Public Health Agency of Canada | Agence de la santé publique du Canada

# Biosafety Directive for *Mycobacterium tuberculosis* Complex (MTBC)

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# ABBREVIATIONS

| | |
|---|---|
| AFB | Acid-fast bacilli |
| BSC | Biological safety cabinet |
| CBH | *Canadian Biosafety Handbook* |
| CBS | *Canadian Biosafety Standard* |
| CFIA | Canadian Food Inspection Agency |
| CL | Containment Level (e.g., CL2, CL3, CL4) |
| CL3-Ag | CL3 large animal containment zone |
| CSF | Cerebrospinal fluid |
| HIV | *Human immunodeficiency virus* |
| LRA | Local risk assessment |
| MDR | Multidrug-resistant |
| MGIT | Mycobacteria Growth Indicator Tube |
| MTBC | *Mycobacterium tuberculosis* Complex |
| NaOH | Sodium hydroxide |
| PPE | Personal protective equipment |
| PHAC | Public Health Agency of Canada |
| ppm | Parts per million |
| RG | Risk group (e.g., RG2, RG3, RG4) |
| SOP | Standard operating procedure |
| TB | Tuberculosis |
| XDR | Extensively drug resistant |

# 1.0 BACKGROUND

The handling or storing of pathogens, toxins, and other regulated infectious material (i.e. animals animal products or by-products [e.g. tissue, serum] or other substances that may carry an animal pathogen or parts thereof) necessitates an awareness and application of biosafety and biosecurity practices. The release of human and animal pathogens and toxins from laboratories or other containment zones may pose a risk to public health, animal health, or both. Personnel can minimize the risks associated with pathogens or toxins through the application of appropriate biosafety and biocontainment principles and practices.

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

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

The pathogens found within the *Mycobacterium tuberculosis* complex (MTBC) are examples of RG3 pathogens where the pathogen risk assessments have been revisited by the PHAC in conjunction with MTBC specialists, based on current risks associated with activities involving these pathogens. It has been determined that CL3 is required for research and other higher risk activities with MTBC, but that certain diagnostic activities can be safely conducted at CL2 with additional biosafety requirements, as specified in this directive. This biosafety directive is intended to provide a comprehensive overview of the risk assessment outcomes, subsequent containment level decisions, and considerations that have been made for those working with MTBC. It describes the activities and MTBC sample types that can be handled at CL2 with additional physical containment and operational practices. The *Biosafety Directive for MTBC* is to be used in conjunction with the CBS.

# 2.0 PATHOGEN DESCRIPTION AND RISK GROUP

## 2.1 The *Mycobacterium tuberculosis* complex

MTBC refers to a group of *Mycobacterium* species known to cause tuberculosis (TB) in humans or animals. MTBC includes the following human and animal RG3 agents:

- *M. tuberculosis* (excluding strain H37Ra);
- *M. bovis* (excluding strain BCG);
- *M. africanum*;
- *M. caprae*;
- *M. canettii*;
- *M. pinnipedii*; and
- *M. microti*.

*M. tuberculosis* strain H37Ra and *M. bovis* strain BCG are classified as human and animal RG2 agents.

Novel MTBC species recently described in the literature include *M. mungi*, *M. orygis*, and *M. suricattae*, isolated from banded mongooses, oryxes, and meerkats, respectively.[6,7,8,9] Furthermore, bacilli isolated from rock hyraxes and chimpanzees are thought to be animal-associated strains related to *M. africanum*, but these species are yet to be named.[6] Most cases of tuberculosis are caused by *M. tuberculosis* and, more rarely, by *M. bovis*, which is the main cause of zoonotic transmission of TB from cattle to humans. The other MTBC organisms are the cause of few cases of TB.

Mycobacteria are aerobic, non-spore forming, non-motile, acid-fast, weakly Gram-positive, and slightly curved or straight rods.[10] They have a thick, lipid-rich cell wall that accounts for the slow growth and resistance of these organisms to many harsh treatments, including alkaline conditions and detergents.

### 2.1.1 *Tuberculosis*

TB has plagued humans for thousands of years. Historically, it was commonly referred to as consumption. Following inhalation of the MTBC bacteria, small lesions develop in the lungs, which usually heal and form calcifications. In 90-95% of cases, host immune responses are sufficient to control the growth and spread of the MTBC bacteria, leading to latent TB infection that is asymptomatic. Less than 10% of healthy individuals with latent TB will develop active TB in their lifetime, with the majority developing it within the first five years after initial infection.[10,11,12,13]

Active TB is a chronic disease that progresses slowly and is characterized by pulmonary infiltrates and the formation of cavities in the lungs. Progression to active TB is associated with underlying medical conditions, recent infection (2% per year for the first 2 years following infection), children under 4 years of age, and the immunocompromised, especially those infected with human immunodeficiency virus (HIV). Active TB can also result from reactivation of latent TB.

Despite the availability of effective treatments, TB remains a major global public health problem due to the limited availability of drugs in some countries, the delayed or improper diagnosis of TB, the emergence of drug resistant TB, the HIV epidemic, and global migration.[11,14,15]

The clinical symptoms of active TB include cough, weight loss, night sweats, low grade fever, dyspnea, and chest pain.[10,16] Positive identification of active TB involves the detection of acid-fast bacilli (AFB) in stained smears from sputum samples or sputum/bronchoalveolar lavage cultures. Approximately 65% of individuals with untreated active TB will die within 5 years.[16]

Pulmonary TB is the most common form of TB worldwide, although extrapulmonary TB can also occur in up to a third of cases, often in conjunction with pulmonary TB.[16] Extrapulmonary TB affects the lymph nodes, pleura, genito-urinary tract, bones, joints, meninges, gastro-intestinal tract, peritoneum, and pericardium. Children and immunocompromised individuals are at higher risk of developing extrapulmonary TB.

Miliary TB is characterized by the wide dissemination of small lesions in the lungs, which then spread to other organs.[15] The clinical symptoms of miliary TB are high and prolonged fever, night sweats, dry cough, malaise, splenomegaly, and occasionally skin lesions.[17] Miliary TB is a more serious form of TB. If left untreated, it will lead to tuberculous meningitis in about 50% of cases, which results in a high mortality rate.

TB caused by *M. bovis* is clinically identical to that caused by *M. tuberculosis*; however, non-pulmonary lesions are more common since the source of infection is most often the consumption of contaminated milk or meat.[10,18] Farmers, veterinarians, abattoir workers, and meat inspectors are at higher risk of pulmonary lesions since they are more likely to be exposed to contaminated droplets from animals.[17]

### 2.1.2 *Drug Resistant TB*

Multidrug-resistant TB (MDR-TB) refers to TB strains with the ability to resist the two most powerful anti-TB drugs: isoniazid and rifampicin. Extensively drug resistant TB (XDR-TB) refers to TB strains with the ability to resist isoniazid and rifampicin, plus any of the fluoroquinolones (e.g., ofloxacin or moxifloxacin) and at least one of three injectable second-line drugs (i.e., amikacin, capreomycin, or kanamycin). The treatment of MDR-TB and XDR-TB infections requires the use of second-line anti-TB drugs and takes substantially longer than non-drug resistant TB. Second-line drugs are more expensive and have more adverse reactions than the first-line drugs used for the treatment of non-resistant strains of TB.[19]

Drug resistance may develop in a patient being treated for active TB due to the inappropriate administration of TB medication (e.g., missed or inappropriate doses, not completing the full course of medication). Drug resistant TB can also occur via transmission from someone infected with a resistant strain.

### 2.1.3 *Laboratory Acquired Infections*

MTBC is a recognized hazard to laboratory personnel, among whom the incidence of tuberculosis has been reported to be three times higher than that of personnel not working with the agent.[20] Given the amount of pulmonary disease in the general population, the mode of transmission of TB, its long incubation period, and opportunities for infection outside of the laboratory, the diagnosis of laboratory acquired TB is difficult. Still, surveys have found that the incidence of TB among laboratory workers processing TB samples is 3-9 times higher than that found in other occupations and the general population.[21,22]

The infectious dose of *M. tuberculosis* is low for humans (i.e., 1-10 bacilli carried in 1-3 droplet nuclei).[7] Although exposure to infectious aerosols is the most probable hazard encountered, incidents of tuberculosis lesions resulting from accidental inoculation of laboratory personnel have been described.[10] Litter from experimentally infected guinea pigs and mice has also served as a source of infectious aerosols.[16] More information on the status of tuberculosis in Canada can be found in the PHAC's *Canadian Tuberculosis Standards,* 7th edition, 2013.[23]

## 2.2 Risk Group Determination

Risk group determination is based on the outcome of a pathogen risk assessment which assesses the inherent risk of a pathogen, considering risk factors such as pathogenicity, communicability, and availability of effective treatment. The categories range from RG1 (low individual and low community risk) to RG4 (high individual and high community risk). A full list of pathogen risk factors and the definitions of the risk groups can be found in the most recent version *Canadian Biosafety Handbook* (CBH).[24]

In collaboration with a group of experts, the PHAC has performed pathogen risk assessments for MTBC and have determined that MTBC species (excluding the strains *M. bovis* BCG and *M. tuberculosis* H37Ra that have been attenuated to the extent that they no longer meet the risk profile of an RG3 pathogen) are RG3 human and animal pathogens. The CBS defines an RG3 pathogen as one that poses a high risk to the health of individuals and/or animals and a low risk to public health. RG3 pathogens are likely to cause serious disease in a human or animal. Effective treatment and preventive measures are usually available and the risk of spread of disease caused by these pathogens is low for the public.[1]

# 3.0 Containment Level Requirements

## 3.1 CBS Containment Zone Overview

The CBS reflects the harmonization of the operational and physical containment requirements for human and terrestrial animal pathogens and toxins. This section will reiterate the types of work that are included for each containment column (e.g., CL2) and the distinction between the different types of containment zones. More detailed information can be found in the CBS.

An animal containment zone refers to a series of co-located animal rooms/cubicles, as well as associated corridors and support rooms (e.g., storage and preparation areas) of equal containment level, serviced by a single entry/exit. In a small animal containment zone (SA zone), animals are housed in primary containment caging. The room encompassing these cages is referred to as an "animal room". Generally, SA zones follow the same requirements as those for laboratory work areas and are represented under the CL2 or CL3 columns.

In a large animal containment zone (LA zone), the room itself provides the primary containment. The room or space housing the animals is referred to as an "animal cubicle". When small-sized animals are not housed in primary containment caging (e.g., chickens in pens), they are considered to be housed in an LA zone where the room and containment requirements provide the appropriate risk mitigation. The CBS provides distinct columns for LA zones at CL2 and CL3, labelled CL2-"Agriculture" (CL2-Ag) and CL3-Ag, respectively.

## 3.2 Containment Level Requirements

The containment level determination provides the end-user with a description of the minimum physical containment and operational practices required for handling pathogens safely in a laboratory setting. Containment levels range from a basic laboratory (CL1) to the highest level of containment (CL4). For the majority of pathogens, the containment level and risk group of the pathogen are the same (e.g., RG2 pathogens are handled at CL2), but there are some exceptions. For example, the PHAC may identify certain activities involving RG3 pathogens not known to be transmissible by inhalation, or activities with lower risk of aerosol production that can be performed with reduced or specific physical and operational requirements.

The risk assessments for MTBC clearly indicate that the pathogens have the ability to produce serious disease in humans. They also indicate that:

- the principle route of MTBC infection is through the inhalation of infectious aerosols;
- mycobacteria are quite resistant and able to survive several months on inanimate objects when protected from light; and,
- the infectious dose for humans is low (1 to 10 organisms).

However, the assessments also indicate that certain diagnostic activities with patient-derived primary specimens and activities with inactivated MTBC specimens and cultures are of lower risk and can be safely performed at CL2 with additional biosafety requirements.

While this directive is based on the risks associated with the types of specimens and laboratory processes, laboratories are encouraged to assess any additional risks based on their own particular situation,[13,25] including:

- laboratory throughput and staff workload: the greater they are, the greater the risks;
- location of TB work relative to other laboratory activities: work in a large diagnostic lab space entails more risk than work in a small separate room;
- number of process steps with the potential for generating aerosols: a single pipetting step to aliquot to a tube is of lower risk than a process requiring several pipetting steps with infectious material; and
- predicted TB prevalence among the sampled population: a laboratory performing workplace screening will be much less likely to come across positive specimens than one testing an at-risk population.

## 3.3 Specimen and Culture Type

The different types of specimens and cultures that facilities working with MTBC may encounter result in different risks. For the purpose of this directive, specimens and activities have each been classified into four broad categories described below.

3.3.1 *Inactivated biological material*: These include products (e.g., pellets, concentrated MTBC) concentrated from primary specimens (e.g., sputum, bronchoalveolar lavage) or propagated MTBC (i.e., cultured) that have been inactivated using a validated method. The inactivation must be performed at the containment level required for the pathogen and type of specimen (e.g., cultures of

MDR TB are inactivated at CL3, while a sealed diagnostic culture can be inactivated at CL2). MTBC inactivation methods can include heat or chemicals (e.g., ethanol-phenol). The ability of a nucleic acid extraction method to inactivate a pathogen must be validated and verified in house.

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

3.3.2 *Primary specimens*: These include specimens collected directly from patients or animals for the purpose of detecting or monitoring MTBC infection (e.g., sputum, CSF, urine, blood, and other body fluids, gastric and bronchial lavage). This type also includes products (e.g., concentrated isolates) resulting from primary specimen processing steps (e.g., NaOH digestion, pelleting, concentrating), up to and including the inoculation of liquid or solid media (e.g., MGIT™, Lowenstein-Jensen). Diagnostic specimens from naturally exposed animals (i.e., *not* resulting from *in vivo* studies) are also included in this category. Primary specimens will generally contain much lower concentrations of bacilli than cultures. Primary specimens containing a human pathogen are excluded from the HPTA and therefore do not require a Pathogen and Toxin Licence issued by the PHAC; however, the importation of primary specimens containing an animal or zoonotic pathogen does require an animal pathogen import permit issued by the CFIA.

3.3.3 *Propagated MTBC*: These include any sample where the pathogen has been propagated by culturing, whether on solid or in liquid media, including stock cultures of clinical isolates or reference strains of MTBC, as well as diagnostic cultures that show visible growth indicative of possible MTBC. Propagated and concentrated pathogens are under the authority of the HPTA, and require a Pathogen and Toxin Licence issued by the PHAC. Also, the importation of cultures of animal pathogens is under the authority of the HAA and HAR, and regulated by the PHAC or the CFIA.

3.3.4 *Multiple Drug Resistant (MDR), Extensively Drug Resistant (XDR) TB and novel resistant strains of MTBC*: Primary specimens or cultures confirmed to be positive for MTBC that is resistant to several or most effective anti-TB drugs. This may include follow-up specimens from a patient infected with MDR-TB or XDR-TB, the remainder of a primary specimen identified as MDR-TB or XDR-TB positive after testing of the material is completed, or a culture confirmed MDR-TB or XDR-TB positive. These strains are of higher risk, and have more stringent handling requirement. Only propagated or concentrated MDR and XDR TB are regulated by the PHAC.

## 3.4 Activities

In addition to specimen type, specific MTBC laboratory procedures can also influence the risks associated with laboratory activities. The following definitions provide examples of laboratory activities that can help classify new activities as they become available.

3.4.1 *Activities with inactivated biological material*: The killing or inactivation process renders the sample essentially free of MTBC organisms such that they are unlikely to be infectious. Inactivation using an effective, validated method must be performed either at the containment level of the specimen or culture (as indicated in Table 1), or before opening the culture tube (i.e., if it has been incubated at CL2 with additional biosafety requirements).

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

Examples of these activities include, but are not limited to, staining and reading inactivated-fixed slides, nucleic acid extraction and DNA probe extraction following heat kill.

3.4.2 *Non-propagative clinical/diagnostic activities with primary specimens*: These include activities performed on primary, patient-derived specimens that have not been cultured (propagated), for the purpose of diagnosing or monitoring MTBC infection. Only activities that aim to propagate, concentrate, or purify pathogens (i.e., MTBC) are regulated by the PHAC; therefore, diagnostic activities that do not increase the number or concentration of MTBC organisms in primary specimens are excluded from this directive. Nonetheless, it is strongly recommended that, at minimum, routine practices are followed in work areas, especially in health care environments, where primary specimens are handled to protect against the potential of exposure to any pathogens that may be present in the specimen.[26]

Examples of diagnostic activities include, but are not limited to, fixing, staining, and reading slides of primary specimens, centrifugation of primary specimens, digestion (e.g., by NaOH method), culture inoculation, and nucleic acid extraction or nucleic acid amplification (NAA) from primary specimens, pre heat kill.

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

Examples of these include, but are not limited to, culture, cultivation, or propagation of specimens likely to contain MTBC, processing of MTBC positive culture for packaging and distribution to laboratories, slide preparation and fixing directly from live cultures, drug susceptibility testing of MTBC probe positive cultures (liquid or solid media), biochemical testing of MTBC, and research activities involving MTBC cultures.

The following table describes the containment level required for containment zones working with MTBC based on sample type and activity. MTBC is categorized as a human and animal RG3 pathogen but work with this pathogen can be carried out safely at either CL2, CL2 with additional biosafety requirements, or CL3, depending on the sample type (e.g., primary specimen, culture) being handled and the activities being performed. See Section 4 for the additional biosafety requirements that are to be followed when working at CL2, and Section 5 for the additional biosafety considerations for working with MTBC. For a complete listing of the physical containment requirements, operational practice requirements, and performance and verification testing requirements, please refer to Chapters 3, 4, and 5 of the CBS.

## Table 1: Containment Levels for MTBC Based on Sample Type and Activity

| Sample Type and Activity | Minimum Containment Level Required | |
|---|---|---|
| | CL2 | CL3 |
| **Non-Propagative Clinical/Diagnostic Activities with Primary Specimens**<br>Examples of these activities include, but are not limited to:<br>• preparing diagnostic specimens with the goal of concentrating or isolating MTBC organisms (e.g., concentration and centrifugation of sample);<br>• inoculating liquid or solid culture medium such as Lowenstein-Jensen and MGIT™ with concentrated primary specimens | ■† | |
| **Propagative *in vitro* Activities**<br>Examples of these activities include, but are not limited to:<br>• culturing of specimens likely to contain MTBC pathogens (including subculturing);<br>• processing of MTBC positive cultures;<br>• preparing and fixing slide;<br>• nucleic acid and DNA probe extraction, prior to inactivation by a validated method (thermal or chemical);<br>• biochemical testing;<br>• drug susceptibility testing;<br>• general *in vitro* research activities; and<br>• preparing (i.e., aliquoting) cultures for shipping.<br><br>Exceptions:<br>– incubating and reading initial diagnostic cultures (i.e., excluding subcultures) in tightly capped/sealed tubes, such as MGIT™, Bactec™, and Lowenstein-Jensen, as well as non-propagative assays on initial diagnostic cultures needed to confirm AFB or TB (e.g., preparation and reading of smears) can be performed at CL2†;<br>– aliquoting diagnostic cultures for inactivation (e.g., for DNA Probe), for packaging, and for shipping can be performed at CL2†; and,<br>– incubating non-diagnostic cultures in tightly capped/sealed tubes must be performed at CL3, but the use of a respirator is not required. | | ■R |
| ***In vivo* Work Activities**<br>Examples of these activities include, but are not limited to:<br>• preparing inoculum;<br>• inoculating animals; and<br>• collecting specimens from experimentally infected animals (e.g., bronchial lavage). | | ■R* |
| **All Activities with MDR-TB or XDR-TB Positive Specimens** | | ■R |

† *With additional biosafety requirements as described in Section 4.0.*

R *A respirator (i.e., N95 or higher, or a Powered Air Purifying Respirator) is required.*

** Work in SA zones must meet the requirements in the CL3 column of the CBS and work in LA zones must meet the requirements in the CL3-Ag column of the CBS.*

# 4.0 ADDITIONAL BIOSAFETY REQUIREMENTS

In addition to the requirements listed for CL2 and CL3 specified in Chapters 3, 4, and 5 of the CBS, the requirements below (designated as 'CBS R' followed by the requirement number) are to be followed for activities listed in Table 1 as requiring CL2 with additional biosafety requirements (■[†]) or CL3 (■[R]). The following requirements apply to all personnel entering the containment zone.

## 4.1 Additional Operational Requirements for Non-Propagative Clinical/Diagnostic Activities with Primary Specimens ([†])

- All activities involving open vessels of infectious material to be conducted in a certified biological safety cabinet (BSC) or other appropriate primary containment device (CBS R4.6.25). BSCs provide effective primary containment for work with infectious material or toxins whose primary route of infection is inhalation. This operational practice is required based on the number of laboratory acquired infections (LAIs) associated with work involving MTBC and the fact that manipulations of this infectious material have the potential to generate infectious aerosols.

- Respirators to be worn where there is a risk of exposure to infectious aerosols (CBS R4.4.9). The need for respiratory protection at CL2 is based on a local risk assessment, and can depend on specimen type and the likelihood of the specimen being positive (i.e., local MTBC prevalence, initial test versus follow up of potential positive). For example, patient specimens collected to monitor response to therapy may contain MTBC organisms for some time, and in such a case, a respirator should be worn.

- An additional layer of protective clothing to be donned prior to work with infectious material (CBS R4.4.7). An additional layer of protective clothing (e.g., solid-front gown with tight-fitting wrists, waterproof apron, second pair of gloves, and head cover) provides additional protection and guards against exposure following a tear that may have compromised, or a spill that may have contaminated, the outer protective layer.

- Personal belongings not required for work to be left outside the containment zone or in change areas outside the containment barrier (CBS R4.5.12).

- Personnel to doff additional layer of PPE when exiting the containment zone (CBS R4.5.17).

## 4.2 Additional Operational Requirements for Propagative Activities, In Vivo Activities, and All Activities with MDR-TB or XDR-TB Positive Specimens ([R])

- Respirators are to be worn when there is a risk of exposure to infectious aerosols (CBS R4.4.9). The principal route of MTBC infection is through the inhalation of infectious aerosols, and the infectious dose is low. Respirators provide added personal protection, given the added risk of handling samples that contain large amounts or high concentrations of MTBC, and the risks associated with handling drug-resistant strains.

# 5.0 OTHER CONSIDERATIONS

## 5.1 Biosafety Considerations

The importance of LRAs in the implementation of requirements is described in the CBS. Many of the requirements in the CBS are risk- and performance-based and as such, are dependent on the LRA. Although these requirements or parts thereof are listed as CL2 and CL3 operational practice requirements in Chapter 4 of the CBS, they are critical for the safe handling of MTBC and are highlighted and expanded upon here to facilitate the development of LRAs and associated procedures (SOPs).

- Good microbiological laboratory practices to be employed (CBS R4.6.18);
- Centrifugation of infectious material where inhalation is the primary route of infection to be carried out in sealed safety cups (or rotors) that are unloaded in a BSC (CBS R4.6.28);
- Personnel to demonstrate knowledge and proficiency in the SOPs on which they were trained (CBS R4.3.7);
- Procedures to be in place to prevent a leak, drop, spill, or similar event during the movement of infectious material within the containment zone, or between containment zones within a building (CBS R4.6.31);
- A medical surveillance program, based on an overarching risk assessment and LRAs, to be developed, implemented, and kept up to date (CBS R4.1.12). The components of a medical surveillance program will vary between facilities and may include routine testing of personnel and immunization requirements;
- Disinfectants effective against the infectious material in use to be available and used in the containment zone (CBS R4.8.2); and
- Decontamination equipment and processes to be validated using representative loads, and routinely verified using application-specific biological indicators, chemical indicators, or parametric monitoring devices (e.g., temperature, pressure, concentration) consistent with technology/method used (CBS R4.8.11, 4.10.7, 5.1.1 and 5.1.4).

## 5.2 Specimen Referral

Containment zones that cannot meet the containment level requirements for the sample type or activities being performed, as outlined in Sections 3 and 4, are to refer the specimen/culture to a containment zone that meets the requirements.

## 5.3 Inactivation Methods

Containment zones that wish to inactivate MTBC samples so they can be handled at a lower containment level must employ inactivation methods for mycobacterium that have been validated and routinely verified in house prior to releasing the samples to lower containment. This includes using a validated inactivation method, whether heat or chemical, when fixing smears or inactivating samples for nucleic acid extraction.[27] Note that several studies have found that temperatures below 100°C did not effectively inactivate *M. tuberculosis*, nor did inactivation by other lab processes (smear preparation) or exposure to chemicals (e.g., as in nucleic acid or protein extraction).[28,29,30]

## 5.4 Disinfection

Mycobacteria are more resistant to chemical disinfectants than most vegetative bacteria and the selection of an effective mycobactericidal product is, therefore, important for containment zones handling MTBC. Disinfectants reported to be effective against mycobacteria include alcohols, aldehydes, some alkalis, halogens, glutaraldehydes, formaldehyde, some peroxygen compounds, and some phenols.[31]

Phenolics are used in mycobacteria laboratories and vary in efficacy, depending on the product. Testing by laboratories is recommended before using a new phenolic product. Chlorine based disinfectants at 10,000 ppm (1%) are effective against MTBC as well as other RG3 organisms and should be considered as an alternative to phenol-based disinfectants. For example, a 1 in 5 dilution of commercial bleach verified at 5.25-6.15% sodium hypochlorite is an effective mycobactericidal disinfectant.[32] Quaternary ammonium compounds are generally ineffective against mycobacteria.

Many commercial disinfectant products are available and laboratories should consult the manufacturer to determine their efficacy against mycobacteria and the recommended directions for use (i.e., application method, contact time, disposal). It is recommended that laboratories conduct their own disinfectant efficacy testing to evaluate a product's performance under specific conditions of use.

# 6.0 CONTACT AND ADDITIONAL INFORMATION

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

Public Health Agency of Canada
Centre for Biosecurity
Biosafety Standards and Guidelines Program
Email: PHAC.pathogens-pathogenes.ASPC@canada.ca

Canadian Biosafety Standards and Guidelines Website: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/second-edition.html

# 7.0 GLOSSARY

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

| | |
|---|---|
| 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/semi-liquid material. |
| Biological safety cabinet (BSC) | A primary containment device that provides protection for personnel, the environment and the product (depending on BSC class), when working with biological material. |
| Biosafety | Containment principles, technologies and practices that are implemented to prevent unintentional exposure to infectious material and toxins, or their accidental release. |
| Containment | The combination of physical design parameters and operational practices that protect personnel, the immediate work environment and the community from exposure to biological material. |
| Containment barrier | The boundary between "clean" and "dirty" areas (i.e., between the laboratory work areas, animal rooms/cubicles, or PM rooms, and outside of that containment area). Where inward directional airflow is provided, a physical containment barrier of air is established to protect against airborne or aerosolized infectious material or toxins from reaching the "clean" areas. |
| Containment level (CL) | Minimum physical containment and operational practice requirements for handling infectious material or toxins safely in laboratory and animal work environments. There are four containment levels ranging from a basic laboratory (CL1) to the highest level of containment (CL4). |
| Containment zone | A physical area that meets the requirements for a specified containment level. A containment zone can be a single room (e.g., 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., CL3 suite comprised of dedicated laboratory areas and separate animal rooms or cubicles). Dedicated support areas, including anterooms (with showers and "clean" and "dirty" change area, where required) are considered to be part of the containment zone. |

| | |
|---|---|
| Culture | The *in vitro* propagation of microorganisms, tissue cells, or other living matter under controlled conditions (e.g., temperature, humidity, nutrients) to generate greater numbers or a higher concentration of the organisms/cells. In the context of the *Canadian Biosafety Standard* and the *Canadian Biosafety Handbook*, "cell culture" refers to cells derived from a human or animal source. |
| Disinfection | Process that eliminates most forms of living microorganisms; disinfection is much less lethal to infectious material than sterilization. |
| Good microbiological laboratory practice | A basic laboratory code of practice applicable to all types of activities with biological material. These practices serve to protect workers and prevent contamination of the environment and the samples in use. |
| Infectious dose | The amount of pathogen required to cause an infection in the host, measured in number of organisms. |
| 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. |
| *In vitro* | Latin for "within glass," *in vitro* refers to experimentation involving components of a living organism within an artificial environment (e.g., manipulation of cells in petri dish), including activities involving cell lines or eggs. |
| *In vivo* | Latin for "within the living," in vivo refers to experimentation conducted within the whole living organisms (e.g., studying the effect of antibiotic treatment in animal models). |
| Local risk assessment (LRA) | Site-specific risk assessment used to identify hazards based on the infectious material or toxins in use and the activities being performed. This analysis provides risk mitigation and risk management strategies to be incorporated into the physical containment design and operational practices of the facility. |
| Medical surveillance program | A program designed to prevent and detect personnel illness related to exposure to infectious material or toxins. The focus of the program is primarily preventative, but provides a response mechanism through which a potential infection can be identified and treated before serious injury or disease occurs. |
| Operational practice requirements | Administrative controls and procedures followed in a containment zone to protect personnel, the environment, and ultimately the community from infectious material or toxins, as outlined in Chapter 4 of the *Canadian Biosafety Standard*. |

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| Pathogen | A microorganism, nucleic acid, or protein capable of causing disease in humans and/or animals. Examples of human pathogens are listed in Schedules 2 to 4 or in Part 2 of Schedule 5 of the *Human Pathogens and Toxins Act* but these are not exhaustive lists. Examples of animal pathogens can be found by visiting the CFIA website. |
| Pathogenicity | The ability of a pathogen to cause disease in a human and/or animal host. |
| Pathogen risk assessment | The determination of the risk group and appropriate physical containment and operational practice requirements needed to safely handle the infectious material or toxins in question. |
| Performance and verification testing requirements | Performance and verification tests that are necessary to demonstrate compliance with the physical containment requirements, as outlined in Chapter 3 of the *Canadian Biosafety Standard,* and, in some cases, the operational practice requirements, as outlined in Chapter 4 of the *Canadian Biosafety Standard*. The performance and verification testing requirements are listed in Chapter 5 of the *Canadian Biosafety Standard*. |
| Personal protective equipment (PPE) | Equipment and/or clothing worn by personnel to provide a barrier from infectious material or toxins, thereby minimizing the risk of exposure. PPE may include, but is not limited to, lab coats, gowns, full-body suits, gloves, protective footwear, safety glasses, safety goggles, masks and respirators. |
| Physical containment requirements | Physical barriers in the form of engineering controls and facility design used to protect personnel, the environment, and, ultimately, the community from infectious material or toxins, as outlined in Chapter 3 of the *Canadian Biosafety Standard*. |
| Primary containment | The first level of physical barriers designed to contain pathogens and toxins and prevent their release. This is accomplished by the provision of a device, equipment, or other physical structure situated between the infectious material or toxins and the individual, the work environment, or other areas within the containment zone. Examples include biological safety cabinets, glove boxes, and animal microisolators. In animal cubicles, the room itself provides primary containment, and personal protective equipment serves as primary protection against exposure. |
| Primary containment device | Apparatus or equipment that is designed to prevent the release of infectious material or toxins and to provide primary containment (i.e., provide a physical barrier between the individual and/or the work environment and the biological material). Examples of primary containment devices include biological safety cabinets, isolators, centrifuges with sealable cups, process equipment, fermenters, microisolator cages, and ventilated cage racks. |

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| Risk group (RG) | The classification of biological material based on its inherent characteristics, including pathogenicity, risk of spread, and availability of effective prophylactic or therapeutic treatments, that describes the risk to the health of individuals and the public as well as the health of animals and the animal population. |
| Virulence | The degree/severity of a disease caused by a pathogen. |

# 8.0 REFERENCES and RESOURCES


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