CBS Requirement Number	CBS Requirement
4.1.3	Where non-indigenous terrestrial animal pathogens are handled or stored, changes in program intent and changes to the physical structure of the facility, to equipment, or to SOPs that could affect biocontainment to be submitted to the CFIA for approval prior to implementation.
Given the risks to the animal population, food supply, and economy associated with non-indigenous terrestrial animal pathogens, any changes to program intent (which describes the scope of a facility's activities, including **infectious material** and animal species handled), or changes that could affect biocontainment in containment zones where non-indigenous terrestrial animal pathogens are handled or stored are submitted to the CFIA prior to their implementation. This allows the CFIA to confirm the changes are acceptable and that the infectious materials and animals will be effectively contained. Changes that may affect biocontainment include changes to the physical structure of the facility (e.g., changing the location of walls, doors, or ductworks, unsealing floor drains), to equipment (e.g., changing the type of filter used in a BSC), or to SOPs (e.g., a new procedure performed outside a BSC).	
4.3.3	Gloves to be worn when handling regulated materials or **regulated animals**.
Appropriate gloves (e.g., latex, nitrile, vinyl) that protect hands from contamination are always worn when handling regulated materials and regulated animals to protect personnel from exposure and prevent the spread of contamination.	
4.3.4	Respirators to be worn where there is a risk of exposure to infectious aerosols that can be transmitted by inhalation, or to aerosolized toxins as determined by a local risk assessment (LRA).
In the context of this Directive, a respirator is required when performing specific activities (e.g., inoculating animals), as the primary route of infection for influenza A virus is inhalation of virus particles. Some influenza A viruses remain viable in aerosols for up to 24 hours, presenting a risk for infection following aerosol-generating activities. Certain activity types, including *in vivo* work, can increase the risk of aerosol production. Respirators are especially important when performing animal work as transmission studies in ferrets indicate that genetically manipulated influenza A virus H5N1 can become airborne transmissible in mammals without reassortment through as few as five amino acid mutations. This can result in the creation of strains with pandemic potential.[41] The risk of infection may also be increased during work involving *in vitro* or *in vivo* propagation of the virus, wherein the concentration or quantity of pathogen is increased. Therefore, wearing a fitted and appropriate respirator can help protect individuals from exposure to influenza A virus particles and prevent infection. The type of respirator (e.g., N95, N100, powered air purifying respirator) must be suited to its use, according to an LRA. Where respirators are worn, occupational health and safety regulations require that a fit-testing program be in place.	

CBS Requirement Number	CBS Requirement
4.4.10	Personal belongings and items for personal use not required for work to be left outside the containment zone or in change areas outside the **containment barrier**.
In the context of this Directive, personal belongings and other items for personal use (e.g., backpack, notebook, purse, cell phone) are to be left outside the containment zone or in change areas outside the containment barrier to prevent the contamination of these items with influenza A. As some influenza A viruses can survive on surfaces for several hours, this measure protects individuals from exposure to and prevents the spread of influenza A outside of the containment barrier.[50]	
4.4.16	Activity-specific **personal protective equipment (PPE)** or an additional layer of PPE to be donned prior to beginning the activity in the containment zone.
For the context of this Directive, LRAs determine the PPE required to protect personnel from exposure to influenza A when performing specific activities. This PPE may be different or in addition to the PPE required to enter the containment zone. Higher risk activities (e.g., creating infectious aerosols, work associated with the possibility of splashes, work with animals) may require the use of additional PPE (e.g., respirator, solid-front gown, apron), while lower risk activities may allow for a lower level of protection. Additional considerations are to be made when conducting an LRA for *in vivo* activities (e.g., increased risk of aerosol production, unpredictability of animals, increased risk of spills). For example, working with animals can increase the risk of **laboratory-acquired infections** (LAIs) from exposure of mucous membranes to contaminated material when handling tissues or secretions from infected animals. For diagnostic laboratories, the epidemiology of the disease, the prevalence of influenza A among the sampled patient population being served by the laboratory, and the seasonality should be considered when selecting PPE.	
4.4.18	Dedicated and activity-specific PPE to be doffed in a manner that minimizes contamination of the skin, hair, and personal clothing (where worn), and stored or disposed of within the containment zone or containment barrier.
For the context of this Directive, removing dedicated and activity-specific PPE in a particular order and in a manner that prevents the contamination of skin, hair, and personal clothing with influenza A (as described in SOPs) reduces the potential of creating aerosols and of contaminated PPE coming into contact with unprotected skin, hair, or personal clothing. Reducing such risks protects personnel from exposure to influenza A. Doffed PPE is stored or disposed of within the containment zone (or within the containment barrier, where applicable).	

CBS Requirement Number	CBS Requirement
4.4.21	Dedicated and activity-specific PPE to be doffed when exiting the containment zone, containment barrier, animal room, **animal cubicle**, or PM room. [*Not required for CL4 zones where positive-pressure suits are worn.*]
For the context of this Directive, removing dedicated PPE or activity-specific PPE (e.g., scrubs, boots, coveralls, lab coats, aprons, gowns, full body suits, shoe covers, head and face protection) when exiting the containment zone, containment barrier, animal room, animal cubicle, or PM room protects individuals from exposure by preventing the spread of influenza A contamination outside these areas. Where it is supported by SOPs, PPE worn within animal cubicles or PM rooms may be worn in "dirty" corridors and removed when exiting the containment zone or containment barrier, based on an LRA.	
4.5.21	All activities involving open vessels of regulated materials to be performed in a BSC or other **primary containment device**. [*Not required when inoculating or collecting samples from **regulated animals** housed in an **animal cubicle**.*]
In the context of this Directive, BSCs and other primary containment devices provide effective **primary containment** to protect personnel from exposure to, and prevent release of, influenza A aerosols. As the primary route of infection for influenza A virus is inhalation of virus particles, performing all activities involving open vessels containing influenza A in a primary containment device can prevent infection. Additionally, influenza A viruses can survive on surfaces for several hours and in aerosols for up to 24 hours.[50] When aerosol particles generated during work activities settle on a surface and inadequate disinfection is performed, infection may result from contact with the contaminated surface.	
4.5.24	Centrifugation of regulated materials to be carried out in sealed safety cups or rotors that are unloaded in a BSC or other primary containment device using a mechanism that prevents their release.
In the context of this Directive, sealed safety cups or rotors for centrifugation are to be used to prevent the release of influenza A aerosols that may be created during centrifugation. As the primary route of infection for influenza A virus is inhalation of virus particles, sealed safety cups or rotors are to be unloaded in a BSC or other primary containment device using specific mechanisms (e.g., **standard operating procedures** [SOPs], equipment, devices) to prevent infection.	

CBS Requirement Number	CBS Requirement
4.6.4	Regulated animals in SA zones to be maintained within a **primary containment device** at all times. *[Not required for CL2 large scale production areas.*]

For the context of this Directive, appropriate **primary containment caging** systems designed to contain the potential release of influenza A virus particles (i.e., with HEPA filtration) are required for work with animals infected, or potentially infected, with influenza A. This includes during housing, transfer from cages to BSCs, cage cleaning, husbandry, inoculation, sample collection, surgeries, necropsies, and any other procedures, to protect personnel from exposure and prevent the release of influenza A. Primary containment in an SA zone may be provided by a BSC, primary containment caging or cage system, or ventilated cage changing station.

4.3.1 Additional Considerations

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.[51] To decrease the probability of infection, vaccination against seasonal influenza, or administration of an approved commercial vaccine for a new or emerging strain (if available), based on an LRA and medical surveillance program, could be included in a facility's policy. The National Advisory Committee on Immunization (NACI)'s annual Statement on Seasonal Influenza Vaccine recommends that vaccination be offered annually unless there is a medical contraindication.[51] For large scale production facilities, it is recommended that a vaccine targeting the virus in production be administered to all personnel if available.[43]

For facilities where influenza vaccines are produced, it is recommended for personnel to avoid contact with susceptible animals, (e.g., pigs, birds, cattle) outside of the containment zone for 14 days after leaving the vaccine production facility.[43]

5.0 Contact Information

Please note that this Biosafety Directive is based on currently available scientific evidence and is subject to review and change as new information becomes available. If this Biosafety Directive is amended, the updated information will be communicated to impacted regulated parties and the amended Biosafety Directive will be posted on the Government of Canada website. For more information on this Biosafety Directive or for further biosafety information, please contact:

Public Health Agency of Canada, Centre for Biosecurity
Email: pathogens.pathogenes@phac-aspc.gc.ca
Website: Biosafety and biosecurity - Canada.ca

Public Health Agency of Canada, Licensing Group, Centre for Biosecurity
Email: licence.permis@phac-aspc.gc.ca
Website: Licensing program - Canada.ca

Canadian Food Inspection Agency, Office of Biohazard Containment and Safety
Email: biocon@inspection.gc.ca
Website: Biohazard Containment and Safety - Canada.ca

Canadian Food Inspection Agency, Terrestrial Animal Health Program
Email: cfia.notification-notification.acia@inspection.gc.ca
Website: Reportable diseases: Terrestrial animals - inspection.canada.ca

Canadian Food Inspection Agency, For more information on animal diseases or toxic substances of concern to CFIA.
Website: Contact Us - Canadian Food Inspection Agency

6.0 Glossary

Aerosol	A suspension of fine solid particles or liquid droplets in a gaseous medium (e.g., air) that can be created by any activity that imparts energy into a liquid or semi-liquid material.
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).
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 regulated materials, and 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 WHO Collaborating Centres 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 barrier	The physical structures or barriers that create a boundary between “clean” and “dirty” areas or between areas of lower contamination and higher contamination (e.g., between the laboratory work areas, large scale production areas, animal rooms, animal cubicles, or post mortem rooms, and outside that containment area). The containment barrier itself is created by the walls, doors, and ceilings of a room that physically enclose the areas within containment, as well as inward airflow at critical doors (where inward airflow is required).
Containment level (CL)	Minimum physical containment and operational practice requirements for handling regulated materials safely in laboratory, large scale production, and animal work environments. There are four containment levels ranging from a basic laboratory (i.e., CL1) to the highest level of containment (i.e., CL4).
Containment level assessment (CLA)	An evaluation of the risks associated with a pathogen as detailed in the pathogen risk assessment and whether the minimum containment requirements corresponding to the pathogen’s risk group classification mitigate those risks. It takes into consideration whether additional precautions or exemptions to specific physical containment or operational practice requirements may be required.
Containment zone	A physical area that meets the requirements for a specified containment level. A containment zone can be a single room (e.g., a 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., a 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.

Culture	The *in vitro* propagation of microorganisms, tissues, cells, or other living matter under controlled conditions (e.g., temperature, humidity, nutrients) to generate greater numbers or a higher concentration of the organisms or cells. In the context of the *Canadian Biosafety Standard*, "cell culture" refers to cells derived from a human or animal source.
Dual-use potential	Qualities of a pathogen or toxin, scientific method, intellectual property, or other related asset that allow it to be either used for legitimate scientific applications (e.g., commercial, medical, or research purposes), or intentionally misused to cause harm or disease. Examples of assets with dual-use potential include pathogens or toxins that could be used as a biological weapon (i.e., for bioterrorism), a method that facilitates propagation of such pathogens in a non-traditional laboratory setting, or the discovery that a certain mutation results in resistance to all available treatments.
Emerging animal disease (EAD)	A new infectious disease resulting from the evolution or change of an existing pathogenic agent; a known infectious disease spreading to a new geographic area or population; or a previously unrecognized pathogenic agent or disease diagnosed for the first time which may have a significant impact on animal health, as determined by the Canadian Food Inspection Agency.
Facility	Structures or buildings, or defined areas within structures or buildings, where regulated materials are handled or stored. This could include individual research and diagnostic laboratories, large scale production areas, or animal housing zones. A facility could also be a suite or building containing more than one of these areas.

Foreign animal disease (FAD)	A disease that appears in the World Organisation for Animal Health Listed Diseases (as amended from time to time) that is not considered indigenous to Canada, as determined by the Canadian Food Inspection Agency (CFIA); or any CFIA-regulated Reportable Disease that does not exist in Canada for which the CFIA has an established response strategy; or any other disease which after due consideration is designated as such by the Minister of Agriculture and Agri-Food. Pathogens causing an FAD may also have serious negative health effects on Canadian animal populations.
Handling or storing	"Handling or storing" regulated materials 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 subsection 7(1) of the *Human Pathogens and Toxins Act*. All tenses and variations of "handling or storing" are also used in this context.
In vitro	Latin for "within glass"; describes experimentation involving components of a living organism within an artificial environment (e.g., manipulating cells in a 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).
Infectious material	Any isolate of a pathogen or any biological material that contains human or animal pathogens and therefore, poses a risk to human or animal health.
Laboratory-acquired infection/ intoxication (LAI)	An infection or intoxication resulting from exposure to pathogens, infectious material, infected animals, or toxins being handled or stored in the containment zone.

Large animal containment zone (LA zone)	An 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 serves as 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)	A site-specific risk assessment used to identify hazards based on the regulated materials in use and the activities being performed. This analysis informs risk mitigation and risk management strategies, which are to be incorporated into the physical containment design and operational practices of the facility.
Non-indigenous terrestrial animal pathogen	A pathogen that causes an animal disease listed in the World Organisation for Animal Health's (WOAH) Listed Diseases (as amended from time to time) and that is exotic to Canada, or any other animal disease that is exotic to Canada which has a significant impact on animal health as determined by the Canadian Food Inspection Agency (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 regulated materials, as specified in Chapter 4 of the *Canadian Biosafety Standard*.
Pathogen	A microorganism, nucleic acid, protein, or other infectious agent that is transmissible and capable of causing disease or infection in humans or animals. Classified human and animal pathogens can be found on the Public Health Agency of Canada's ePATHogen – Risk Group Database.

Pathogen and Toxin Licence	An authorization issued by the Public Health Agency of Canada: • under section 18 of the *Human Pathogens and Toxins Act* to conduct one or more controlled activities with human pathogens or toxins; and/or • under paragraph 51(a) of the *Health of Animals Regulations* for the importation into Canada of terrestrial animal pathogens (except for Emerging Animal Disease pathogens and non-indigenous terrestrial animal pathogens). "Licence" is also used in this context.
Pathogen risk assessment (PRA)	An evaluation of the inherent characteristics of a biological agent (i.e., microorganism, protein, nucleic acid, or biological material containing parts thereof), which determines its risk group classification. A pathogen risk assessment involves the analysis of four key risk factors, including pathogenicity (i.e., infectivity and virulence), pre- and post-exposure measures, communicability, and impact on the animal population (i.e., host range, natural distribution, and economic impact).
Pathogenicity	The ability of a pathogen to cause disease in a human or animal host.
Performance and verification testing requirements	Performance and verification tests that are necessary to demonstrate compliance with the physical containment requirements, as specified in Chapter 3 of the *Canadian Biosafety Standard* (CBS) and, in some cases, the operational practice requirements specified in Chapter 4 of the CBS. The performance and verification testing requirements are listed in Chapter 5 of the CBS.
Personal protective equipment (PPE)	Equipment and/or clothing worn by personnel to provide a barrier against regulated materials, 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 regulated material, as specified in Chapter 3 of the *Canadian Biosafety Standard*.
Primary containment	The first level of physical barriers designed to contain regulated materials and prevent their release. This is accomplished by the provision of a device, equipment, or other physical structure situated between the regulated materials and the individual, the work environment, or other areas within the containment zone. Examples include biological safety cabinets, glove boxes, and microisolator cages. In animal cubicles, the room itself serves as 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 regulated materials. Examples include ventilated filter-top cages and ventilated microisolator cage rack systems, with or without high efficiency particulate air filters.
Primary containment device	Apparatus or equipment that is designed to prevent the release of regulated materials, and to provide primary containment (i.e., provide a physical barrier between the regulated materials and the individual or the work environment). Examples include biological safety cabinets, isolators, centrifuges with sealable cups or rotors, process equipment, fermenters, bioreactors, microisolator cages, ventilated cage racks.
Primary specimens	Samples derived directly from a human or animal (e.g., blood, urine, saliva, skin, hair).

Regulated animal	In the context of this Biosafety Directive, regulated animals include: • animals experimentally infected or intoxicated with a human pathogen or toxin (under the *Human Pathogens and Toxins Act* and *Human Pathogens and Toxins Regulations*); and • animals naturally or experimentally infected or intoxicated with a terrestrial animal pathogen or part of one (e.g., toxin), including those known or suspected to be infected or intoxicated (under the *Health of Animals Act* and *Health of Animals Regulations*).
Regulated material	In the context of this Biosafety Directive, regulated material includes: • human pathogens and toxins (under the *Human Pathogens and Toxins Act* and *Human Pathogens and Toxins Regulations*); • terrestrial animal pathogens (under the *Health of Animals Act* [HAA] and *Health of Animals Regulations* [HAR]); and • terrestrial animal pathogens in animals, animal products, animal by-products, or other organisms (under the HAA and HAR).
Release	The discharge of regulated materials from a containment system or containment zone (e.g., resulting from leaking, spraying, depositing, dumping, vaporizing).
Risk group (RG)	The classification of a biological agent (i.e., microorganism, protein, nucleic acid, or biological material containing parts thereof) based on its inherent characteristics, including pathogenicity, virulence, communicability, and the availability of effective prophylactic or therapeutic treatments. The risk group 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* (HPTR). This means all Risk Group 3 (RG3) and RG4 human pathogens that are in the *List of Human and Animal Pathogens and Toxins 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 and Toxins for Export Control* when in a quantity greater than that specified in Section 10(2) of the HPTR.
Small animal containment zone (SA zone)	An 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, or rabbits, provided that they are housed in primary containment caging.
Standard operating procedure (SOP)	A document that standardizes safe work practices and procedures for activities with regulated materials in a containment zone, as determined by an local risk assessment. Examples of SOPs include experimental protocols, entry and exit procedures, decontamination protocols, and emergency response procedures.

Terrestrial animal pathogen	A microorganism, nucleic acid, protein, or other infectious agent that is transmissible and capable of causing disease or infection in terrestrial animals; including those derived from biotechnology. These include pathogens that cause disease in avian and amphibian animals, but exclude those that only cause disease in invertebrates and aquatic animals. This also includes terrestrial animal pathogens or part of one (e.g., toxin) present on or in animal products, animal by-products, or other organisms.
Terrestrial animal pathogen import permit	A permit issued under paragraph 51(a) and (b) of the *Health of Animals Regulations* by the Public Health Agency of Canada or the Canadian Food Inspection Agency for the importation into Canada of terrestrial animal pathogens or part of one (e.g., toxin); or animals, animal products, animal by-products (e.g., tissue, serum), or other organisms carrying a terrestrial animal pathogen or part of one (e.g., toxin).
Terrestrial animal pathogen transfer permit	A permit issued under paragraph 51.1(a) of the *Health of Animals Regulations* by the Public Health Agency of Canada or the Canadian Food Inspection Agency for the transfer of terrestrial animal pathogens or part of one (e.g., toxins); or animals, animal products, animal by-products (e.g., tissue, serum), or other organisms carrying a terrestrial animal pathogen or part of one (e.g., toxin).
(Microbial) Toxin	A poisonous substance that is produced by or derived from a microorganism and can lead to adverse health effects in humans or animals. Human toxins are listed in Schedule 1 and Part 1 of Schedule 5 in the *Human Pathogens and Toxins Act*.

Validation	The act of confirming that a method achieves its objective and is suitable for its intended purpose through the provision of objective evidence. This can be achieved by observing that specific conditions have been met (e.g., using biological indicators, chemical integrators, or parametric monitoring devices placed in challenging locations within the load to confirm that a given autoclave cycle can decontaminate a representative load of waste).
Verification	The routine monitoring of equipment and processes to confirm continued efficacy between validations (e.g., testing the performance of an autoclave using biological indicators, viewing airflow gauges to confirm fan function in a biological safety cabinet). Verification includes comparing the accuracy of a piece of equipment to an applicable standard or standard operating procedure.
Virulence	The degree or severity of a disease caused by a pathogen.

7.0 References

1 *Human Pathogens and Toxins Act (S.C. 2009, c. 24).*

2 *Human Pathogens and Toxins Regulations (SOR/2015-44).*

3 *Health of Animals Act (S.C. 1990, c. 21).*

4 *Health of Animals Regulations (C.R.C., c. 296).*

5 Government of Canada. (2022). *Canadian Biosafety Standard* (3rd ed.). Ottawa, ON, Canada: Government of Canada. Available from https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/third-edition.html

6 Government of Canada. (2018). *Canadian Biosafety Guideline—Pathogen Risk Assessment*. Ottawa, ON, Canada: Government of Canada. Available from https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance/pathogen-risk-assessment.html

7 Government of Canada. *Canadian Biosafety Guidelines*. Ottawa, ON, Canada: Government of Canada. Available from https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance.html

8 Government of Canada. *Exclusions from the Human Pathogens and Toxins Act*. Ottawa, ON, Canada: Government of Canada. Available from https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/human-pathogens-toxins-act/exclusions-human-pathogens-toxins-act.html

9 Government of Canada. *Exemptions from the Licensing Requirements of the Human Pathogens and Toxins Act and Human Pathogens and Toxins Regulations*. Ottawa, ON, Canada: Government of Canada. Available from https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/human-pathogens-toxins-act/exemptions-licensing-requirements-human-pathogens-toxins-act-human-pathogens-toxins-regulations.html

10 International Committee on Taxonomy of Viruses. (2023). *Virus Taxonomy: 2023 Release*. Retrieved 2024-07-29 from https://ictv.global/taxonomy

11 Centers for Disease Control and Prevention (United States). (2023). *Types of Influenza Viruses*. United States Department of Health & Human Services. Retrieved 2026-02-04 from https://www.cdc.gov/flu/about/viruses-types.html?CDC_AAref_Val=https://www.cdc.gov/flu/about/viruses/types.htm

12 Matsuzaki, Y., Katsushima, N., Nagai, Y., Shoji, M., Itagaki, T., et al. (2006). Clinical Features of Influenza C Virus Infection in Children. *Journal of Infectious Diseases*, 193(9): 1229-1235.

13 Taubenberger, J. K., & Morens, D. M. (2008). The Pathology of Influenza Virus Infections. *Annual Review of Pathology*, 3: 499-522.

14 Quast, M., Sreenivasan, C., Sexton, G., Nedland, H., Singrey, A., et al. (2015). Serological Evidence for the Presence of Influenza D Virus in Small Ruminants. *Veterinary Microbiology*, 180(3-4): 281-285.

15 Buckrell, S., Moussa, M. B., Bui, T., Rahal, A., Schmidt, K., et al. (2022). National Influenza Annual Report, Canada, 2021–2022: A Brief, Late Influenza Epidemic. *Canada Communicable Disease Report*, 48(10): 473-483.

16 Schanzer, D. L., McGeer, A., & Morris, K. (2013). Statistical Estimates of Respiratory Admissions Attributable to Seasonal and Pandemic Influenza for Canada. *Influenza and Other Respiratory Viruses*, 7(5): 799-808.

17 Schanzer, D. L., Sevenhuysen, C., Winchester, B., & Mersereau, T. (2013). Estimating Influenza Deaths in Canada, 1992-2009. *Public Library of Science One*, 8(11): e80481.

18 Public Health Agency of Canada. (2023). *Seasonal Influenza Vaccination Coverage in Canada, 2022–2023*. Available from https://www.canada.ca/en/public-health/services/immunization-vaccines/vaccination-coverage/seasonal-influenza-survey-results-2022-2023.html

19 Glezen, W. P., Schmier, J. K., Kuehn, C. M., Ryan, K. J., & Oxford, J. (2013). The Burden of Influenza B: A Structured Literature Review. *American Journal of Public Health*, 103(3): e43-51.

20 Food and Agriculture Organization of the United Nations, World Health Organization, & World Organization for Animal Health. (2024). *Joint FAO/WHO/WOAH Preliminary Assessment of Recent Influenza A(H5N1) Viruses*. Retrieved 2024-07-29 from https://cdn.who.int/media/docs/default-source/global-influenza-programme/2024_04_23_fao-woah-who_h5n1_assessment.pdf?sfvrsn=3ca3dba6_2&download=true

21 García-Sastre, A. (2010). Influenza Virus Receptor Specificity. *The American journal of pathology*, 176(4): P1584-1585.

22 Hampson, A. W., & Mackenzie, J. S. (2006). The Influenza Viruses. *Medical Journal of Australia*, 185(S10): S39-43.

23 Centers for Disease Control and Prevention (United States). (2022). *How Flu Viruses Can Change: "Drift" and "Shift"*. United States Department of Health & Human Services. Retrieved 2026-02-04 from https://www.cdc.gov/flu/php/viruses/change.html?CDC_AAref_Val=https://www.cdc.gov/flu/about/viruses/change.htm

24 Centers for Disease Control and Prevention (United States). (2024). *About Influenza in Swine (Pigs)*. United States Department of Health & Human Services. Retrieved 2024-

07-29 from https://www.cdc.gov/swine-flu/about/influenza-in-swine.html?CDC_AAref_Val=https://www.cdc.gov/flu/swineflu/keyfacts_pigs.htm

25 United States Department of Agriculture, & Animal and Plant Health Inspection Service. (2024). Guidelines for Avian Influenza Viruses. United States Department of Agriculture. Retrieved 2024-11-18 from https://www.selectagents.gov/compliance/guidance/avian/docs/AIV-Guidelines-Exemption-2024-August_508.pdf

26 World Health Organization. (2018). *Human Infection with Avian Influenza A(H7N9) Virus – China: Update*. World Health Organization. Retrieved 2024-07-29 from https://www.who.int/emergencies/disease-outbreak-news/item/05-september-2018-ah7n9-china-en

27 Government of Canada. (2024). *Avian influenza A(H5N1): Canada's Response*. Government of Canada. Available from https://www.canada.ca/en/public-health/services/diseases/avian-influenza-h5n1/canada-response.html#a2

28 Pasick, J., Berhane, Y., Joseph, T., Bowes, V., Hisanaga, T., et al. (2015). Reassortant Highly Pathogenic Influenza A H5N2 Virus Containing Gene Segments Related to Eurasian H5N8 in British Columbia, Canada, 2014. *Scientific Reports*, 5: 9484.

29 World Organisation for Animal Health. (2024). *High Pathogenicity Avian Influenza (HPAI) – Situation Report 61*. World Organisation for Animal Health. Retrieved 2024-07-29 from https://www.woah.org/en/document/high-pathogenicity-avian-influenza-hpai-situation-report-61/

30 Lee, D. H., Kwon, J. H., Park, J. K., Lee, Y. N., Yuk, S. S., et al. (2012). Characterization of Low-Pathogenicity H5 and H7 Korean Avian Influenza Viruses in Chickens. *Poultry Science*, 91(12): 3086-3090.

31 Hayden, F., & Croisier, A. (2005). Transmission of Avian Influenza Viruses to and Between Humans. *The Journal of Infectious Diseases*, 192(8): 1311-1314.

32 Liu, Q., Lui, D., & Yang, Z. (2013). Characteristics of Human Infection with Avian Influenza Viruses and Development of New Antiviral Agents. *Acta Pharmacologica Sinica*, 34: 1257-1269.

33 Woolhouse, M. E. (2002). Population Biology of Emerging and Re-emerging Pathogens. *Trends in Microbiology*, 10(10 Suppl): S3-S7.

34 Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., et al. (2008). Global Trends in Emerging Infectious Diseases. *Nature*, 451(7181): 990-993.

35 Jia, X., Huang, L., & Liu, W. (2013). Biosafety Issues and Public Concerns on Recombinant Influenza Viruses Generated in the Laboratories. *Sheng Wu Gong Cheng Xue Bao*, 29(12): 1736-1742.

36 Ganti, K., Bagga, A., Carnaccini, S., Ferreri, L. M., Geiger, G., et al. (2022). Influenza A Virus Reassortment in Mammals Gives Rise to Genetically Distinct Within-Host Subpopulations. *Nature Communications*, 13(1): 6846.

37 de Silva, U. C., Tanaka, H., Nakamura, S., Goto, N., & Yasunaga, T. (2012). A Comprehensive Analysis of Reassortment in Influenza A Virus. *Biology Open*, 1(4): 385-390.

38 Sedova, E. S., Shcherbinin, D. N., Migunov, A. I., Smirnov, I. A., Logunov, D., et al. (2012). Recombinant Influenza Vaccines. *Acta Naturae*, 4(4): 17-27.

39 Ito, T., Couceiro, J. N., Kelm, S., Baum, L. G., Krauss, S., et al. (1998). Molecular Basis for the Generation in Pigs of Influenza A Viruses with Pandemic Potential. *Journal of Virology*, 72(9): 7367-7373.

40 Ma, W., Kahn, R. E., & Richt, J. A. (2008). The Pig as a Mixing Vessel for Influenza Viruses: Human and Veterinary Implications. *Journal of Molecular and Genetic Medicine*, 3(1): 158-166.

41 Wei, K., Sun, H., Sun, Z., Sun, Y., Kong, W., et al. (2014). Influenza A Virus Acquires Enhanced Pathogenicity and Transmissibility after Serial Passages in Swine. *Journal of Virology*, 88(20): 11981-11994.

42 Government of Canada. *ePATHogen—Risk Group Database*. Ottawa, ON, Canada: Government of Canada. Available from https://health.canada.ca/en/epathogen

43 World Health Organization. (2019). *Guidelines for the Safe Development and Production of Vaccines to Human Pandemic Influenza Viruses and Influenza Viruses with Pandemic Potential, Annex 3, TRS No 1016*. World Health Organization. Retrieved 2023-10-16 from https://www.who.int/publications/m/item/human-pandemic-influenza-viruses-annex-3-trs-no-1016

44 Government of Canada. (2018). *Canadian Biosafety Guideline—Local Risk Assessment*. Ottawa, ON, Canada: Government of Canada. Available from https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance/canadian-biosafety-guidelines.html

45 Government of Canada. (2021). *Canadian Biosafety Guideline—Human Diagnostic Activities*. Ottawa, ON, Canada: Government of Canada. Available from https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance/human-diagnostic-activities.html

46 Government of Canada. (2018). *Canadian Biosafety Guideline—Conducting a Biosecurity Risk Assessment*. Ottawa, ON, Canada: Government of Canada. Available from https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance/conducting-biosecurity-risk-assessment.html

47 Public Health Agency of Canada. (2015). *Plan for Administrative Oversight for Pathogens and Toxins in a Research Setting—Required Elements and Guidance*. Ottawa, ON, Canada: Government of Canada. Available from https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/licensing-program/plan-administrative-oversight-pathogens-toxins-a-research-setting-required-elements-guidance.html

48 Public Health Agency of Canada. *Guide to Services—Influenza, Respiratory Viruses and Coronaviruses*. Ottawa, ON, Canada: Government of Canada. Available from https://cnphi.canada.ca/gts/laboratory/1013

49 Government of Canada. (2024). *National Centre for Foreign Animal Disease (NCFAD) Winnipeg*. Available from https://inspection.canada.ca/en/science-and-research/our-laboratories/ncfad-winnipeg

50 Otter, J. A., Donskey, C., Yezli, S., Douthwaite, S., Goldenberg, S. D., & Weber, D. J. (2016). Transmission of SARS and MERS Coronaviruses and Influenza Virus in Healthcare Settings: The Possible Role of Dry Surface Contamination. *Journal of Hospital Infection*, 92(3): 235-250.

51 Public Health Agency of Canada. (2023). *Statement on Seasonal Influenza Vaccine for 2023-2024*. Available from https://www.canada.ca/en/public-health/services/publications/vaccines-immunization/national-advisory-committee-immunization-statement-seasonal-influenza-vaccine-2023-2024.html