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Canada

Biosafety Directive

for

Risk Group 3 Fungi

March 2023

The *Biosafety Directive for Risk Group 3 Fungi*

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Contents

Abbreviations and Acronyms

BSC	Biological safety cabinet
CBH	*Canadian Biosafety Handbook*
CBS	*Canadian Biosafety Standard*
CFIA	Canadian Food Inspection Agency
CL	Containment Level (i.e., CL1, CL2, CL3, CL4)
CL2-Ag	CL2-Agriculture (i.e., CL2 large animal containment zone)
CL3-Ag	CL3-Agriculture (i.e., CL3 large animal containment zone)
EAD	Emerging animal disease
FAD	Foreign animal disease
HAA	*Health of Animals Act*
HAR	*Health of Animals Regulations*
HPTA	*Human Pathogens and Toxins Act*
HPTR	*Human Pathogens and Toxins Regulations*
LA zone	Large animal containment zone
LRA	Local risk assessment
MALDI-TOF	Matrix-assisted laser desorption ionization time-of-flight (mass spectrometry)
PHAC	Public Health Agency of Canada
PPE	Personal protective equipment
RG	Risk Group (i.e., RG1, RG2, RG3, RG4)
SA zone	Small animal containment zone
SOP	Standard operating procedure
SSBA	Security sensitive biological agent

Executive Summary

- Identification activities for Risk Group 3 (RG3) fungi that involve propagated or concentrated material (e.g., for diagnostic purposes) must be performed in a Containment Level 3 (CL3) facility or, at the very least, in a CL2 facility with additional operational practices (according to the conditions detailed in Section 5.0), unless facilities are exempted from the licensing requirement.
- Where facilities are exempted from the licensing requirement, it is still recommended that CL2 laboratories in which RG3 fungi identification activities are performed (e.g., in diagnostic settings) adopt the additional operational practices detailed in Section 5.0.
- Controlled activities with *Cladophialophora bantiana, Coccidioides immitis, Coccidioides posadasii,* and *Rhinocladiella mackenziei* must always be performed at CL3.
- Controlled activities with *Blastomyces dermatitidis, Blastomyces gilchristii*, *Blastomyces percursus*, *Cryptococcus gattii*, *Histoplasma capsulatum* var. *capsulatum*, *Histoplasma capsulatum* var. *duboisii*, *Histoplasma capsulatum* var. *farciminosum*, *Paracoccidioides brasiliensis*, and *Paracoccidioides lutzii* may be performed at CL2 with additional operational practices under certain conditions (detailed in Section 4.0 and Section 5.0).
- Additional mitigation measures recommended for activities with dimorphic fungi can also be implemented based on local risk assessments (LRAs) where activities pose a higher risk of exposure or release.

1.0 Background

The words in **bold type** are defined in the glossary found in Section 8.0.

In Canada, facilities where **Risk Group** 2 (RG2), RG3, and RG4 human **pathogens** or **toxins** are **handled or stored** 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). Unless a facility meets the licensing exemption criteria indicated in the HPTA and the HPTR, a **Pathogen and Toxin Licence** (hereafter "licence") is required to perform **controlled activities** with **regulated materials**.[1, 2] The importation of animal pathogens or part of one (e.g., toxin), animals naturally or experimentally exposed to an animal pathogen or part of one (e.g., toxin), and animal products or by-products (e.g., tissue, serum), or other organisms carrying an animal pathogen or part of one (e.g., toxin) is regulated under the *Health of Animals Act* (HAA) and the *Health of Animals Regulations* (HAR). A **terrestrial animal pathogen** may be imported and subsequently transferred under a **terrestrial animal pathogen permit** issued by the PHAC (as part of a licence), provided that:

- it is not a pathogen that causes an **emerging animal disease** (EAD) or a **foreign animal disease** (FAD), or an aquatic animal, plant or bee pathogen;
- it is in an environment in which it *does not* naturally occur (e.g., **cultures**, proficiency panels);
- it is in a primary specimen from a human, a plant, or the environment.

The importation of any other type of primary specimen that contains an animal pathogen (e.g., primary specimens from animals, animal products or by-products), or any type of material that contains a pathogen that causes an EAD or an FAD, or an aquatic animal, plant or bee pathogen, requires an import permit issued by the Canadian Food Inspection Agency (CFIA).[3,4] As a condition of their licence and/or terrestrial animal pathogen permit, facilities must comply with the applicable **physical containment requirements**, **operational practice requirements**, and **performance and verification testing requirements** specified in the *Canadian Biosafety Standard* (CBS).[5]

The PHAC issues licences according to a pathogen's risk group (e.g., RG3 licence for an RG3 human or terrestrial animal pathogen). In most cases, the risk group of a pathogen matches the **containment level** at which it can be handled (i.e., an RG2 pathogen handled at Containment Level 2 [CL2]). When certain activities can be safely performed at a containment level that does not align with a pathogen's risk group, the PHAC and the CFIA may develop **biosafety** directives to clarify **containment** requirements for specific activities with such pathogens. Note that **security sensitive biological agents** (SSBAs) are not covered by this directive, and activities with SSBAs (e.g., *Coccidioides immitis*, *Coccidioides posadasii*) must always meet the requirements specified in the CBS for SSBAs handled at CL3.

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 to safely work with RG3 fungi. This Biosafety Directive was developed in consultation with experts in the field of mycology from research and clinical diagnostic settings to provide a comprehensive overview of the risk assessment outcomes, subsequent containment level decisions, and considerations for individuals working with RG3 fungi.[1,6,7] This Biosafety Directive is to be used in conjunction with the CBS, as a condition of licence or terrestrial animal pathogen permit.

2.0 Introduction

Fungi are eukaryotic microorganisms that can be easily distinguished from bacteria and other prokaryotes by their greater size and the presence of organelles, including nuclei, vacuoles, and mitochondria. Most fungi exist in one of two morphological forms (phenotypes): **yeasts** that normally grow as single cells, or **moulds** (filamentous fungi) that consist of apically growing, tandemly attached cells arranged in long branching filaments (hyphae). In contrast to yeast, moulds form copious spores (i.e., conidia) on the surfaces of their filaments that can easily dislodge and become airborne (i.e., readily aerosolized material).[8,9] Exposure to fungal cells and spores can occur through inhalation of airborne material (i.e., aerosolized material), ingestion (e.g., contaminated food or water), percutaneous inoculation (e.g., via wounds, skin puncture), or direct skin contact, depending on the species.[10]

Dimorphic fungi have the inherent ability to manifest in different forms during growth (e.g., yeast and mould), depending on environmental conditions. Typically, thermally dimorphic fungi exist as yeast forms at 37°C, while incubation at lower temperatures can yield spore-producing filamentous forms.[11] Consequently, these different forms of dimorphic fungi have distinctive characteristics that can influence their infectivity. For example, the spores produced by mould forms of certain fungi may readily become aerosolized and result in infection when inhaled by a susceptible host.[12,13] Yeast-like forms of dimorphic fungi, by contrast, only become aerosolized upon mechanical disruption or mixing.

Spores are readily aerosolized material by nature; therefore, exclusively working with the yeast form of a dimorphic fungus can present a lower exposure risk than working with the mould form.[14] Nonetheless, working with yeast forms still presents a risk of exposure; material that appears to only be present in yeast form can still have the potential of producing readily aerosolized material depending on the growth conditions and the integrity of the isolate, as the conversion of some dimorphic fungi in laboratories can be incomplete (i.e., both mould and yeast forms can be present in the same sample). **Laboratory-acquired infections/intoxications** (LAIs) with yeast forms of dimorphic fungi have also been reported due to accidental percutaneous inoculation. These include LAIs resulting from inoculation with the yeast forms of species of *Blastomyces*, *Histoplasma,* and *Paracoccidioides*.[15,16,17]

2.1 Risk Group and Containment Level

The risk group classification of a microorganism is based on the outcome of a **pathogen risk assessment**. This process considers the inherent characteristics of the pathogen that contribute to the risk it poses to an individual human or animal, to public health, and to the animal population. The PHAC has performed pathogen risk assessments and classified certain species of fungi as RG3 human pathogens. RG3 fungi pose a high risk to the health of individuals since they are likely to cause serious disease in humans, and a low risk to public health due to their low communicability. Risk group classifications are subject to change. Since risk assessments are influenced by the most recent reports on fungal disease treatments and prognostics, consulting the ePATHogen – Risk Group Database is necessary prior to any activity with fungi to confirm their classification.[18] Table 1 indicates the RG3 fungi to which this Biosafety Directive may apply. As fungal taxonomy continues to evolve, the names indicated in Table 1 may be changed in future versions of this Biosafety Directive.

Table 1: RG3 fungi to which this Biosafety Directive may apply

Genera	Species	Human Risk Group Classification	Animal Risk Group Classification
Blastomyces	*dermatitidis* *gilchristii* *helicus* *percursus*	3	3
Cryptococcus	*gattii*	3	3
Histoplasma	*capsulatum* var. *capsulatum* *capsulatum* var. *duboisii* *capsulatum* var. *farciminosum*	3	3
Paracoccidioides	*brasiliensis* *lutzii*	3	1

The PHAC can be consulted to determine whether a fungus (e.g., novel species, genetically modified) with characteristics similar to the examples listed in Table 1 may be included in future versions of this Biosafety Directive. Until approval by the PHAC is given, the containment requirements described in this Biosafety Directive for RG3 fungi cannot be applied to novel species or strains that are not well characterized.

Handling or storing an RG3 fungus (e.g., fungi listed in Table 1) requires an RG3 licence issued by the PHAC. This RG3 licence specifies which controlled activities with RG3 fungi are permitted, and under which conditions (e.g., additional operational practice requirements). Controlled activities with RG3 fungi can be safely performed at CL3 (i.e., facilities that meet the minimum applicable requirements for CL3, as specified in the CBS). Some activities with certain RG3 fungi may be performed at CL2, if they are performed in accordance with an RG3 licence that specifies the additional operational practice requirements described in Section 5.0 of this Biosafety Directive as conditions of the licence. In some cases, the facility's **local risk assessment** (LRA) may need to be reviewed by the PHAC to determine the conditions of the licence for activities performed at CL2 or at lower containment. For example, the PHAC may review LRAs of diagnostic facilities for licences that allow storage of identified concentrated or propagated material of an RG3 fungus in a CL2 or CL1 work area.

2.2 Factors to Consider in a Local Risk Assessment

The *Canadian biosafety guideline - Local Risk Assessment* describes best practices for conducting an LRA in an organization where human or animal pathogens, toxins, or other regulated infectious material are handled or stored.[19] There are many factors that may significantly increase the risk of personnel exposure during laboratory procedures with dimorphic fungi. In order to implement appropriate risk mitigation measures that can prevent exposure or release incidents, it is important to take such factors into consideration when conducting LRAs for RG3 fungi.[19]

While some risk mitigation measures may not appear necessary for fungi that do not easily convert to a filamentous form at room temperature, they can serve as additional precautionary measures for activities that present a higher biosafety risk, such as activities that may produce infectious aerosols.

Factors to consider when conducting an LRA include:

- Form of the fungus:

  The yeast form, which exists at 37°C, carries the lowest risk of exposure by aerosol transmission.[11] For certain fungi, the risk of conversion from yeast to non-yeast forms can be mitigated by limiting the time that the fungi-containing material remains at temperatures below 37°C. Mitigation measures can be developed into standard operating procedures (SOPs) to prevent storage of materials at room temperature between procedures and require the use of sealed containers and refrigeration (e.g., at 4°C) for any long-term storage (e.g., waste).

- Waste management:

  The risk of inadvertently growing non-yeast forms during waste storage is important to take into consideration.[20,21,22] SOPs can be developed to describe the frequency (e.g., immediately following procedures, on a regular basis) of surface **decontamination** and the appropriate, validated, and routinely verified method to use. SOPs can also be implemented to prevent contaminated waste from being stored at room temperature and require infected animal carcasses to be kept in sealed bags.

- Genetic alterations:

  Genetic recombination or mutation that results from natural methods (e.g., mating experiments) or biotechnology (e.g., recombinant DNA, genetic modification) can produce new strains of fungi for which the risks are difficult to assess. When there is an increased possibility of genetic recombination (i.e., due to research procedures or the fungus having the ability to reproduce sexually), SOPs can be implemented to reduce the probability of producing a new strain.[23,24,25] While some fungi can self-fertilize (e.g., *Cryptococcus gattii* in special laboratory conditions), risks of recombination while working with other fungi may be mitigated with spatial or temporal segregation.[26] For example, SOPs can include avoiding the **propagation** of different strains of fungi simultaneously within the same work area.

- Aerosol production:

  While the natural route of infection for dimorphic fungi is inhalation of aerosolized fungal spores or hyphae fragments, yeast forms can also become aerosolized during experimental procedures.[27] Of note, *Cryptococcus gattii* is known to infect humans and animals via dried yeast cells in the air.[27] Aside from inhalation, aerosols can increase the risk of infection resulting from contact with mucous membranes, wounds, and broken skin.[28,29] SOPs that limit activities with a potential for creating aerosols, or that have these activities performed in a **primary containment device** (e.g., **biological safety cabinet** [BSC]), can reduce the risk of exposure or release.

- ***In vivo*** activities:

  Additional **personal protective equipment** (PPE) (e.g., respirators, head covers, solid-front gowns with tight-fitting wrists, disposable sleeves) is worn based on LRAs. For example, additional PPE is worn for *in vivo* activities that involve exposing animals to infectious forms of a fungus and in **post mortem rooms** when working with **regulated animals** and tissues.

- Training and experience:

  Personnel training raises awareness of the hazards related to dimorphic fungi; however, the ability to differentiate certain dimorphic fungi is only achieved through their extensive study. Less experienced personnel may have difficulty distinguishing dimorphic fungi when they are in certain forms; therefore, it is important to take into consideration the level of personnel expertise and experience (e.g., assessed by the frequency at which they work with such fungi) when conducting LRAs.

- Medical surveillance:

  The long incubation periods of certain fungi and their low prevalence in non-endemic areas can lead to misdiagnosis.[30,31] The facility's medical surveillance program can determine the mitigation measures that can be implemented to facilitate diagnosis of an LAI, and develop post-exposure response plans, including medical follow-up. Facilities where samples containing RG3 fungi are routinely handled or stored can also inform personnel of the symptoms of infection during training. Facilities where high-risk activities are performed can implement additional measures such as assessing the immune status of the personnel for non-indigenous fungi (e.g., frequency of testing is based on the LRA) and informing the local healthcare facility if the fungus routinely handled or stored can cause a disease that requires special treatment. Most infections with fungi can be treated with antifungal therapies. Nevertheless, the medical surveillance program may include documenting the health status of facility personnel, as immunocompromised, human immunodeficiency virus (HIV)-positive, and diabetic individuals are at greater risk of severe disease following exposure to certain fungi.[32,33]

3.0 Activity Types

In addition to the risk group classification and the risks associated with certain forms of fungi (e.g., mould, spores), the containment level required to safely work with an RG3 fungus is determined based on the types of activities planned. The following activity descriptions are used in this Biosafety Directive to classify which activities can be performed at CL2 or CL3.

3.1 Activities with Inactivated Biological Material

Inactivated biological material is any primary specimen, product, or culture (e.g., pellets, blood, soil) that has been rendered non-infectious by an inactivation method. Inactivation methods must be validated to be effective against the specific fungus and its form (i.e., yeast, mould, spores) and must be routinely verified. Validation must be performed using a **representative load** and procedures must be designed to demonstrate the effectiveness of the method. Evidence such as manufacturer's claims, peer-reviewed articles, or protocols from professional associations, may be sufficient to serve as validation for certain methods (e.g., autoclaving, use of fungicidal concentrations of lactophenol cotton blue). Following validation, in-house verification of these methods (e.g., with biological indicators) must be routinely performed using representative loads and materials.

Inactivation methods can include nucleic acid extraction, heat, and chemicals, as well as irradiation for certain fungi.[34,35] The inactivation must be performed at the containment level required for the fungus and/or the type of specimen. If the inactivation cannot be performed at the required containment level, the inactivation procedure must be performed in a sealed container (e.g., irradiation, addition of chemical through a rubber septum cap) or the material must be sent to a facility where inactivation can be safely performed.

Examples of activities with inactivated biological material include antigen assays, nucleic acid extractions, and extraction procedures that are effective in eliminating viable cells for matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry.

Once biological material has been inactivated, subsequent laboratory procedures with the inactivated material are not regulated by the PHAC or the CFIA.

3.2 Identification Activities

Diagnostic facilities perform identification activities with the intent of identifying a pathogen in a primary specimen for diagnosing and monitoring infection and disease. Primary specimens may be collected from the environment (e.g., soil sample, tree bark, air filter) or collected directly from humans or naturally exposed animals (i.e., not resulting from *in vivo* research studies). In a diagnostic setting, identification activities with primary specimens often allow the pathogen to remain in its natural environment; however, some identification activities with primary specimens may result in cultivating, collecting, or extracting the pathogen. The following section provides examples for both of these types of activities, as well as regulatory implications and containment considerations in both circumstances. Regulatory implications and containment considerations for quality control samples and proficiency panels are also discussed. Biosafety recommendations for human diagnostic activities can be found in the Canadian Biosafety Guideline *Human Diagnostic Activities*.[36]

3.2.1 Identification Activities Involving Primary Specimens Only

Identification activities with primary specimens that do not involve cultivation, collection, or extraction of a human pathogen are excluded from the HPTA [HPTA 4]. As a result, diagnostic facilities where these types of activities are performed do not require a licence issued by the PHAC [HPTR 27(1)(a)]; however, if the identification activities involve imported material containing a terrestrial animal pathogen they require:

- a terrestrial animal pathogen permit from the PHAC under section 51(a) of the HAR if the pathogen is in a primary specimen from a human and is considered a terrestrial animal pathogen; or
- a terrestrial animal pathogen permit from the CFIA under section 51(b) of the HAR if the pathogen is in an animal sample or is also considered a pathogen that causes an EAD or an FAD, or an aquatic animal, plant or bee pathogen.

Where identification activities with primary specimens are performed, it is recommended that **good microbiological laboratory practices**, routine practices, and universal precautions be followed.[37] It is also recommended that identification activities be performed at minimum in a CL2 facility when possible. While activities with primary specimens generally present a lower risk of exposure than handling concentrated or propagated material, handling primary specimens containing certain forms of fungal species still poses a risk. For example, primary specimens sampled from the environment (e.g., soil) can potentially contain a high concentration of sporulating moulds that are easily transmissible through inhalation of the released spores. In such a circumstance, the use of a primary containment device or PPE is recommended to reduce the risks associated with the handling of environmental samples.[38] Such protection may also be required in certain laboratories under federal and/or provincial/territorial Occupational Health and Safety legislation.[39,40,41,42]

Examples of identification activities involving primary specimens include centrifugation of primary specimens (e.g., to separate plasma while not creating a pellet or concentrating a pathogen), enzyme-linked immunosorbent assay, antigen or antibody detection, sample preparation for direct microscopic examination, nucleic acid amplification test, initial inoculation of culture media, inactivation of non-propagated and non-concentrated samples in open vessels, and storage of primary specimens for future reference.

Identification activities with primary specimens where RG3 fungi have not been intentionally propagated, concentrated, or extracted are not regulated by the PHAC.

3.2.2 Identification Activities Involving Propagated or Concentrated Material

Identification activities that involve the cultivation, collection, or extraction of a pathogen are regulated under the HPTA; therefore, diagnostic facilities where such identification activities are performed require a licence issued by the PHAC, unless the production of a human pathogen is done using a sealed container that prevents the release of the human pathogen and that is decontaminated before its disposal or reuse [HPTR 27(1)(b)]. This exemption from the licensing requirement does not apply to prions or SSBAs.

If an RG3 fungus is unintentionally cultivated, collected, or extracted during identification activities in a facility that does not possess a licence for the RG3 fungus, the facility is considered to be in possession of an inadvertently produced RG3 fungus. In such cases, the PHAC must be notified and the facility will have 30 days to transfer or dispose of the material [HPTR 4(1)(f)]. Depending on the type of RG3 fungus, the activities conducted, and the mitigation measures used, personnel may have been exposed during the identification process. In licensed facilities, exposure incidents must be reported to the PHAC [HPTA 13]. Facilities exempted from the licensing requirement are also encouraged to submit a voluntary notification report should an exposure take place.

To avoid routinely having to notify the PHAC for inadvertent possession, facilities may apply for a licence listing the RG3 fungi most likely to be identified during routine activities (e.g., when diagnostic laboratories are located in endemic areas). This RG3 licence will include a condition stipulating that identification activities involving concentrated or propagated material can be safely performed at CL2 when facilities meet the additional biosafety requirements specified in Section 5.1 of this Biosafety Directive.

Facilities where identification activities involving the cultivation, collection, or extraction of RG3 fungi are performed are regulated by the PHAC and require an RG3 licence, unless they are exempted from the licensing requirement [HPTR 27(1)(b)].

3.2.3 Quality Control Samples and Proficiency Panel Specimens

Identification activities often involve the handling of non-inactivated quality control samples and proficiency panel specimens that mimic primary specimens containing pathogens. Such materials are often used to monitor a laboratory's continuing proficiency; more specifically to calibrate instruments, to determine the performance of laboratory tests or measurements, or to confirm the continued accuracy of diagnostic assays. Their importation always requires a permit issued under the HAR by the CFIA or the PHAC. While these materials are also regulated under the HPTA, their handling does not require a licence issued under the HPTA if they do not contain a prion or an SSBA (e.g., *Coccidioides* spp.), if the activities do not include propagation or culture, or if propagation or culture is performed in a sealed container [HPTR 27(1)].[43] If quality control samples and proficiency panel specimens contain a prion or an SSBA, a licence is always required for their handling or storing, and all applicable SSBA requirements are to be met when the material contains an SSBA.[43]

A licence is required when quality control samples and proficiency panel specimens for RG3 fungi are propagated or concentrated in a container that is not sealed, or contain a prion or an SSBA such as *Coccidioides* spp.

3.3 *In Vitro* and *In Vivo A*ctivities

In vitro activities that include the intentional cultivation, collection, extraction, propagation, or culture of RG3 fungi are regulated by the PHAC under the HPTA and require an RG3 licence, unless they are exempted from the licensing requirement [HPTR 27(1)]. Examples of *in vitro* activities are provided in Table 3 of this Biosafety Directive.

In vivo activities include experimentally exposing an animal to an RG3 fungus and the subsequent handling of the animal (and specimens obtained from it) in a research setting. *In vivo* activities are to be performed in accordance with a licence issued under the HPTA. Examples of *in vivo* activities are provided in Table 3 of this Biosafety Directive.

The importation of an animal carrying a terrestrial animal pathogen (i.e., an animal naturally or experimentally exposed to a terrestrial animal pathogen) or an animal-derived sample carrying a terrestrial animal pathogen requires an import permit issued by the CFIA under the HAR. The handling of animals that have been naturally exposed to an RG3 fungus or of samples obtained from naturally infected animals does not require a licence if the animal or sample is not imported and the RG3 fungus is not intentionally propagated or concentrated. Biosafety recommendations for diagnostic activities involving exposed animals and animal-derived samples can be found in the Canadian Biosafety Guideline *Veterinary Practices: Physical Design and Operational Practices for Diagnostic Activities*.[44]

4.0 Containment Level Requirements for the Safe Handling of Risk Group 3 Fungi

The containment level requirements for the safe handling of RG3 fungi depend on several factors:

- the fungal species;
- the form of the fungus and the potential for aerosolization of spores; and
- the activities being performed.

In this Biosafety Directive, containment level requirements for identification activities involving propagated or concentrated material are specified in a separate table from containment level requirements for *in vitro* and *in vivo* activities.

4.1 Minimum Containment Level for Identification Activities Involving Propagated or Concentrated Material

When identification activities are performed at CL2 and involve propagated or concentrated material, additional biosafety requirements are implemented to mitigate the risk of exposure. As indicated in Table 2, the operational practice requirements outlined in Section 5.1, such as the use of PPE or a BSC, are particularly important when there is a high risk of exposure to infectious aerosols.

Activities with a high risk of exposure to infectious aerosols include:

- laboratory procedures with a high potential of generating infectious aerosolized particles or liquid droplets;
- activities that involve a biological material that is at risk of being, or producing, a readily aerosolized material (e.g., spores, filamentous forms) based on:
    - its source (e.g., environmental sample);
    - its incubation conditions (e.g., that may generate sporulating mould forms);
    - the laboratory procedures.

Containment requirements for activities with *Cryptococcus gattii* were determined based on evidence that conversion from a yeast to a filamentous form is unlikely to occur during routine identification activities.[26,45,46]

Table 2: Minimum containment level for identification activities with propagated or concentrated material

Examples of activities	Minimum containment level			
	Cryptococcus gattii		Other RG3 fungi covered by this Biosafety Directive *	
	Low risk of infectious aerosols [a]	High risk of infectious aerosols [b]	Low risk of infectious aerosols [a]	High risk of infectious aerosols [b]
Non-propagative identification activities • Procedures with human or animal diagnostic specimens to concentrate an RG3 fungus (e.g., centrifugation).	CL2	CL2 with additional biosafety requirements [c]	CL2 with additional biosafety requirements [c]	CL2 with additional biosafety requirements [c]
Propagative identification activities • Handling cultures of an unknown RG3 fungus from primary specimens; • Performing a validated inactivation procedure on diagnostic cultures for analysis (e.g., microscopy, DNA diagnostic method, MALDI-TOF mass spectrometry) or before disposal; • Processing sealed diagnostic cultures for packaging and distribution to other facilities (e.g., to reference laboratories).	CL2	CL2 with additional biosafety requirements [c]	CL2 with additional biosafety requirements [c]	CL2 with additional biosafety requirements [c]

a Activities with a low risk of infectious aerosols are those that only involve non-readily aerosolized materials such as yeasts, spherules, and yeast-like cells in a state unlikely of becoming readily aerosolized material (e.g., when incubation conditions are kept above 37°C) and that only include procedures with a low potential of generating infectious aerosolized particles or liquid droplets.

b Activities with a high risk of infectious aerosols include laboratory procedures with a high potential of generating infectious aerosolized particles or liquid droplets and/or activities that involve a material that is, or might produce, a readily aerosolized material (e.g., spores, filamentous forms) based on the source (e.g., environmental sample), the incubation conditions (e.g., below 37°C), and the laboratory procedures.

c With additional biosafety requirements as described in Section 5.1.

* Note that SSBAs are not covered by this directive, and activities with SSBAs (e.g., *Coccidioides immitis*, *Coccidioides posadasii*) must always meet the requirements specified in the CBS for SSBAs handled at CL3.

4.2 Containment Level Requirements for *In Vitro* and *In Vivo* Activities

Where the risk of exposure to infectious aerosols is low, *in vitro* activities and *in vivo* activities with *Cryptococcus gattii* and RG3 species of *Blastomyces*, *Histoplasma*, and *Paracoccidioides* may be safely performed at CL2 with additional biosafety requirements (outlined in Section 5.0). Containment level requirements for *in vitro* and *in vivo* activities with *Cryptococcus gattii* and RG3 species of *Blastomyces*, *Histoplasma*, and *Paracoccidioides* are described in Table 3.

Activities with a low risk of exposure to infectious aerosols are activities that:

- only include procedures with a low potential of generating infectious aerosolized particles or liquid droplets;
- only involve materials that are not readily aerosolized such as yeasts, spherules, and yeast-like cells :
    - for which complete conversion (i.e., absence of filamentous forms) is confirmed;
    - that are unlikely of becoming aerosolized based on incubation conditions (e.g., cultures are kept above 37^{o}C), procedures, and the state of the material (e.g., not a powder).

In vitro and _in vivo_ activities must be performed at CL3 (or CL3-Agriculture [CL3-Ag], based on the activity) if they involve handling _Cladophialophora bantiana_, _Coccidioides immitis_, _Coccidioides posadasii_, _Rhinocladiella mackenziei_ or any other RG3 fungus not listed in Table 3.

Table 3: Minimum containment level for *in vitro* and *in vivo* activities with *Cryptococcus gattii* and RG3 species of *Blastomyces*, *Histoplasma*, and *Paracoccidioides*

Examples of activities		Minimum containment level			
		Cryptococcus gattii		**RG3 *Blastomyces* spp., RG3 *Histoplasma* spp., RG3 *Paracoccidioides* spp.**	
		Low risk of infectious aerosols [a]	**High risk of infectious aerosols [b]**	**Low risk of infectious aerosols [a]**	**High risk of infectious aerosols [b]**
***In vitro* activities** • Preparing cultures and subcultures (e.g., control extracts for DNA probe tests, antifungal susceptibility testing); • Research activities (e.g., mating experiments); • Processing identified cultures for packaging and distribution to other facilities; • Preparatory work for *in vivo* activities (e.g., preparing inoculum).		CL2	CL2 with additional biosafety requirements[c]	CL2 with additional biosafety requirements[c]	CL3
***In vivo* activities** • Inoculating or exposing animals (e.g., via aerosol, contact); • Collecting specimens from experimentally exposed animals (e.g., nasal/throat swab, blood, bronchial lavage).	**Small animal containment zone**	CL2	CL2 with PHAC-approved LRA [d]	CL2 with additional biosafety requirements [c]	CL2 with PHAC-approved LRA [d]
	Large animal containment zone	CL2-Ag with PHAC-approved LRA [e]	CL2-Ag with PHAC-approved LRA [e]	CL2-Ag with PHAC-approved LRA [e]	CL2-Ag with PHAC-approved LRA [e]

a Activities with a low risk of infectious aerosols are activities that only involve non-readily aerosolized material such as yeasts, spherules, and yeast-like cells in a state unlikely of becoming readily aerosolized material (e.g., when incubation conditions are kept above 37°C) and that only include procedures with a low potential of generating infectious aerosolized particles or liquid droplets.

b Activities with a high risk of infectious aerosols include laboratory procedures with a high potential of generating infectious aerosolized particles or liquid droplets and/or activities that involve a material that is, or may produce, a readily aerosolized material (e.g., spores, filamentous forms) based on the source (e.g., environmental sample), the incubation conditions (e.g., below 37°C), and the laboratory procedures.

c With additional biosafety requirements as described in Section 5.1.

d Following approval of the LRA by the PHAC, work in CL2 **small animal containment zones** (SA zones) may be authorized with additional biosafety requirements as described in Section 5.2.

e Following approval of the LRA by the PHAC, work in **large animal containment zones** (LA zones) may be authorized in CL2-Agriculture (CL2-Ag) with additional biosafety requirements as described in Section 5.2.

5.0 Additional Operational Practice Requirements

The CBS requirements listed below (designated "CBS3 R" followed by the requirement number) are reasonable precautions to be taken for activities listed in Table 2 and Table 3 that can be performed at CL2, including CL2 SA zones with additional biosafety requirements, or CL2-Ag with additional biosafety requirements. As a condition of licence or terrestrial animal pathogen permit, these requirements are to be followed in addition to the applicable minimum physical containment requirements, operational practice requirements, and performance and verification testing requirements specified in Chapters 3, 4, and 5 of the CBS for CL2 or CL2-Ag. The following requirements apply to all personnel entering the **containment zone**.

5.1 Additional Operational Practice Requirements for Containment Level 2

- CBS3 R4.5.21 – All activities involving open vessels of regulated materials to be performed in a BSC or other primary containment device.

  This operational practice is required since manipulations with regulated materials have the potential to generate infectious aerosols. BSCs and other primary containment devices provide effective **primary containment** to protect personnel from exposure to, and prevent release of, infectious aerosols (e.g., readily aerosolized material, aerosolized particles, liquid droplets), as well as help to limit dispersal in the event of a spill.

- CBS3 R4.5.24 – 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.

  Sealed safety cups and rotors for centrifugation prevent the release of infectious aerosols that may be created during centrifugation. To protect individuals from exposure to any aerosolized material (e.g., readily aerosolized material, aerosolized particles, liquid droplets) and prevent the spread of contamination, sealed safety cups and rotors are also unloaded in a BSC or other primary containment device using specific mechanisms (e.g., SOPs, equipment, devices).

- CBS3 R4.3.4 – Respirators to be worn where there is a risk of exposure to infectious aerosols that can be transmitted by inhalation, as determined by an LRA.

  Wearing a fitted and appropriate respirator (e.g., N95, N99, N100) protects personnel from exposure via the inhalation route when an LRA determines there is a risk of infectious aerosols (e.g., readily aerosolized material, aerosolized particles, liquid droplets) that are not contained in a primary containment device (e.g., BSC, HEPA-filtered cage).

5.2 Additional Operational Practice Requirements for Containment Level 2 Animal Containment Zones Following Approval of the Local Risk Assessment

- CBS3 R4.5.21 – 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*.]

  This operational practice is required since manipulations with regulated materials have the potential to generate infectious aerosols. BSCs and other primary containment devices provide effective primary containment to protect personnel from exposure to, and prevent release of, infectious aerosols (e.g., readily aerosolized material, aerosolized particles, liquid droplets), as well as help to limit dispersal in the event of a spill.

- CBS3 R4.5.24 – 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.

  Sealed safety cups and rotors for centrifugation prevent the release of infectious aerosols that may be created during centrifugation. To protect individuals from exposure to any aerosolized material (e.g., readily aerosolized material, aerosolized particles, liquid droplets) and prevent the spread of contamination, sealed safety cups and rotors are also unloaded in a BSC or other primary containment device using specific mechanisms (e.g., SOPs, equipment, devices).

- CBS3 R4.3.4 – Respirators to be worn where there is a risk of exposure to infectious aerosols that can be transmitted by inhalation, as determined by an LRA.

  Wearing a fitted and appropriate respirator (e.g., N95, N99, N100) protects personnel from exposure via the inhalation route when an LRA determines there is a risk of infectious aerosols (e.g., readily aerosolized material, aerosolized particles, liquid droplets) that are not contained in a primary containment device (e.g., BSC, HEPA-filtered cage).

- CBS3 R4.4.16 – Activity-specific PPE or an additional layer of PPE to be donned prior to beginning the activity in the containment zone.

  Specific activities with regulated materials may result in specific risks. LRAs determine the PPE required to protect personnel from exposure to regulated materials when performing specific activities. This PPE may be different or in addition to the PPE required to enter the containment zone. Wearing an additional layer of protective clothing (e.g., second pair of gloves, back closing waterproof gown) protects the personnel handling a fungus from being exposed if the outer layer of protective clothing becomes compromised or contaminated.

6.0 Additional Biosafety Recommendations

In addition to the containment level requirements, facilities can implement additional biosafety recommendations based on LRAs to mitigate the risks of exposure to pathogens and release from containment. For example, diagnostic laboratories may implement additional mitigation measures based on the probability of routinely identifying an RG3 fungus. The following is a non-exhaustive list of mitigation measures that can be implemented and are highly recommended in areas where RG3 fungi are handled.

- Implement operational procedures to reduce the risk of aerosol production and limit or substitute procedures more likely to produce infectious aerosols (e.g., avoid slide cultures, use the safest technique possible when performing a tease mount or a scotch tape transfer protocol for microscopy analysis);[47,48]

- Require that all personnel wear additional PPE (e.g., respirators) in areas where procedures with a potential for generating infectious aerosols are taking place;

- Work within a primary containment device (e.g., BSC) when activities or sample types have a potential for creating infectious aerosols (e.g., spores) or have the potential for increased pathogenicity (e.g., new strain);

- Always open primary containers and secondary containers (e.g., sealed cups or rotors) of fungus cultures in a primary containment device (e.g., BSC);

- Perform packaging of material into secondary containers for transportation in a primary containment device (e.g., BSC), with surface decontamination of the secondary container prior to removal from the BSC;

- Perform all inactivation procedures of moulds within a BSC or sealed container;

- Culture fungi in sealed containers (e.g., agar slants with screw caps) to prevent accidental opening or spore release;

- Culture fungi in receptacles or containers made of plastic instead of glass to reduce the risks of exposure resulting from accidental breakage;

- Enclose primary containers of fungal cultures in a closed (e.g., gas-permeable) secondary container (e.g., plastic container with gas exchange membrane) to provide an additional physical barrier against release (e.g., when moving samples between a BSC and an incubator within the containment zone, when moving samples between containment zones);

- Avoid storing materials (e.g., tissues, bronchoalveolar lavage, fungi-containing samples, waste) at room temperature for extended periods of time, even within a primary containment device, as some RG3 fungi can grow even on routine bacteriology media in a short period of time;

- Refrigerate (at 4^{o}C) or freeze (at -20^{o}C) in sealed containers any material or waste (e.g., infected animal carcasses) that is stored long-term to prevent mould growth and sporulation of fungi;

- Implement procedures for temperature monitoring of equipment (e.g., incubators) to maintain required temperatures to prevent conversion to spore-producing non-yeast forms (e.g., mould forms);

- Prepare slides for morphological studies using mounting liquid or staining procedures that have been previously validated (i.e., in-house or with body of evidence) to inactivate the fungus handled (e.g., fungicidal concentrations of lactophenol cotton blue, DNA extraction with guanidium lysis buffer);

- Disinfect work areas with an effective (i.e., validated) high-level fungicidal disinfectant (e.g., 0.5%-1.0% sodium hypochlorite [bleach] solution, accelerated 0.5% hydrogen peroxide) and allow an appropriate contact time. Phenolic-based germicides and 70% ethanol are not effective against all forms of fungi (e.g., ascospores).[49,50] As a result, contact transmission may occur when infectious aerosols generated during work activities settle on a surface that is inadequately decontaminated afterward;[51]

- Develop and implement SOPs for spills and accidents involving specimens containing fungi (e.g., allowing sufficient time for aerosols to settle or be cleared prior to re-entering, posting warning signs on doors, and decontaminating with 0.5% bleach for an appropriate contact time).

7.0 Contact and Additional Information

Please note that this Biosafety Directive is based on current scientific evidence and is subject to review and change as new information becomes available. If this Biosafety Directive is amended, the PHAC will communicate the updated information to the impacted regulated parties and post the amended Biosafety Directive 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

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

8.0 Glossary

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 *Canadian Biosafety Handbook* (CBH); therefore, some definitions may not be applicable to facilities that fall outside of the scope of the CBS.

Biological safety cabinet (BSC)	A primary containment device that provides protection for personnel, the environment, and the product (depending on the 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.
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 regulated materials safely in laboratory, large scale production, and animal work environments. There are four containment levels ranging from a basic laboratory (i.e., Containment Level 1 [CL1]) to the highest level of containment (i.e., 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., 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 Containment Level 3 [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.
Controlled activities	Controlled activities include knowingly producing (e.g., cultivating, extracting), handling, possessing, storing, using, transferring, importing, exporting, or disposing of a human pathogen or toxin (i.e., extracted, purified, concentrated, or propagated human pathogen or toxin) [Section 7(1) of the *Human Pathogens and Toxins Act*].
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 this Biosafety Directive, “cell culture” refers to cells derived from a human or animal source.

Decontamination	The process by which materials and surfaces are rendered safe to handle and reasonably free of microorganisms, toxins, or prions; this may be accomplished through disinfection, inactivation, or sterilization.
Dimorphic fungus (fungi)	A fungus that can exist in different forms, one being yeast spherule, or muriform cells, and the other being hyphae, mould or spores.
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.
Foreign animal disease (FAD)	A disease that appears in the World Organisation for Animal Health (WOAH) 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.
Good microbiological laboratory practices	A basic laboratory code of practice applicable to all types of activities with biological material. These practices serve to protect workers and prevent contamination of the environment and the samples in use.
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 organism (e.g., studying the effect of antibiotic treatment in animal models).
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 one 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.
Mould	Filamentous fungi with a phenotype of branching chains of cells (hyphae) that can produce spores.
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: a) under section 18 of the *Human Pathogens and Toxins Act* to conduct one or more controlled activities with human pathogens or toxins; and/or b) under paragraph 51(a) of the *Health of Animals Regulations* for the importation into Canada of terrestrial animal pathogens (except for emerging animal disease [EAD] pathogens and non-indigenous terrestrial animal pathogens). "Licence" is also used in this context.
Pathogen risk assessment	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).

Performance and verification testing requirements	Performance and verification tests that are necessary to demonstrate compliance with the physical containment requirements 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 biological material, 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 materials, as specified in Chapter 3 of the *Canadian Biosafety Standard*.
Post mortem room	A room within the containment zone where necropsies and dissections are conducted on animals outside a primary containment device.
Primary containment	The first level of physical barriers designed to contain biological material, and prevent its release. This is accomplished by the provision of a device, equipment, or other physical structure situated between the biological material 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 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 and ventilated cage racks.
Propagation	The act of multiplying pathogens under controlled laboratory conditions.
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 the *Human Pathogens and Toxins Regulations*); • 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 the *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 the *Human Pathogens and Toxins Regulations*); • terrestrial animal pathogens (under the *Health of Animals Act* [HAA] and the *Health of Animals Regulations* [HAR]); • terrestrial animal pathogens in animals, animal products, animal by-products, or other organisms (under the HAA and HAR).
Representative load	A simulation batch of materials of a particular load type (e.g., plastics, waste, liquids, carcass), including mixed load types (e.g., containing pipette tips, agar plates and gloves), used to validate a decontamination method for routine loads. The quantity that would be decontaminated in a single load can be a defined amount (e.g., 6 lab coats), size (e.g., an autoclave bag 2/3 full) or weight (e.g., 5 kg).
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 subsection 10(2) of the HPTR. *Coccidioides* spp. fungi are considered SSBAs.
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.

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 permit	A permit issued by the Public Health Agency of Canada or the Canadian Food Inspection Agency for the importation into Canada [under paragraph 51(a) and (b) of the *Health of Animals Regulations* (HAR)] or transfer [under paragraph 51.1(a) of the HAR] 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).
(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*.
Yeast	Single-celled fungi (spherules and muriform cells) that reproduce asexually by budding or fission.

9.0 References and Resources

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