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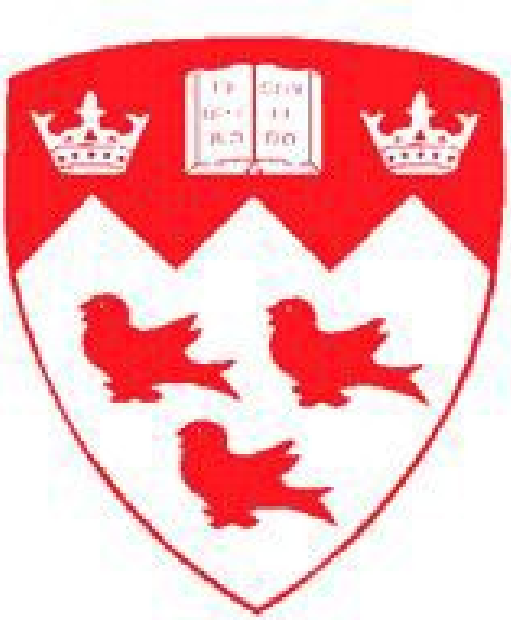

McGill University Biosafety Manual

EHS-BIOS-001 VERSION 1.3

April-2023

Contents

Part 1 – ORGANIZATION

Section 1.1 Program Intent and Goals

The McGill University Biosafety Manual is designed as an informational guide to those who handle or work in close proximity with potentially **infectious** materials. This Laboratory Biosafety Manual addresses the safety requirements for working with biological materials as defined below:

> Pathogenic and non-pathogenic **microorganisms**, proteins, and nucleic acids, as well as any biological matter that may contain microorganisms, proteins, nucleic acids, or parts thereof. Examples include but are not limited to: (Canadian Biosafety Standard, 3rd edition (CBS)):

- microorganisms such as **viruses**, fungi, parasites, **prions** and bacteria and their toxic metabolites
- animal blood and body fluids
- unfixed and fixed tissues and diagnostic specimens
- cell lines and other tissue cultures
- nucleic acids, such as DNA derived from **pathogenic organisms**, human oncogenes or transformed cell lines
- genetically modified organisms
- zoonotic agents

A biohazard exists when the use of these materials poses a potential risk to humans, animals or the environment. Exposure to biohazardous materials may occur via puncture wounds or as a result of absorption through the respiratory tract, digestive system, skin and mucous membranes: such exposures may result while handling microorganisms, animals, cell cultures and tissues or diagnostic specimens. Investigators who are uncertain as to whether a material is biohazardous or not should consult the Biosafety Officer (**BSO**) at ehs@mcgill.ca.

A glossary of commonly used terms is available at the end of this Manual (section 4.2), all terms in the glossary are in bold in their first appearance in the Manual. A list of commonly used acronyms is provided (section 4.3), all acronyms appearing in the Manual are in bold throughout the text of the Manual.

As this manual does not address the chemical and physical hazards commonly encountered in the lab, it is to be regarded as an addendum to the Laboratory Safety Manual. The hazards presented by radiation are of physical rather than biological origin and thus are not covered in the Biosafety Manual; information on working safely with radiation can be obtained by consulting the Radiation Safety Officer at **EHS**.

Section 1.2 Administrative requirements

1.2.1 Acts and Regulations Governing the Use of Biological Materials

Various federal and provincial Acts and Regulations govern the use of biological materials based on the types of hazard present. The following are the major agencies responsible for the enforcement of this legislation:

Agency	Legislation	Agents covered	Supporting documentation
PHAC	**HPTA** & **HPTR**	Human pathogens, toxins and prions	**CBS** & Canadian Biosafety Handbook (**CBH**)
	HAA & **HAR**	Terrestrial Animal Pathogens	
CFIA	**HAA** & **HAR**	Foreign and Emerging Animal Diseases	**CBS** & **CBH**
		Aquatic Animal Pathogens	Containment Standards for Facilities Handling Aquatic Animal Pathogens
	Plant Protection Act & Regulations	Plant Pests and Materials Containing Plant Pests	Containment Standards for Facilities Handling Plant Pests

1.2.2 Roles and Responsibilities of Supervisory Personnel

Responsibility for biosafety begins with the individual handling biological materials in the laboratory and extends upwards, through a chain comprising the Permit Holder, the **BSO**, **EHS** and the University Laboratory Safety Committee (**ULSC**).

1.2.2.1 Permit Holder

A Permit Holder (PH) is an individual responsible for the safe procurement, storage, use and disposal of biological materials. He/she is usually in charge of research or teaching operations. In particular, a Permit Holder must:

- Submit the Application to Use to Biohazardous Materials (EHS-FORM-014) to **EHS** for approval and to receive an Internal Biohazard Permit.
- Advise **EHS** of any changes affecting the Internal Biohazard Permit.
- Adhere to conditions stated in the Internal Biohazard Permit.
- Ensure the laboratory meets all the containment requirements for the biological materials present as described in Section 1.3.1.
- Ensure the laboratory follows all procedures and policies described by the **ULSC**.
- Ensure that personnel read and understand the sections of this Manual relevant to their work in the lab. Document their competency in the Documentation of Training Form (EHS-FORM-108).
- Ensure that all users of biological materials under their supervision receive adequate instruction in the safe handling of the biological materials and equipment used in the performance of their duties. Document in the Documentation of Training Form (EHS-FORM-108)
- Allow only authorized persons to access areas designated for use and storage of biological materials. Ensure guests and visitors are accompanied by an authorized person.
- Ensure that any biological materials present in their laboratory are located in a locked area, room or enclosure, (magnetic cards, code locks, keys) when not in use or not under the direct supervision and control of an authorized user.
- Provide special instruction and/or precautionary measures for students, clerical personnel and others who are authorized to enter or work in areas where there is a possibility of exposure to biological materials, but who are not classified as Users

- Report incidents of loss, unauthorized use or theft involving biological materials to the **BSO**.
- Ensure that an inventory of biological materials is maintained.

- Ensure that all transfers of biological material (receiving or shipping) are reported to the **BSO**, by completing the Biohazardous Agent Transfer Notification form (EHS-FORM-101) or equivalent documentation and sending it to the **BSO** for approval or providing another proof of purchase to the **BSO**.

1.2.2.2 Biosafety Officer

The **BSO** shall:

- Act as the primary liaison officer between McGill University and outside authorities such as the Public Health Agency of Canada (**PHAC**), Canadian Food Inspection Agency (**CFIA**) and the Commission des normes, de l'équité, de la santé et de la sécurité du travail (CNESST) of Québec in all matters relating to Biosafety.
- Ensure the information on the McGill University Human Pathogens and Toxins Regulations (**HPTR**) license is accurate and complete.
- Ensure the preparation and dissemination of information on biosafety.
- Conduct and/or supervise the commissioning of laboratories where biohazardous materials will be handled or stored to ensure all laboratories are in compliance with the relevant regulations.
- Supervise the use, transfer and disposal of biological materials.
- Develop and provide application forms for the Internal Biohazard Permit.
- Maintain the Internal Biohazard Permit management system and keep records and forms of all related information, including: lists of Permit Holders, Permit Modifications and areas where biological materials are stored or used.
- Identify, investigate and report occupational biohazard exposures to **PHAC**.
- Ensure appropriate biosafety training is provided for all personnel and students.
- Certify all biological materials have been removed and safely discarded or transferred during laboratory decommissioning (EHS-SOP-010).
- Investigate the loss of all biological materials, used, stored or transferred to the laboratory.
- Participate in the development of emergency/incident response planning for incidents involving biological materials.
- Periodically review and propose any amendments to the Biosafety Manual to the **ULSC**.
- Prepare and submit summaries of biosafety services and the results of inspections, follow-ups and decommissioning projects to the **ULSC**.
- Complete and submit, in consultation with the **ULSC** and the License Holder, the renewal application of McGill University's **PHAC** licenses for human and animal pathogens and toxins in accordance with the Human Pathogens and Toxins Act (**HPTA**) and **HPTR**.
- Present information on accidents, incidents or releases of biological materials to the **ULSC** and relevant regulating agencies (ie. **PHAC, CFIA**).

1.2.2.3 Environmental Health and Safety

EHS has the mandate to plan, organize, co-ordinate and implement University programs in occupational health and safety in conformity with applicable laws, regulations, codes and standards.

The objectives of **EHS** are to establish and maintain a high standard of safety in all University activities, to recognize and minimize occupational hazards and to prevent accidents and injuries of all kinds. The principal functions of **EHS** are:

- To provide information and training to McGill staff and students in matters related to **EHS** safety programs.

To perform specialized Occupational hygiene and environmental health measurements and assessments.

- To serve as a liaison between the University and regulatory agencies, such as the Canadian Nuclear Safety Commission, the CNESST, the **PHAC** and the **CFIA**.
- To assist Researchers in maintaining compliance with all applicable regulations and standards based on the hazards present in the laboratory.
- To support Researchers in establishing and maintaining a safe and healthy working environment in the laboratory.
- To administer the McGill University Occupational Health Program (OHP and provide medical surveillance to laboratory personnel. See the Occupational Health Program website for more information.

The **BSO** is an employee of **EHS**, reporting to the Operations Manager.

1.2.2.4 University Laboratory Safety Committee

The **ULSC** is the designated committee responsible for Biosafety as per **HPTA** requirements. More information on the mandate and makeup of the Committee is available on the EHS website.

Section 1.3 Physical Containment Requirements

The term "containment" is used in describing measures used to provide a barrier between the infectious organism(s) or biological materials being handled and the worker and/or the environment where a **local risk assessment (LRA)** indicates that a release of the materials could have harmful consequences. Containment is achieved through the use of appropriate safety equipment, facility design and lab procedures and practices.

1.3.1 Containment Requirements

When working with biohazardous materials, careful consideration must be given to both facility design and work practices to ensure protection of laboratory personnel, their colleagues and the community. Containment standards have been described for work with human (CL), terrestrial (CL) and aquatic animal pathogens (AQC) as well as plant pests (PPC) (see section 1.2.1). Containment requirements are described for laboratory, animal and field work. The containment requirements for work involving biological materials are determined by a **LRA**. **LRAs** are discussed in Section 2.2.2.

1.3.1.1 Containment Level 1 (CL1, AQC1 or Plant Basic)

Level 1 containment is required when working with biological materials that pose no risk to healthy adults or will have minimal impact on plants. In general it includes the following practices:

- The laboratory may be near a public area but doors should be kept closed.
- Work may be carried out on an open bench top.
- Lab surfaces (walls, ceilings, furniture and floors) should be cleanable.
- Open windows should have insect screens.
- Eyewash stations and handwashing facilities should be available.
- Street clothes and lab coats should not be kept together.
- Disinfection should be carried out as required, using effective concentrations and contact times; solutions should be replaced regularly.

1.3.1.2 Containment Level 2 (CL2, AQC2 or PPC1)

Level 2 containment is appropriate for work with biological materials that can cause disease in human and/or animals but, under normal circumstances, are unlikely to pose a serious hazard (risk group 2) or when determined by an **LRA**. The following precautions, in addition to those for level 1 containment, are recommended:

- The facility should be away from public areas and should have self-closing doors.
- A biohazard sign with relevant information should be posted at the entrance.
- Service and custodial staff should be informed of the hazards; the latter should be expected to clean floors and pick up non-lab waste garbage.
- Items should be autoclaved or chemically decontaminated before removal from the facility.
- Use Class I or II biological safety cabinets (**BSC**) for procedures that generate aerosols.
- Procedures should be carried out such that aerosol generation is minimized.
- An emergency spill response plan should be in place and posted in a visible location.
- Vacuum lines should be equipped with HEPA filters.
- Lab coats may be front-closing, but should not be worn outside the lab.
- Wear gloves to prevent skin contamination.

For a complete list of the requirements for a CL2 laboratory, consult the appropriate containment standards documents as listed in Section 1.2.1.

1.3.1.3 Containment Level 3 (CL3, AQC3 or PPC2)

Level 3 containment is recommended for work with human and animal pathogens known to cause severe disease in humans (risk group 3) and/or animals or as determined by an **LRA**. Measures should include the recommendations outlined for levels 1 and 2, plus the following:

- The lab should be away from general work areas, with controlled access.
- There should be a change and shower area within the containment facility perimeter.
- The area should be kept at negative pressure relative to surrounding areas.
- Supply and exhaust air should be HEPA-filtered or provided by dedicated systems.
- A hands-free handwashing sink should be located near the exit.
- Lab windows should be unbreakable and sealed shut.
- Lab personnel should be trained in handling, disposal, and emergency procedures. Written protocols for these procedures should be developed and posted in a visible location.
- Personnel should wear solid-front lab clothing, which should be autoclaved before laundering or disposal.
- A medical surveillance program is recommended.

For a complete list of the requirements for a CL3 laboratory, consult the appropriate containment standards documents as listed in Section 1.2.1., or the McGill University CL3 Biosafety Manual.

1.3.1.4 Containment Level 4 (CL4, PPC3)

Level 4 containment is recommended for work with human and animal pathogens known to cause fatal disease in humans (risk group 4) and/or animals or as determined by an **LRA**. Measures should include the recommendations outlined for levels 1 and 2 and 3, plus the following:

- Physical isolation of the laboratory, with an airlock for access.
- Entry restricted to authorized personnel and recorded in a log book: no one should work alone.
- Use of Class III **BSC** and/or positive-pressure protective suits.
- Additional safety measures for ventilation, waste treatment, and gas and water services.

For a complete list of the requirements for a CL4 laboratory consult, the appropriate containment standards document as listed in Section 1.2.1. There are no CL4 facilities available at McGill University.

1.3.2 Biological Safety Cabinets

Natural or mechanical aerosolization of biological materials poses a serious risk to laboratory workers. The degree of penetration and retention of airborne pathogens in the respiratory tract is determined primarily by size: particles which are 5-10 µm in diameter or smaller are most efficiently inhaled, deposited and retained in the upper respiratory tract or in lung alveoli. Smaller particles can be inhaled into the lungs. Larger particles (100 µm or greater diameter) are also of concern because they can settle and contaminate work surfaces, equipment and personnel. **BSCs** reduce the risk of exposure to aerosolized biological materials by reducing the escape of aerosolized infectious agents into the laboratory environment. **BSCs** minimize contact between the operator and biological materials through the use of directional airflow, HEPA filtration of supply and/or exhaust air, and, in some cases, a physical barrier such as a plastic or glass shield. For more detailed information on the maintenance and operation of **BSCs** see Section 3.2.

1.3.2.1 HEPA Filters

BSCs operate through the principal of directional airflow of HEPA (High Efficiency Particulate Air) filtered over the work surface. The particle removal efficiencies of HEPA filters is 99.97% or better for a particle size of 0.3 µm. This size particle is used as the basis for filter definition because it is considered the most difficult to remove. Thus, a filter that can trap 0.3-µm diameter particles can easily eliminate other sizes.

HEPA filters consist of continuous sheets of glass fiber paper pleated over rigid corrugated separators and mounted in a wooden or metal frame. The filter medium is delicate and should never be touched. As well, the gaskets used to seal the filter frame to the cabinet must not be disturbed; thus the biological safety cabinet should not be moved without subsequently being tested and certified.

While HEPA filters remove particulates from an airstream, they are not effective at collecting chemical gases or vapours. Thus, it is inadvisable to use recirculating Class II cabinets with agents which have significant amounts of hazardous volatile or radioactive components. Although Class III and 100% exhaust Class II cabinets can be used in experiments which involve use of chemicals of moderate toxicity, it should be remembered that these cabinets are not explosion-proof. Use of flammable or explosive products is to be avoided unless the cabinet has been specifically designed for their use.

1.3.2.2 Classes of Biological Safety Cabinets

There are three classes of **BSCs**, defined by the pattern of re-circulated HEPA-filtered air. This Biosafety Manual provides a brief description of the three classes of **BSCs** and their uses in the laboratory. **EHS** provides training on the "Safe Use of Biological Safety Cabinets"; this training is mandatory for any person operating a **BSC**.

NOTE: horizontal and vertical clean benches are not **BSCs**: HEPA- filtered air is directed over the work surface and then discharged directly into the room. These units do not protect the operator from exposure to the materials being handled and they must not be used for work with potentially infectious or toxic materials.

1.3.2.2.1 Class I

- open-fronted
- protects operator and environment

- for work with low and moderate risk agents (Risk Groups 2 and 3) where product protection is not critical

General principle of operation: An inward flow of room air through the work opening, away from the operator, prevents the escape of airborne pathogens into the laboratory. Negative cabinet pressure is created by a blower that exhausts the air, either into the room or to the outside, through a HEPA filter, providing environmental protection. For Class I **BSCs**, the supply air is not HEPA-filtered. The products that are inside the **BSC** are therefore exposed to contaminants that are pulled in from the room environment and internal air turbulences may result in cross-contamination between products within the cabinet. The use of Class I **BSCs** should be reserved to work where sterility is not required (e.g. cage dumping).

1.3.2.2.2 Class II

- open-fronted
- protects operator, product and environment from particulate contamination
- for work with low to moderate risk agents (Risk Groups 2 and 3)

General principle of operation: Escape of pathogens into the worker's environment is prevented by an inward flow of room air which enters the front opening without crossing the work area and by HEPA filtration of exhaust air (this provides environmental protection), while downward flow of HEPA-filtered air through the work area removes work zone contaminants and protects the product. The amounts of room air drawn into the intake grille and the amount of air exhausted through the exhaust filter are equal. This balance is critical: positive pressure will allow the outflow of pathogens, while negative pressure will result in inflow of room contaminants.

Class II **BSCs** are divided into 3 Types, A, B or C based on the following:

- airflow velocities
- amount of cabinet air recirculated (from 0 to 70%)
- amount of cabinet air exhausted (from 30 to 100%)
- destination of exhaust air (back to lab or outside)
- exhaust ducting (building system versus dedicated ducts)

Volatile and radiolabeled chemicals must not be handled in Class II, type A cabinets that exhaust into the laboratory. When working in a class II, type B2 **BSC**, the **BSC** must be installed and the ventilation balanced in order to protect the user from a reversal of airflow ("puff-back") in the intake window in the event of a failure in the exhaust ventilation for the **BSC**. A class II, type C can be converted between type A mode that exhausts directly into the laboratory and type B mode connected to an HVAC system. When in type B mode a class II, type C BSC offers protection from volatile or radiolabeled chemicals.

1.3.2.2.3 Class III

- totally enclosed, gas tight, with glove ports for manipulation of pathogens
- provides the greatest level of operator and product protection
- for work with high risk pathogens (Risk Group 4)

General principle of operation: These cabinets form a physical barrier between the operator and microbiological agent. Internal negative pressure confines any leaks to the inside of the cabinet. Supply and exhaust air is HEPA-filtered; a dedicated exhaust fan, separate from that of the facility ventilation system, discharges directly to the outdoors. There is no recirculation of air within the cabinet.

A Class III cabinet system must be designed to allow for the safe introduction, handling and removal of all materials throughout the procedure. Equipment such as the incubator, refrigerator, centrifuge, autoclave and chemical dunk tank are connected to the cabinet system.

Part 2 – User Requirements

Section 2.1 Access

2.1.1 Authorized Personnel

Access to the laboratories must be limited to trained authorized personnel who are aware of the risks and risk mitigation strategies required for working in the laboratories handling biological materials. Environmental Health and Safety provides the following laboratory safety trainings for working with biohazardous materials:

- **Introduction to Biosafety** – mandatory for all personnel handling biohazardous materials
- **Safe Use of Biological Safety Cabinets** – mandatory for all personnel using a Biological Safety Cabinet as a primary containment device for biological hazards
- **Transport of Dangerous Goods Class 6.2** – mandatory for all personnel who will be shipping or receiving regulated materials
- **WHMIS 2015** – mandatory for all personnel who handle and store WHMIS controlled products
- **Hazardous Waste Management** - This training is mandatory for all laboratory users that handle hazardous materials

Labs are required to keep a list of all persons who are authorized to access any lab space handling risk group 2 materials or above. Any person entering the laboratory who has not completed the necessary training for authorization must be accompanied at all times. This includes, but is not limited to:

- Couriers making deliveries
- External contractors performing equipment maintenance

Housekeeping personnel may enter the laboratory space to empty non-hazardous waste once they have viewed video, *Custodial Work in Laboratories: WHMIS and Safety Awareness* prepared by Environmental Health and Safety.

2.1.2 Security Sensitive Biological Agents

Pathogens and toxins with the potential for dual-use (ie. where the inherent qualities of a pathogen or toxin allow for its use in legitimate scientific applications, as well as for intentional and malicious misuse as a biological weapon) are a biosafety concern. The **PHAC** has identified a list of selected pathogens and toxins which are of particular concern. These pathogens and toxins are considered Security Sensitive Biological Agents (**SSBAs**). The identified toxins are considered **SSBAs** when present in the laboratory in quantities above the threshold amount. Work with **SSBAs** is strictly controlled and the **HPTA/HPTR** sets out additional security requirements for work with **SSBAs**. Any work with these materials must be reported directly to the McGill **BSO** who will provide further guidance on the requirements for work with **SSBAs.**

Section 2.2 Application to Use Biohazardous Materials:

2.2.1 Classification of Biohazardous Materials

Criteria for classification of infectious agents are outlined in the **CBS** published by the **PHAC**. Essentially, microbiological pathogens are classified according to their impact upon the individuals who manipulate them, upon their colleagues, and upon the surrounding community. Agents that pose little or no risk are assigned to Risk Group 1, while those with the greatest hazardous potential are in Risk Group 4. The **PHAC** has classified the risk group of the most common human and terrestrial animal pathogens and published the information on the ePathogen database. If a pathogen has not been assigned a risk group, the Researcher, in collaboration with the **BSO**, must perform a risk assessment based upon the following:

- Pathogenicity/virulence

- Route of infection
- Mode of transmission
- Environmental survival
- Infectious dose
- Availability of prophylaxis/treatment
- Host Range

While this classification is designed to assess the risks associated with conventional pathogens, it can be expanded to assess the risks of most biohazardous materials. For many pathogens, a risk assessment has been performed and documented in the form of a Pathogen Safety Data Sheet (**PSDS**) provided by the **PHAC** or by the supplier.

An outline of the characteristics of agents in each Risk Group is presented in Table 1.

TABLE 1 - Risks and characteristics associated with pathogens from Risk Groups 1 to 4, and recommended containment level and class of **BSC**.

Risk group	*Risk assessment*	*Characteristics*	*Examples*	*Biosafety cabinet*
1	Low individual; low community	Unable or unlikely to cause disease in animals or humans	*Lactobacillus spp.*, *Bacillus subtilis*, *Naegleria gruberi*, *Micrococcus spp.*, *E. coli* K12	Not required
2	Moderate individual; low community	Rarely causes serious human or animal disease; effective prevention and treatment available; limited risk of spreading	Hepatitis B virus, *Toxoplasma spp*., HIV (non-cultured), *Ascaris*, *Salmonella typhimurium*	Class I *or* Class II – For manipulations creating aerosols
3	High individual; low community	May cause serious disease in humans or animals; effective prevention and treatment available; unlikely to be spread by casual contact	Lassa fever virus, Hantavirus, *Yersina pestis*, *Histoplasma capsulatum*, *Bacillus anthracis*, cultured isolates of HIV*	Class I *or* Class II
4	High individual; high community	Likely to cause very serious disease in humans or animals; readily transmitted from one individual to another, or between animals and humans; preventative vaccines or effective treatment not available	Marburg virus, Ebola virus, Crimean-Congo hemorrhagic fever virus, *Herpesvirus simiae*	Class I *or* Class II plus positive pressure suits *or* Class III

2.2.1.2 Prions

Infectious proteins, called prions, can cross the blood-brain barrier following ingestion, causing degenerative neurological diseases such as Creutzfeld-Jakob disease or kuru in humans or chronic wasting disease in deer and elk. Prions have a long incubation period (decades) and are resistant to destruction, with exposure overnight to 1N sodium hydroxide being the only documented means of inactivating prions. Prion diseases can be transmitted through the ingestion of infected neuronal tissues prior to the development of symptoms of disease. As such, precautions must be taken when handling all neuronal tissues, to avoid accidental ingestion, even when there is no known presence of a prion.

The **CBS** outlines the physical and operational containment practices necessary for working with prions.

2.2.1.3 Genetically Modified Organisms

Many methods have been used to alter the genetic materials of a biological organism (i.e. natural selection, cross-breeding, conjugation and transformation). Recombinant DNA technology is the *in vitro* incorporation of segments of genetic material from one cell into another and has resulted in altered organisms that can manufacture products such as vaccines, hormones, interferons and enzymes. This new field of science has been dubbed as "Biotechnology" and has resulted in a new class of organisms called genetically modified organisms (**GMO**). **GMO**s are used for treatment of waste and spills, and can be used to make plants resistant to cold, disease, pests and drought.

However, biotechnology carries with it the potential for harm. A **GMO** may be directly pathogenic, toxic or, if released into the environment, crowd out beneficial organisms, transfer undesirable genetic traits to wild species or mutate into a pathogenic form.

The risks associated with recombinant DNA technology are to be assessed by the investigator when submitting the Application to Use Biohazardous Materials form (EHS-FORM-014) to McGill **EHS** using the pathogen risk assessment format as is used for all biological material where the hazard information is unavailable. Additional factors to consider when assessing a **GMO** are:

- source of the DNA to be transferred
- vector
- host

When assessing the risk of, and containment level required for a protocol involving a **GMO**, the following approach is recommended; if the components of a genetic manipulation are not hazardous, then the altered organism is unlikely to present a risk, and no restrictions are needed. However, if one of the components is potentially hazardous, a risk level appropriate for the known hazard is assigned and modified as required. Subsequent modifications depend on factors such as:

- expression of the transferred gene in the recombinant organism
- ability of the vector to survive outside the laboratory
- expected interactions between transferred gene, host and other factors

2.2.1.4 Viral vectors

Viral vectors are used to deliver genetic material into host cells for expression of genes and can be used for research and gene therapy applications. Viral vector systems are modified from viruses present in the human population ie. adenoviruses, herpesviruses, retroviruses etc.) to improve gene delivery efficiency and enhance their safety.

Risks associated with viral vectors are assessed using the same considerations as for other **GMO**s (section 2.2.1.3), along with the choice of vector system, the safety features engineered into the system, and the nature of the transgene insert(s) encoded by the vector. The risk assessment tool in the Application to Use Biohazardous Materials (Appendix VI – Viral vector risk assessment) includes all elements required for this risk assessment. An Application to Use Biohazardous materials, with Appendix VI must be completed for all *in vitro* and *in vivo* research performed with viral vectors.

2.2.1.5 Tissue Cultures

Cell cultures derived from humans or animals known to be infected with a pathogen, as well as cultures known or suspected to contain infectious microorganisms (e.g., herpesvirus or EBV-transformed cultures) should be assigned to the risk group appropriate for the suspected or known pathogen and handled using the relevant containment level and work practices. Risk groups and containment levels for specific pathogens can be obtained from the **PHAC PSDS** and ePathogen databases available on-line or from the manufacturer.

In addition, cell cultures may carry unsuspected oncogenic, allergenic or infectious particles. It is impractical, if not impossible, to screen such cultures for all potentially harmful microorganisms. Well characterized lines with a history of safe use can become contaminated by adventitious, possibly infectious, microorganisms. For this reason, it is prudent to treat all eukaryotic cells as moderate risk agents (i.e., Risk Group 2) and to use containment level 2 facilities and work practices whenever working with them.

2.2.1.6 Biological Toxins

Special precautions must be taken when working with biological toxins. While biological toxins are not considered infectious materials, they are capable of affecting an individual in much the same way as the pathogen that produces the toxin. A full list of toxins governed under the **PHAC** are listed in the **HPTA** Schedule 1 and Part 1 of Schedule 5. As a general rule, containment level 2 is required to work with toxins. Included below is a list of the criteria for a risk assessment involving work with biological toxins:

- Exposure risk (manipulation)
- Routes of exposure
- Concentration/quantities
- Indicators of toxicity (lethal dose and effective dose)
- Rate of action
- Severity and duration of illness
- Availability of prophylaxis/treatment
- Use of chemical safety practices

2.2.1.7 Human blood, tissue and bodily fluids

Human blood, tissue and bodily fluids are a potential source of pathogenic micro-organisms, such as the Human Immunodeficiency Virus, hepatitis B and C and Syphilis (Blood-borne Pathogens). All McGill faculty, students and staff working with human blood, tissue and bodily fluids have access to the McGill Occupational Health Program (see section 2.5.4 for more information). While these organisms in their native environment are exempt from the **HPTR** licensing requirements it is important to note that the rest of the provisions **HPTA** apply and all reasonable efforts must be made to ensure the safety of personnel working with human blood, tissue and bodily fluids. Any work with pathogens isolated from human blood, tissue or bodily fluid must be done in a CL2 laboratory covered under the McGill HPTR license. An **LRA** should be performed on the materials to determine the potential for infection. The following factors should be considered when performing an **LRA** on human blood, tissue and bodily fluids:

- Source of material (ie. blood, feces, urine)
- Origin of materials (ie. blood from a region where HIV is endemic)

In general, Universal Precautions are followed when working with human blood, tissue and bodily fluids. The requirements for Universal Precautions mirror the requirements for a Containment Level 2 laboratory (see Section 1.3.1.2), as such labs handling these materials at McGill will be evaluated to the CL2 standard.

2.2.2 Local Risk Assessments

Risk assessments must be performed on tasks involving biological materials in the laboratory. This risk assessment is to include the likelihood and consequences of an accidental exposure to or release of the biological materials in the laboratory. This is done by completing a **Local Risk Assessment (LRA)**.

The following must be considered when performing an **LRA**:

- Risk assessment of the biological materials
- Aerosolgeneration
- Quantity
- Concentration
- Type of work
- Pathogen shedding (with animal work)

An **LRA** is performed by the Principal Investigator and must be approved by **EHS** before any work involving biohazardous materials is initiated or modified. The information required for the **LRA** is captured in the Application to Use Biohazardous Materials (EHS-FORM-014). The **LRA** requirement is not restricted to research activities, but also includes biological agents used for testing, diagnostic or teaching purposes. **EHS** approval is required for all containment levels, including Level 1 and for laboratories working with "Universal Precautions". Investigators must complete the *Application to Use Biohazardous Materials* form and submit it for approval to ehs@mcgill.ca prior to:

- starting new projects
- changing a protocol (i.e., use of a new biohazardous material)
- expiry of a previously approved application

The information provided in the *Application to Use Biohazardous Materials* must be reviewed on an annual basis and the Annual Review for Biohazards Permit Holders (EHS-FORM-099) submitted to ehs@mcgill.ca. A Biohazards permit is valid for 5 years, to renew a new Application must be submitted.

It is the responsibility of the investigator to send a copy of the first page of the approved license to the Research Grants Office and to the granting agency.

For further information, review the McGill Biohazards Policy and section D of the McGill University Administrative Handbook, or contact ehs@mcgill.ca.

Section 2.3 Emergency/Incident Response Plan

McGill University uses the Incident Command System (ICS) model for emergency response. The University Emergency Management Plan (UEMP) is available to all response personnel. In the event of an emergency requiring ambulance, fire or police, University personnel are required to call 9-1-1. Security Services (514) 398-3000 (downtown) or (514) 398-7777 (Macdonald Campus) monitors all 9-1-1 calls made from a McGill landline. If a cell phone is used, Security Services should be notified of the emergency and that 9-1-1 was contacted. The Security Operations Centre (SOC) maintains contact information and a list of hazards present in all laboratories in McGill operated facilities. The SOC director will decide if the UEMP needs to be implemented. The University Safety Emergency Guide (USEG) is a general hand-out freely available to all McGill personnel. All laboratories should have this guide available in the laboratory at all times along with

a Laboratory Emergency Plan identifying the hazards present. This section of the manual will discuss the measures taken to protect biohazardous materials and maintain CL2 containment during an emergency. These measures should be identified in the Laboratory Emergency Plan where hazardous materials are used.

Section 2.4 Sterilization and Disinfection in the Laboratory

There is an important distinction between **sterilization** and disinfection. Whereas sterilization results in the destruction of all forms of microbial life, disinfection results in the destruction of specific pathogenic microorganisms. Disinfection can be divided into 3 categories based on the efficacy of the method as follows:

- "High level disinfection" inactivates fungi, viruses and bacteria. High level chemical disinfectants may be ineffective against bacterial spores if they are present in large numbers. Extended exposure times may be required.
- "Intermediate level disinfection" destroys fungi, some viruses (lipid and most non-lipid medium-size and small viruses), mycobacteria and bacteria.
- "Low level disinfection" kills vegetative forms of bacteria, some fungi, and some medium-size and lipid-containing viruses. Low level disinfectants do not reliably kill bacterial spores, mycobacteria or small or non-lipid viruses.

McGill University Hazardous Waste Management provides a biological waste pick-up service available to all McGill University laboratories handling biological materials. Waste is shipped off-site to an accredited facility for sterilization and disposal. For more information refer to the Hazardous Waste Management website. Alternatively laboratories may choose to autoclave their biological waste, for information on the requirements for the use of an Autoclave for the sterilization of biological waste see Section 3.4.8.

2.4.1 Microbial Resistance to Physical and Chemical Agents

Microorganisms vary in their resistance to destruction by physical or chemical means. A disinfectant that destroys bacteria may be ineffective against viruses or fungi. There are differences in susceptibility between gram-negative and gram-positive bacteria, and sometimes even between strains of the same species. **Bacterial spores** are more resistant than vegetative forms, and non-enveloped, non-lipid- containing viruses respond differently than do viruses which have a lipid coating.

Information on the susceptibility of a particular microorganism to disinfectants and physical inactivation procedures can be found in the **PSDS** for that agent. A disinfectant should be chosen that has been shown to be effective against the pathogen in use. Information on efficacy is available from the manufacturer.

2.4.2 Physical Sterilization and Disinfection

2.4.2.1 Heat Sterilization and Decontamination

Generally, sterilization is best achieved by physical methods such as steam or dry heat, which are less time-consuming and more reliable than chemical **germicides**. A summary of physical agents that employ heat for control of microorganisms can be found in Appendix A – Heat decontamination methods. Of these physical procedures, steam autoclaving is the most practical option for the majority of laboratories for both sterilization and **decontamination** purposes.

Details on the use of an autoclave are given in Section 3.4.8.

2.4.2.2 Ultraviolet (UV) Light (Germicidal Lamps)

McGill University **EHS** discourages use of UV light for the purposes of decontamination. The light (approximately 260 nm wavelength) emitted by UV lamps is germicidal, and can reduce the number of pathogenic micro-organisms on exposed surfaces and in air. However, UV light has poor penetrating power; accumulations of dust, dirt, grease or clumps of microorganisms may shield microorganisms from the direct exposure required for destruction. UV light can cause burns to skin and eyes, and factors such as lamp age and poor maintenance can reduce performance. For safe and reliable use of germicidal lamps:

- Clean the bulb at least every 2 weeks; turn off power and wipe with an alcohol-moistened cloth.
- Blue light output is not an indication of the lamp's effectiveness; measure radiation output at least twice yearly with a UV meter or replace the bulb when emission declines to 70% of its rated output.
- Post warning signs to discourage personnel from entering areas where there is risk of exposure to UV light. Wear UV protective goggles, caps, gowns and gloves in rooms with UV installations.
- Use only as an adjunct to another reliable means of decontamination (ie. Chemical decontamination).

2.4.2.3 Miscellaneous Physical Methods

The procedures listed below are included for the reader's interest:

- Infrared radiation: used for heat treatment of small metal and glass items.
- Microwaves: used for treatment of liquids, non-metallic objects, and biohazardous waste.
- Gamma irradiation: disrupts DNA and RNA in living organisms, and is used by hospital and laboratory suppliers for materials that do not tolerate heat and pressure (i.e., autoclaving) or chemical treatments.
- Membrane filtration: physically removes particulates (e.g., microorganisms) from heat-sensitive pharmaceutical and biological fluids. The size of the particles removed is determined by the pore size of the filter membrane.

2.4.3 Chemical Sterilization and Disinfection

Instruments or materials that cannot withstand sterilization in a steam autoclave or dry-air oven can be sterilized with a gas such as ethylene oxide or a broad spectrum liquid chemical germicide. Chemical decontamination of surfaces may also be necessary for very large or fixed items. Since liquid chemical germicides generally require high concentrations and several hours of exposure time for sterilization purposes, they are usually used for disinfection rather than for sterilization purposes. The majority of chemical disinfectants have toxic properties: follow the manufacturer's directions for use and wear the appropriate personal protective equipment (e.g., gloves, eye protection, apron), especially when handling stock solutions and follow all relevant Workplace Hazardous Materials Information Systems (WHMIS) requirements.

Choice of a chemical germicide for use on contaminated equipment, supplies, laboratory surfaces or biohazardous waste depends upon a number of factors, including:

- Number and nature of microbes to be destroyed (e.g., spores vs vegetative cells, bacteria vs viruses)
- Type and configuration of item to be disinfected (fissures, crevices and enclosures may shield organisms)
- Purpose of treatment (e.g., disinfection vs sterilization)
- Interaction with other active chemicals
- Whether the item is covered with soil which might inactivate the disinfectant and increase the contact time required for disinfection
- Toxicity to individuals, culture systems, environment, residual toxicity on items

- pH, temperature, hardness of available dilution water
- Cost

Direct contact between germicide and microorganism is essential for disinfection. Microorganisms can be shielded within air bubbles or under dirt, grease, oil, rust or clumps of microorganisms. Agar, nutrients and other cellular material can directly (through inactivation of the germicide) or indirectly (via physical shielding of microorganisms) reduce the efficacy of some liquid germicides.

No one chemical germicide is effective for all disinfection or sterilization purposes. A summary of chemical germicides, their use, effective concentrations, advantages and disadvantages are outlined in Appendix B – Chemical disinfection.

Section 2.5 Biosafety Concerns for Animal Handling

2.5.1 Zoonoses

Zoonoses are diseases that can be transmitted from animals to humans. Laboratory acquired infections can occur when animals used for research are either naturally or experimentally infected with a zoonotic pathogen. The standards for working with experimentally infected animals are described in the **CBS**. Zoonoses may be acquired through:

- animal bites and scratches
- contact with animal tissues and cultures, blood, body fluids and excreta
- exposure to aerosols produced as a result of activities such as cleaning of cages

Individuals whose work involves exposure to or handling of animals and animal tissues, body fluids and cell cultures should be aware of the possibility of acquiring a **zoonosis** and the risk mitigation strategies to avoid contacting a zoonosis. Over 150 diseases have been classified as zoonoses, some of which are listed in Table 2 below. A more complete listing can be found in the "Guide to the Care and Use of Experimental Animals", published by the Canadian Council on Animal Care.

2.5.2 Laboratory Acquired Allergies to Animals

Exposure to laboratory animals can result in allergic responses in susceptible individuals. Allergies can develop following inhalation of airborne animal allergens or after eye or skin contact with hair, dander, urine, saliva, and serum or body tissues of laboratory animals. Estimates of the prevalence of animal allergy among laboratory workers range from ten to thirty percent. Symptoms of allergy can be mild (itchy eyes, runny nose, sneezing, red raised itchy patches on skin) to severe (wheezing, chest tightness, shortness of breath). Consult a physician if you experience allergy symptoms when working with laboratory animals.

Measures to reduce exposure to laboratory animal allergens include:

- engineering controls (e.g., ventilation)
- filtered cage systems
- respiratory protection (face mask)
- protective clothing such as gloves, gowns and shoe covers, which are reserved for use inside the animal facility
- regular handwashing and showering after handling laboratory animals, their serum or other body tissues
- regular cleaning and decontamination of animal facilities

TABLE 2 - Examples of laboratory-acquired zoonoses, causative microorganisms, and animals most commonly associated with transmission to humans.

DISEASE	AGENT	MEANS OF SPREAD	HOST ANIMALS
BACTERIAL			
ANTHRAX	*Bacillus anthracis*	Contact, inhalation & ingestion	Farm animals
BRUCELLOSIS	*Brucella spp.*	Contact & ingestion	Swine, dogs, cattle & sheep
Q FEVER	*Coxiella bernetii*	Contact, inhalation & ingestion	Cattle, sheep & goats
TUBERCULOSIS	*Mycobacterium spp.*	Contact, inhalation & ingestion	Primates
SALMONELLOSIS	*Salmonella spp.*	Contact, inhalation & ingestion	Farm animals, rodents, reptiles & amphibian
TETANUS	*Clostridium tetani*	Bite and soil-contaminated puncture wounds	Horses, other equinae (also carried by other mammals, and present in soil)
VIRAL			
RABIES	*Rabies virus*	Bites & saliva contact	Dogs, bats & other feral animals
MONKEY B VIRUS	*Herpesvirus simiae*	Bite wounds & contact	Old world monkeys
LYMPHOCYTIC CHORIOMENINGITIS (LCM)	*Lymphocytic choriomeningitis virus*	Contact & inhalation	Mice, guinea pigs, hamsters & monkeys
FUNGI, PROTOZOAN			
TOXOPLASMOSIS	*Tosoplasma gondii*	Ingestion of oocytes & inhalation	Cats
RINGWORM	Dermatophytes	Contact	Dogs, cats, guinea pigs & cattle
HISTOPLASMOSIS	*Histoplasma capsulatum*	Inhalation of fungi	Dogs, other domestic & wild species

2.5.3 Theory and Practical Training Requirements for Animal Users

The University Animal Care Committee (UACC), in conjunction with the Office of the V-P-Research and International Relations, provides online theory training in animal use for research and teaching. This training is mandatory for all individuals who intend to work with animals at McGill and its affiliated hospitals. In addition, everyone who plans to work with live wild or laboratory animals is required to attend and pass a practical Animal Methodology Workshop specific to the species which he or she will handle. The practical training is provided at the Comparative Medicine and Animal Resources Centre, as well as several McGill-affiliated institutions. Certifications for both courses are valid for a period of 5 years. Detailed course information is available on the UACC website.

2.5.4 The Occupational Health Program

While prevention is the most desirable means of minimizing the risk of transmission, it is not always completely effective. Thus, verifying the health status of those involved in animal studies or animal care is a necessary safety check. In recognition of this, McGill University has developed an occupational health program that includes medical monitoring for students and staff who, as part of their duties, are potentially exposed to zoonotic agents. Table 3 outlines the steps involved in medical monitoring.

The program is free of charge for McGill employees and students who are involved in animal studies or animal care. Participation in the program is mandatory for persons in contact with non-human primates, and is optional for those in contact with all other species.

Medical records remain confidential, will be maintained only by the administering physician, and will be shared only with the patient. The physician will inform the University only in instances where active zoonoses are diagnosed: such cases will be handled no differently than any other illness that compromises the safety of the individual or that of others.

TABLE 3 – Steps Involved in Medical Monitoring

Procedure	Persons eligible
Pre-placement assessment; medical history questionnaire; and, if clinically indicated, medical examination	Everyone in direct[1] or indirect[2] contact with animals
Tetanus immunization (booster every 10 years);	Everyone in direct contact with animals
Pre-placement PPD skin testing (2-step)	Everyone in direct or indirect contact with non-human primates
Hepatitis A vaccination; (2 doses)	Everyone in direct contact with non-human primates
Measles titer (and booster if necessary)	Everyone in direct or indirect contact with marmosets

[1]Direct contact: handling live animals, unpreserved tissues or body fluids, animal cages, cage accessories, animal waste or carcasses

[2]Indirect contact: working in areas where animals are used or housed

All procedures are specific to the species of animal involved and the nature of contact, are designed to be relevant only to the diagnosis of zoonoses, and are not used for any other purpose.

A detailed description of McGill's Occupational Health Program, as well as registration instructions and links, is available at the website below:

https://www.mcgill.ca/ehs/laboratory/ohs

Persons working in affiliated hospitals or Research Institutes should contact their local Occupational Health Offices, which have their own programs.

Section 2.6 Reporting of Accidents/Incidents

All accidents, dangerous incidents, workplace exposures to infectious material, or suspected occupational diseases should be reported using Online Accident, Incident & Occupational Disease Form (EHS-FORM-001).

All Accidents/Incidents should be reported to **EHS** within 24 hours. These reports aid in determining the cause of the accident/incident and in developing measures for preventing recurrence. Any near-accidents or incidents which could have resulted in an accident should also be reported, as these reports are useful in evaluating hazards for prevention of future accidents. All accidents and incidents involving a potential exposure to a risk group 2 or above pathogen will be reported by the **BSO** to the **PHAC**.

Section 2.7 Biomedical Waste Disposal

To protect individuals and the community from unnecessary exposure to biohazardous agents, biomedical waste must not be disposed of with regular waste. Disposal of biomedical waste is governed by the Regulation Respecting Biomedical Waste (Quebec), and encompasses the following categories:

- human anatomical waste (body parts or organs)
- animal anatomical waste (carcasses, body parts, organs)
- non-anatomical waste, which includes:
    - **sharps** which have contacted animal or human blood, biological fluids or tissues
    - tissue or microbial cultures, and material contaminated by such cultures
    - live vaccines
    - containers or materials saturated with blood products

Biomedical waste should be disposed of frequently to reduce accumulation of these materials in work areas. Disposal service for solid biomedical waste is provided to users in McGill buildings by Hazardous Waste Management. The service is provided at no charge and includes provision of waste containers and regular pick-ups. Waste boxes are filled by those who generate the waste and must be packed and labeled as follows:

- Line boxes with a biohazard plastic bag.
- Affix user identification to the outside.
- Place sharps in a plastic puncture-proof container prior to disposal in the biomedical waste box. Refer to Section 6.3.3.1 of the Laboratory Safety Manual for a definition of Sharps.
- Store the box at 4°C or lower in a locked refrigerator.
- Use separate boxes for each category of waste, e.g., human anatomical should not be mixed with animal anatomical or non-anatomical waste.

Do not dispose of liquid waste in the biomedical waste boxes. Liquid waste should be decontaminated using an appropriate physical or chemical method (Section 2.4). Once decontaminated, liquid waste is no longer considered biomedical waste and can be disposed of accordingly.

Section 2.8 Transport of Containment Levels 1 and 2 Material

Whenever biohazardous materials are moved, whether it be within the lab, between labs or buildings or by public carrier, precautions must be taken to control the risks associated with a spill or leak.

Arrangements should be made to:

- Limit the number of moves,
- Reduce the possibility of breakage, and
- Contain the material in the event of a leak or spill.

2.8.1 Transport Within or Between Labs

When transporting within or between laboratories:

- Place specimens in leak-proof and breakage-resistant receptacles. Close with screw caps rather than snap caps whenever possible.
- Use unbreakable leak-proof secondary containers; for light loads that are to be carried, ensure that the secondary containers have solid handgrips. Small tubes can be sealed inside zipper-lock freezer bags, which are inexpensive, leak-proof and will not break if dropped.
- For heavier items, use a cart with guard rails or raised edges. Load so that the contents will not dislodge if the cart should bump into a wall or door.

2.8.2 Transport Between Buildings

When moving biohazardous substances from one building to another:

- Ensure that the substance is in a closed and sealed primary receptacle such as a test tube, vial or flask.
- Place cushioning absorbent material around the primary container.
- Use a secondary leak-proof container that can withstand dropping or crushing while in transit.
- If the material must be kept refrigerated or frozen during transport, place the coolant (e.g., dry ice, crushed ice) inside an insulated tertiary vessel. To prevent rupture of the package, ensure that dry ice is able to release carbon dioxide gas.
- Any movement of biological materials regulated by the **Transport of Dangerous Goods (TDG)** legislation must be done according the **TDG** regulations (section 2.8.3).

2.8.3 National and International Transportation Regulations

In Canada, the transport of biohazardous substances from one institution to another institution, is regulated by federal and provincial laws and acts. Use of regular mail for shipment of material that is known to be infectious is prohibited by Canada Post. If transport is done by a person, or using a courier company, agencies and associations such as the World Health Organization, the United Nations Committee of Experts on the Transport of Dangerous Goods (TDG), the Universal Postal Union (UPU), the International Civil Aviation Organization (ICAO) and the International Air transport Association (IATA) and Transports Canada have developed standards for the safe international shipment of infectious substances. It is the responsibility of the sender, carrier and the recipient to ensure that all regulations are enforced and that all requirements have been met and proper documentation is provided. Contact **EHS** for additional information. Prior to transferring any biological materials, the Principal Investigator must notify the **BSO** of the transfer using the Biohazardous Agent Transfer Notification form (EHS-FORM-101).

2.8.3.1 Shipping Requirements

Biohazardous materials are classified as Class 6, Division 6.2 Infectious Substances under Transport of Dangerous Goods Regulations. The regulations stipulate that all individuals involved in the transport of hazardous materials must be trained, tested and certified.

All biological material must be packaged so that there will be no leakage during transport. Packaging requirements may differ according to destination, carrier, mode of transport and whether the material is fully or partially controlled. Contact your carrier and **EHS** for assistance. The norms generally stipulate that biohazardous substances must be packaged as described below:

- Place the specimen inside an appropriately labeled leak-proof primary (inner) container; close with screw caps or seal with stoppers and tape or other suitable material.
- Wrap the container in enough absorptive material (e.g. paper towels, tissue, cotton wool) to absorb all fluid in the event of a leak.
- Several samples or cultures can be sent together provided each is inside a primary container, packed to prevent contact with each other, and surrounded by sufficient absorbent in case of breakage.
- Place the wrapped container inside a secondary watertight receptacle, using enough absorbent material to cushion the primary container.
- Place the secondary container inside a third (outer) package for protection from physical damage and water while in transit.
- If the shipment must be kept cold or frozen, notification to that effect should appear on the accompanying documents and on the outer package. Containers shipped with dry ice must be able to release carbon dioxide gas that could otherwise build up and cause the package to rupture.

Infectious substances should not be sent until arrangements have been made with the sender (the "consignor"), the carrier and the recipient (the "consignee"). To ensure that the material is transported safely and as quickly as possible, the sender should:

- Observe national and international transport regulations.
- Communicate with carrier and consignee to coordinate transport and receipt.
- Obtain and complete shipping documents and declaration forms.
- Arrange dispatch by direct route whenever possible.
- Send all transportation documentation to the receiving lab.

2.8.3.2 Importation Requirements

Importation of animal and human pathogens is overseen by the **CFIA** Office of Biohazard Containment Safety and the **PHAC** Pathogen Regulation Directorate.

2.8.3.2.1 Human and Terrestrial Animal Pathogens

Transfer of human and terrestrial animal pathogens are controlled by the **HPTA** and **HPTR** and the Health of Animals Act (**HAA**) and Regulations (**HAR**), respectively. The **CBS** outlines the requirements for labs working with human and animal pathogens. All laboratories wishing to import human pathogens, materials containing human pathogens and terrestrial animal pathogens must be in compliance with the **CBS**. The **BSO** must be notified of all transfers of these materials, within McGill, Canada and the international community.

When transferring materials to another facility, it is the responsibility of the **BSO** to communicate with the facility to ensure they have a valid **HPTR** license for the materials being transferred. No human pathogen, materials containing human pathogens or animal pathogens should be shipped or received without the prior approval of the **BSO**.

The procedure for importing biohazardous substances is summarized below:

- Obtain permission from the **BSO** for the materials to be transferred.
- Provide McGill University **HPTR** license number to the sender.
- Ensure that the sender packs and labels the infectious materials according to regulations.
- Arrange to have someone available on the delivery day to accept and examine the package.
- Have the necessary supplies and equipment on hand for decontamination and disposal in case of leakage during transport.
- Acknowledge receipt to the sender.

Note that both sender and receiver are required to keep copies of shipping documents for at least 2 years.

Other Regulated Biological Materials Import Permits for the following are issued through CFIA:

- Pathogens causing foreign animal and emerging animal diseases (i.e. pathogens not established or indigenous to Canada);
- Animals, animal tissues, sera and blood infected with animal pathogens;
- Aquatic animal pathogens;
- Plant pathogens;
- Foreign soil.

Part 3 – Standard Operating Procedures

One of the aspects of a strong safety program is the creation of standard operating procedures (SOP) for all routine tasks in the laboratory. These SOPs help identify the risks associated with the tasks performed as well as the mitigation strategies in place to minimize the risk and impact of an exposure to hazardous materials. This section outlines the basic safety requirements that should be included in laboratory SOPs when handling biohazardous materials. A Standard Operating Procedures (SOP) Template is available for developing project specific SOPs.

Section 3.1 Good Microbiological Practices

Basic requirements for a laboratory using infectious materials are:

- Ensure that all laboratory personnel, including service and custodial staff and visitors, understand the chemical and biological dangers associated with the lab. The Laboratory Information Card must include the biohazards symbol and be posted on doors outside laboratories where biohazardous material is handled or stored.
- Post the spill response protocol in a visible location in the laboratory.
- Facility doors must be lockable and access restricted to authorized personnel.
- The facility must be clean and free of clutter. Emergency safety devices (e.g., fire extinguishers, eyewashes, etc.) must be easily accessible and in working order.
- Personnel, students and visitors must adhere to University policies for eye and face protection and for protective clothing (Refer to Section 11 of the Lab Safety Manual). Remove lab coats or gowns and gloves before leaving the laboratory; never wear lab clothing in eating facilities.
- Avoid eating, drinking, smoking, storage of food and food utensils, application of cosmetics or lip balm, insertion or removal of contact lenses and inserting or removing ear buds in the laboratory.
- Restrain long hair. Avoid wearing loose clothing or jewelry, shorts and open-toed shoes or sandals.
- Observe "**Universal Precautions**" when collecting, processing, storing, shipping or transporting human blood and body fluids; i.e., handle such specimens as if infected with a **blood borne pathogen** such as hepatitis B or C or human immunodeficiency virus (HIV).
- Use **aseptic procedures** so as to minimize risks of splashes, spills and generation of aerosols.
- Refrain from pipetting by mouth.
- Use hypodermic needles only when absolutely necessary. Do not bend, break, shear or recap used needles.
- Wash hands after handling infectious material (even when gloves have been worn) and before leaving the laboratory.
- Decontaminate all contaminated materials before disposal or reuse.
- Decontaminate laboratory surfaces following any spill of biohazardous materials and at the end of each workday.
- Report all spills and accidents/incidents to **EHS** using the Accident/Incident Reporting Form (Annex E).

Section 3.2 Biological Safety Cabinets

3.2.1 Placement of the Biological Safety Cabinet in the Lab

Since an uninterrupted curtain of inward air flow at the front of the **BSC** is critical to its performance, the **BSC** should be situated in an area where there will be no interference with this airflow. Here is a list of common DO's and DON'Ts for the placement of a **BSC** in the lab to avoid interference with the airflow:

DO	DON'T
Maintain an undisturbed space of 40" around **BSC**	Place **BSCs** near an entryway. If necessary, maintain a distance of 60" to doorways behind the workspace and 40" from an adjacent doorway
Maintain a distance of 12'' to adjacent walls	
Place **BSCs** at least 80'' from opposing walls	
Place **BSCs** at least 60'' to opposing bench tops or areas with occasional traffic	
Maintain a distance of 40" between **BSC** and bench top along perpendicular wall	
Maintain a distance of 12" to columns to avoid disturbance to **BSC** airflow	Crowd together bench tops and **BSCs**
Maintain a distance of 120" between opposing **BSCs**	Place **BSCs** directly near benchtops
Maintain a distance of 40" between **BSCs** along same wall	
Maintain a distance of 48'' between **BSCs** when placed along perpendicular walls	

For more information on the placement of a BSC in the lab, refer to the National Institutes of Health Biosafety Cabinet (**BSC**) Placement Requirements for New Buildings and Renovations.

3.2.2 Working Safely in the Biological Safety Cabinet

- Procedures for working in a **BSC** are described in the Standard Operating Procedure: Safe Use of a Class II Biological Safety Cabinet (EHS-BIOS-201) available on the EHS website: (https://www.mcgill.ca/ehs/laboratory/biosafety/forms-tools-and-resources).

3.2.3 Decontamination of Biological Safety Cabinets

- The HEPA filter must be decontaminated by a certified company prior to changing the HEPA filter or prior to moving a BSC (within a laboratory, to a new laboratory or to a different building).
- It is the responsibility of the Principal Investigator to ensure that all new information regarding a BSC is forwarded to EHS.
- Examples of information to be forwarded to EHS include:
  - A new BSC that is purchased
  - A BSC that is discarded
  - Decontamination certificates
  - Certification certificates (if covered by the Principal Investigator)

Section 3.3 Emergency Response

3.3.1 Emergency Spill Response

All individuals who work in a lab where pathogens are used must know how to handle these agents safely and what to do in case of a spill. An emergency spill response protocol specific for the microorganisms in use should be prepared and posted in a visible location within the laboratory. All spills involving biohazardous materials must be reported to **EHS** via the online Accident/Incident Report Form (EHS-FORM-001). The **BSO** will be required to notify the **PHAC** of any incidents and possible exposures involving biohazardous materials.

3.3.1.1 Prevention

An accident prevention plan should be the first priority. General safety precautions include:

- Limit access to rooms where microbiological agents are used.
- Wear appropriate protective clothing.
- Use the appropriate **BSC**.

- Use plastics rather than breakable glassware to reduce likelihood of puncture wounds, cuts and generation of aerosols in the event of an accident.
- Transport materials on carts that have lipped shelves, using secondary containers (i.e. tubs) to catch spills.
- Disinfect waste.

3.3.1.2 The Spill Response Plan

Response procedures should be established before a spill occurs. Assessment of the hazards presented by the pathogen(s) in use should be based upon:

- Virulence and infectivity of the agent
- Viability - e.g., does the organism become inactive when dried?
- Route of entry - e.g., can the organism enter the body via aerosols or splash to the eye?
- Quantity and location of possible spill
- Immune status of the individuals at risk

The necessary clean-up materials should be available on site. In preparing a spill response kit, ascertain that it contains the appropriate clean-up materials, protective clothing and equipment. The kit should be stored in a visible and accessible location immediately outside the facility and should include:

- Disposable protective clothing (e.g., long-sleeved coat or gown, mask, gloves)
- Absorbent paper
- Autoclavable container and bags
- Disinfectant appropriate for the pathogen(s) handled: be sure to replace the disinfectant before it expires
- Autoclavable squeegee or forceps and dustpan

3.3.1.3 Spill Response Procedures

The appropriate spill response depends on the nature of the spilled organism and on the size of the spill. The following sections outline suitable approaches to handling minor and major spills. All spill response plans should include the possibility of the exposure of laboratory personnel to the biohazardous materials present in the laboratory.

3.3.1.3.1 Minor Spills

Small spills can be cleaned up immediately by lab personnel, provided that the organism does not pose a health risk (i.e., if the spill consists of low to moderate risk agents). Take the following steps:

- Remove contaminated gloves, lab-coat and clothing
- Evacuate the area if there is a risk of exposure to aerosols
- Allow aerosols to settle (~30 minutes)
- Cover with a disinfectant-soaked towel (using a spray bottle for distributing the disinfectant generates aerosols and is to be avoided).
- Apply disinfectant in a circular pattern working from the outer perimeter to the centre of the spill
- Allow to sit for an appropriate time as determined by the disinfectant in use and the contaminated materials
- Autoclave or discard contaminated material in a biomedical waste container.
- Report spill to **EHS** using the Accident, Incident and Occupational Disease Report Form (EHS-FORM-001).

3.3.1.3.2 Major Spills

For spills of large volumes of moderate risk agents or small volumes of high risk agents, proceed as follows:

- If you are able to clean the spill yourself follow the procedures as for a minor spill
- Treat serious injuries before attempting to contain the spill.

- Evacuate the area immediately if exposure to the aerosolized microorganism presents a potential health hazard; close the facility door(s) and allow aerosols to settle for 30 minutes. Remove contaminated clothing and place it in an autoclave bag or other sealed container; disinfect and wash exposed skin. If additional assistance is required contact Security Services at **514 398-3000** (Downtown) or **514 398-7777** (MacDonald Campus).
- If the spilled material has leaked through the grilles of a **BSC**, leave the cabinet running and pour in enough disinfectant (avoid alcohol due to explosion hazard) to dilute the spill tenfold. Drain the catch tray after the time interval appropriate for the disinfectant.
- Wipe down any adjacent walls, cabinets, furniture and equipment that may have been splashed.
- Use forceps/squeegee and dustpan to pick up and transfer the contaminated material into an autoclave bag or biomedical waste container.
- Decontaminate the waste and cleaning utensils.
- Report spill to **EHS** using the online Accident, Incident and Occupational Disease Report Form (EHS-FORM-001).

3.3.1.3.3 Personnel Exposure

In the event of exposure of personnel to a biohazardous material it is important to follow the following steps:

- Ask for help
- Serious injury: dial **911** followed by Security
    - Downtown : 514 398-**3000**
    - Macdonald Campus: 514 398-**7777**
    - Montreal Neurological Institute: **55555**
    - **Note:** When dialing 911 from a McGill phone, Security will monitor the call and dispatch an operator
- Needle sticks and cuts: wash with soap and water
- Splashes: flush eyes, mouth, nose
- If required, seek medical attention:
    - Student Health Services
        - **Downtown:** https://www.mcgill.ca/wellness-hub/access-care
        - **Macdonald Campus:** https://www.mcgill.ca/macdonald-studentservices/health-wellness
    - Nearest hospital or clinic.
- Notify supervisor
- Complete online Accident, Incident & Occupational Disease Report (EHS-FORM-001)
    - Page 1: 'victim'
    - Page 2: supervisor/safety representative
    - Submit to **EHS** (MNI Rm 778 if working at MNI)
- Monitor for symptoms – See pathogen **PSDS** for information

McGill personnel working at locations off the McGill Downtown or MacDonald Campuses should complete the online Accident, Incident & Occupational Disease Report (EHS-FORM-001) and submit to EHS as a record to the Incident. All local forms should be completed for investigation and follow-up.

3.3.2 *Evacuation*

All facilities must be aware of their department emergency plan and be aware of the primary and back- up evacuation routes. Each building on campus has a volunteer evacuation team whose role is to help evacuate in a quick and orderly manner to ensure everyone's safety. It is important to follow the evacuation team's instructions. In addition to the recommendations in the University Safety Emergency Guide (USEG),

a plan to secure biohazardous materials should be written into the laboratory and departmental emergency plans. This plan should include:

- Location of all biohazardous materials storage areas.
- Procedures for securing biohazardous materials prior to evacuation.
- Roles of various personnel in the securing of biohazardous materials.

In certain emergencies you may be asked to shelter in place instead of evacuating a building. Take all reasonable precautions to secure biohazardous materials in this instance.

3.3.3 Fire

If you detect fire or smoke, follow the directions in the University Safety Emergency Guide (USEG). When safe to do so, secure all biohazardous materials according to the evacuation plans outlined in the laboratory and departmental emergency plans prior to evacuating the facility. All laboratory facilities should have an evacuation plan in place. Evacuation drills are performed campus wide on an annual basis.

3.3.4 Natural disaster

A natural disaster is defined as a natural event, such as an earthquake or flood, with catastrophic consequences. Severe weather; such as rain, snow or ice storms, or an earthquake are the natural disasters most likely to have a catastrophic effect on McGill University infrastructure. The USEG gives general instructions in the event of a natural disaster. When not in use, all biohazardous materials should be stored in a manner so as to minimize the risk of damage and spill in the event of damage caused by a natural disaster.

The Montreal region has experienced severe ice, snow, rain and wind storms in the past. While there is the potential for longer term power failures and infrastructure damage during these storms, they do not appear at random. Most catastrophic damage can be avoided by the simple precaution of verifying the weather forecasts and warnings for the regions and taking measures in the days leading up to the potential severe weather to ensure all biohazardous materials are secure. Prior to resuming laboratory work after a severe weather storm, all critical equipment and infrastructure should be inspected to ensure it is in proper working order. All critical equipment and infrastructure should be identified in the laboratory and departmental emergency plans and the requirements for resuming work after a natural disaster should be well defined. Critical equipment/infrastructure can include, but is not limited to:

- BSCs
- Freezer, refrigerators and incubators
- Gas and vacuum lines

During an earthquake, take every reasonable precaution to secure all biohazardous materials not already in storage. An inventory of all biohazardous materials must be maintained for reference. In the recovery phase of a natural disaster, all biohazardous materials must be accounted for. Also, based on an **LRA**, all critical equipment and infrastructure must be inspected and certified prior to resuming work in the laboratory.

3.3.5 Power Interruption

All equipment critical to maintaining containment should be identified and placed on an outlet that will provide emergency power during a power interruption. During a power outage, follow the instructions in the USEG. During a power interruption, immediately cease all work with biohazardous materials as vital safety equipment may not be functional at this time. Keep a flash light on hand to secure all biohazardous materials. If lighting is interrupted for more than 10 minutes, notify security at (**514) 398- 3000** (Downtown) or (**514) 398-7777** (Macdonald Campus). Minimize movement in the dark. Security will evaluate the need to evacuate the building.

3.3.6 Extended Laboratory Closure

An extended shut-down of the laboratory can occur for a number of external factors including but not limited to:

- Global pandemic
- Natural disaster
- External threat
- Building damage

Depending on the nature of the emergency a shut-down could last for any amount of time with minimal time to plan for the shut-down. In order to ensure that during an extended shut-down biohazardous materials are contained and secure, the labs emergency response plan should include the following information:

- Process for quickly ramping down research activities
- Projects should be given a priority level to help determine which projects will continue with the available resources
- Identify tasks that must occur in order to ensure containment and security of biological materials during an extended shut-down

Section 3.4 Safe Handling of Laboratory Equipment

Whenever lab equipment is purchased, preference should be given to equipment that:

- Limits contact between the operator and the infectious agent.
- Is corrosion-resistant, easy to decontaminate and impermeable to liquids.
- Has no sharp edges or burrs.

Every effort should be made to prevent equipment from becoming contaminated. To reduce the likelihood of equipment malfunction that could result in leakage, spill or unnecessary generation of aerosolized pathogens:

- Review the manufacturer's documentation. Keep for future reference.
- Use and service equipment according to the manufacturer's instructions.
- Ensure that anyone who uses a specific instrument or piece of equipment is properly trained in the setup, use and cleaning of the item.
- Decontaminate equipment before it is sent out for repairs or discarded.

The following sections outline some of the precautions and procedures to be observed with some commonly used laboratory equipment.

3.4.1 Centrifuges

Improperly used or maintained centrifuges can present significant hazards to users. Failed mechanical parts can result in release of flying objects, hazardous chemicals and biohazardous aerosols. The high- speed spins generated by centrifuges can create large amounts of aerosol if a spill, leak or tube breakage occurs.

To avoid contaminating your centrifuge:

- Check glass and plastic centrifuge tubes for stress lines, hairline cracks and chipped rims before use. Use unbreakable tubes whenever possible.
- Avoid filling tubes to the rim.
- Use caps or stoppers on centrifuge tubes. Avoid using lightweight materials such as aluminum foil as caps.
- Use sealed centrifuge buckets (safety cups) or rotors which can be loaded and unloaded in a **BSC**. Decontaminate the outside of the cups or buckets before and after centrifugation.
- Inspect O-rings regularly and replace if cracked or dry.
- Ensure that the centrifuge is properly balanced.

- Do not open the lid during or immediately after operation, attempt to stop a spinning rotor by hand or with an object or interfere with the interlock safety device.
- Decant supernatants carefully and avoid vigorous shaking when re-suspending packed cells.
- Clean spills promptly.

When using high-speed or ultra-centrifuges, additional practices should include:

- Connect the vacuum pump exhaust to a disinfectant trap.
- Record each run in a log book: keep a record of speed and run time for each rotor.
- Install a HEPA filter between the centrifuge and the vacuum pump.
- Never exceed the specified speed limitations of the rotor.

3.4.2 Lyophilizers (Freeze-Driers)

Aerosols may be produced during operation of a freeze drier and when material is being removed from the chamber. When lyophilizing biohazardous materials:

- Load samples in a **BSC**.
- Check glass vacuum containers for nicks and scratches.
- Use only glassware that was designed for high vacuum use.
- Use a disinfectant-containing trap for the vacuum pump exhaust.
- After completion of the run, decontaminate all accessible surfaces.

3.4.3 Mixing Apparatus

Homogenizers, shakers and sonicators can release significant amounts of aerosols during their operation and should be operated in a **BSC** if possible.

When using any mixing equipment, remember to:

- Check condition of gaskets, caps and bottles before using.
- Allow aerosols to settle for at least one minute after use before opening containers, opening in a **BSC** if possible.
- Cover tops of blenders with a disinfectant-soaked towel during operation.
- Immerse sonicator tip into solution to a depth sufficient to avoid creation of aerosols.
- Disinfect all exposed surfaces after use.

3.4.4 Freezing Apparatus

Spills inside freezing equipment may place laboratory and maintenance personnel at risk; for safe use of such equipment:

- Periodically check freezers, liquid nitrogen tanks and dry ice chests for broken ampoules, tubes etc.
- To minimize breakage and leaks, place primary containers such as test tubes inside secondary containers prior to storage in freezing units.
- For electrical safety, remember to shut down units before proceeding with decontamination.

3.4.5 Vacuum/Aspirating Equipment

Glass vacuum vessels may rupture and shower laboratory personnel with glass fragments and flask contents. To reduce these risks:

- Use metal flasks and vacuum traps whenever possible.
- Tape glass containers with duct or adhesive tape to contain glass shards in case of rupture or, use a secondary metal container that is at least as tall as the vacuum flask.

To prevent exposure of lab personnel or maintenance employees who may be required to repair the central vacuum system, vacuum line connections that draw biohazardous aerosols or fluids should be fitted with:

- A HEPA filter in the line leading into the vacuum line: cartridge-type in-line filters provide an effective barrier to escape of aerosols into vacuum systems, and are commercially available for this purpose (discard used filters as biomedical waste)
- An overflow flask in case of accidental aspiration of liquids out of the collection vessel. This flask should:
  - be of sufficient capacity
  - be placed between the collection flask and the air filter
  - contain the appropriate disinfectant
  - contain an antifoam agent whenever air bubbling generates excessive foam

3.4.6 Needles and Syringes

Hypodermic needles and syringes present hazards of spill, autoinoculation and aerosol generation, and should be used only when absolutely necessary, such as for parenteral injection or withdrawal of bodily fluids. When working with syringes and needles, the following precautions are recommended:

- Perform all operations with infectious material in a **BSC**.
- Fill syringes carefully; avoid frothing or introduction of air bubbles.
- Shield needles with disinfectant-soaked cotton pledgets when withdrawing from stoppers.
- Use luer-lock needles and syringes or units in which needles are integral to syringes. Better still, use one of the newer "safe" alternatives to needles and syringes.
- Do not bend, shear by hand, or recap needles.
- Place used needles and syringes in puncture-resistant containers and decontaminate before disposal.
- When withdrawing liquids from septum-capped or diaphragm bottles, consider using an opener made especially for this type of bottle; this allows for use of a pipette rather than a syringe/needle assembly.
- Use cannulas or blunt-end needles for introduction or removal of fluids through small apertures in equipment.

3.4.7 Pipettes

Improper handling of pipettes can lead to contamination of the user and/or to generation of hazardous aerosols. Mechanical pipetting aids should be used for all pipetting procedures: never pipette by mouth.

Selection of a pipetting device should be based upon:

- Intended use
- Ease of handling
- Delivery accuracy
- User preference
- Quality of seal formed with pipettes to be used; liquid should not leak from the pipette tip
- Whether the pipetting aid can be sterilized

If infectious aerosols are likely to be generated, perform pipetting operations in a **BSC**. Handling pipettes as described below will reduce splashing and aerosols:

- Plug pipettes with cotton.
- Check pipettes before using; cracked or chipped suction ends may damage the seals of the pipetting aid.
- Keep pipettes upright while in use and between steps of a procedure to prevent contamination of the mechanical aid.
- Gently expel contents close to the surface of a liquid or allow to flow down the side of the container.
- Avoid mixing fluids by alternate suction and blowing, or by bubbling air from the pipette.

- Avoid forceful ejection of the contents; use TD (short for "to deliver", also referred to as "mark-to-mark") rather than TC ("to contain") pipettes, as the last drop of fluid does not have to be expelled with TD pipettes.
- Use easier-to-handle shorter pipettes when working inside a **BSC**.
- Submerge used non-disposable pipettes horizontally in disinfectant solution; dropping them in vertically may force out any liquid remaining in the pipette.

3.4.8 Autoclaves

Autoclaves are ideal for decontaminating biohazardous waste and for sterilizing surgical dressings, glassware and microbiological media and liquids. They must be loaded carefully to allow for steam penetration, since steam must contact pathogens in order to destroy them. Longer times are needed for larger loads, large volumes of liquid and denser materials. Proper loading and packing procedures include the following precautions:

- Wrap packages to allow for steam penetration; aluminum foil does not allow steam penetration, and should not be used for wrapping.
- Do not overload the chamber.
- Fill autoclave bags no more than ⅔ full
- Do not seal bags or close bottles and other containers tightly.
- Do not stack containers.
- Place materials inside autoclave-safe secondary container

The changes that are seen on autoclave indicator tapes following an autoclave cycle do not guarantee that the contents of containers are sterile: they indicate only that the tape on the outside of the packages has been exposed to a certain amount of heat or steam. The time required for effective sterilization depends on the size of the load, volumes of liquid and density of materials to be autoclaved. **Validation** of chosen cycle parameters must be done for all decontamination autoclave cycles using a biological indicator in a non-contaminated representative load. Routine **verification** of the autoclave should be done at least monthly (or more frequently as determined by an **LRA**) using a biological indicator. Biological indicators, such as *Geobacillus stearothermophilus,* are available and consist of a heat resistant bacterial spore in a vial format with a colour indicator growth media which are kept separate until needed. The indicator is placed in the area least likely to reach sterilizing conditions, such as in the middle of the largest or densest package. A subsequent colour change upon incubation in the colour indicator media (usually 37^{O}C for 24-48 hours) indicates that the load has been exposed to the required conditions for a sufficient length of time. Results of the biological indicator and a control must be kept on file, see Autoclave Efficacy Testing Log form (EHS-FORM-096) for a template to record results.

Safe work practices when using an autoclave include the following:

- Read the operating manual and post proper work procedures near the autoclave.
- Never autoclave hazardous chemicals or radioactive materials.
- Always ensure autoclave chamber pressure gauge reads 0 psi.
- Open the door slightly to allow escape of steam before unloading.
- Wear insulated gloves or mitts when unloading.

3.4.9 Liquid Nitrogen Storage

Explosion of vials stored in liquid nitrogen can occur when liquid nitrogen seeps into the vial during storage in the liquid phase of the tank. Liquid nitrogen in the vial rapidly expands as the liquid transforms to gas expanding to about 700 times its original volume, causing the sealed vial to explode. Liquid nitrogen may also seep into vials due to factors such as the use of inappropriate or faulty vials, lids or caps that were over-tightened, and vials that were over filled. Vials should be selected that are:

- Designed or certified for liquid nitrogen storage (follow manufacturers' specifications for use)

- Made of polypropylene
- Which have a male cap preferably with large threads
- Which contain a silicone O-ring

Safety considerations for storage of biological materials in liquid nitrogen include:

- Examine vial (including O-ring) for defects prior to use
- Never reuse vials
- Do not over-tighten vial (may compromise integrity of the seal)
- Do not over-fill vial (expansion when freezing may compromise integrity of vial or seal)
- Vial can be wrapped (e.g., heat shrink tube wrap)
- If possible, store vials in gas phase instead of in liquid phase of the tank
- If stored in the liquid phase, move vial to gas phase prior to removing from the tank (e.g., 24-48 hours prior to removal)
- Thaw or transport vial in thick-walled container
- Avoid over-filling liquid nitrogen storage units
- Use appropriate personal protective equipment (PPE) such as:
    - Face shield
    - Thermal gloves
    - Thermal insulated apron

3.4.10 Miscellaneous Equipment

- Microscopes: disinfect the stage, eyepieces, knobs and any other contaminated parts. Select a disinfectant that will be effective on the pathogens and non-corrosive to the microscope.
- Microtomes: disinfect knives and anti-roll plates after use.
- Water baths: Clean regularly; add disinfectant, such as a phenolic detergent, to the water. Avoid using sodium azide to prevent growth of microorganisms (sodium azide forms explosive compounds with some metals).
    - Raise the temperature to 90^{O}C or higher for 30 minutes once a week for decontamination purposes.
    - To prevent electrical shocks, unplug the unit before filling or emptying and have the continuity-to-ground checked on a regular basis.
- Tissue grinders: use in a **BSC**; wrap glass grinders in a wad of absorbent paper and wear gloves. Polytetrafluoroethylene (PTFE, "Teflon") grinders are safer, as they will not break.
- Microbiological transfer loops: to eliminate the spattering and aerosols associated with flaming of loops, char the material before fully inserting the loop into the flame: i.e., before flaming, hold the loop close to (but not into) the flame. Alternatively, use disposable loops or a micro- incinerator.

Part 4 – Resource Information

Section 4.1 References

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Shematek G and W Wood. Biological Hazards. In: Laboratory Safety, CSLT guidelines, 7th edition. Canadian Society of Laboratory Technologists, Hamilton, Ontario, (2013).

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Section 4.2 Glossary

Aerosol: A suspension in air of liquid or solid microscopic particles.
Antiseptic: Acting against sepsis. An antiseptic agent is formulated for use on living tissue such as mucous membranes or skin to prevent or inhibit growth or action of organisms. Antiseptics should not be used to decontaminate inanimate objects.
Aseptic procedure: A procedure carried out in a manner that prevents contamination of material.
Autoclave: An apparatus which employs physical means (moist heat under pressure) to sterilize or decontaminate.
Bacterial spore: A bacterial spore is a resistant body formed as part of the life cycle of some bacteria. Bacterial spores are able to withstand severe environmental conditions (e.g., heat, drying, and chemicals) for many years. When conditions are favourable, spores germinate into vegetative bacterial cells capable of replication.
Bacteriostatic: Inhibiting growth of bacterial organisms without necessarily killing them or their spores.
Bacteria: Single-celled microorganisms, ranging in size from 0.4 to 2.0 microns, which multiplies by subdivision.
Biocide: An agent that can kill all pathogenic and non-pathogenic living organisms, including spores.
Blood borne pathogen: Infectious microorganisms that are carried in the blood of infected humans or animals and that can be transmitted through contact with infected blood, body fluids, tissues or organs. Blood borne pathogens are implicated in diseases such as malaria, syphilis, brucellosis, tuberculosis, hepatitis B and acquired immunodeficiency syndrome (AIDS).
Decontamination: Removal of microorganisms to a lower level, such that there is no danger of infection to unprotected individuals. Sterilization and disinfection are decontamination procedures.
Disinfectant: An agent used to kill microorganisms on inanimate objects such as instruments or surfaces.
Disinfection: Use of physical or chemical agents to destroy pathogens and potential pathogens on inanimate objects. Disinfection does not necessarily result in sterilization.
Germicide: An agent which destroys microorganisms, especially pathogenic microorganisms ("germs"). Sterilants, disinfectants and antiseptics are germicides.
Infectious: Able to cause disease in a susceptible host. A biological organism that can establish a process of infection is an **Infectious agent**.
Iodophor: An "iodine-carrying" compound. An iodophor is a combination of iodine and a solubilizing surface-active agent, or carrier.
Microorganism: A microscopic organism, such as a bacteria, protozoan, yeast, virus or alga.
Pathogenic organisms: Organisms capable of causing disease, either directly (by infecting) or indirectly (by producing a toxin that causes illness).
ppm: Abbreviation for parts per million, used to describe concentrations in liquids or gases, e.g., 10,000 ppm is approximately equivalent to 10 g/liter or a 1% W/V solution.
Prion: Virus-like proteinaceous infectious agent. Prions differ from viruses in that they are not known to contain either DNA or RNA.
Protozoa: Nucleated microorganisms, some of which are large enough to be detected with the naked eye. Sizes range from .01 to 200 microns.
psi: Abbreviation for pounds per square inch, a unit of pressure equal to the pressure exerted on an area of one square inch. 1 psi = 7.03 x 10-2 kilograms per square centimeter.
Sharps: Sharp objects such as needles, scalpel blades, broken glass, pasteur pipettes or any other object that can penetrate skin.
Sporicide: An agent that destroys bacterial and fungal spores.
Sterilization: Use of physical or chemical means to bring about the total destruction of all viable microbes, including resistant bacterial spores.

Universal Precautions: Precautions taken when handling, storing, transporting or shipping items or specimens containing or contaminated with human blood and body fluids: all such materials are treated as if infectious.
Validation: The act of confirming that a method achieves its objective by observing that specific parameters have been met (ie. using biological indicators to confirm that a given autoclave cycle can decontaminate a representative load of waste). Validation infers that a method is suitable for its intended purpose.
Vector: An agent, such as an insect, that can carry a disease-producing organism from one host to another.
Vegetative form: In bacteria, a stage of active growth, as opposed to a resting state or spore formation.
Verification: The routine monitoring of equipment and processes to ensure continued efficacy between validations. This includes comparing the accuracy of a piece of equipment to an applicable standard or standard operating procedure (ie. testing of a class I biological safety cabinet in accordance with the manufacturers specifications).
Viable: Able to grow and multiply.
Virucide: An agent that destroys or inactivates viruses.
Virus: A microorganism, ranging in size from .01 to .25 microns (10 - 250 nanometers), that can reproduce only within living cells.
Virulence: The disease-producing power of a microorganism.
Zoonosis: A disease that can be transmitted from animals to humans.

Section 4.3 List of Acronyms

BSC: Biological Safety Cabinet or Biosafety Cabinet
BSO: Biosafety Officer
CBH: Canadian Biosafety Handbook
CBS: Canadian Biosafety Standard
CFIA: Canadian Food Inspection Agency
EHS: Environmental Health and Safety
HAA: Health of Animals Act
HAR: Health of Animals Regulations
HPTA: Human Pathogens and Toxins Act
HPTR: Human Pathogens and Toxins Regulations
PHAC: Public Health Agency of Canada
PSDS: Pathogen Safety Data Sheet
SSBA: Security Sensitive Biological Agent
ULSC: University Laboratory Safety Committee

Section 4.4 Directory of Useful Contact Agencies

Public Health Agency of Canada
Centre for Biosecurity Ottawa, Ontario, K1A 0K9
Tel: (613) 957-1779; Fax: (613) 941-0596
Email:licence-permis@phac-aspc.gc.ca

Canadian Food Inspection Agency
Biohazards Containment and Safety
1400 Merivale Road, Ottawa, ON K1A 0Y9 Tel: (613) 773-6520; Fax: (613) 773-6521
E-mail: importzoopath@inspection.gc.ca
http://www.inspection.gc.ca/animals/biohazard-containment-and-safety/eng/1300121579431/1315776600051
For Importation of Animal or Plant pathogens
2001 Robert-Bourassa Boulevard, Room 671, Montreal, QC H3A 3N2 Tel: (514) 283-8888; Fax: (514) 283-3143

To order WHO publications

World Health Organization Canadian Public Health Association 1335 Carling Avenue, Suite 210 Ottawa, Ontario, K1Z 8N8
Tel: (613) 725-3769

Transports Québec
For information on transport of biohazardous materials 700, boul. René-Lévesque Est, 27e étage
Québec (Québec) G1R 5H1 Tel: (514) 873-2605
Transport Canada, Surface 685 Cathcart Street, Suite 701 Montréal, Québec, H3B 1M7 Tel: (514) 283-5722
Transport Canada, Air Carrier Operations, Dangerous Goods 700 Leigh Capreol Street
Dorval, Québec, H4Y 1G7 Tel:1-800-305-2059
For information on mailing non-infectious bloods, diagnostic specimens and biological products, contact Canada Post Corporation General Inquiries, Customer Service Tel: 1-866-607-6301

Section 4.5 Revision history

Revision number	Date initiated	Initiated by	Reason for update	Approved by	Approval date
Version 1.0		Ruth Blanchette	Updated to reflect the Human Pathogens and Toxins Regulations and add document tracking	ULSC	
Version 1.1	2-Oct-17	Ruth Blanchette	Updated to add validation and verification of autoclave, updated "Application to Use Biohazardous Materials" and a template Standard Operating Procedure for laboratories.	ULSC	January 2020
Version 1.2	16-Nov-20	Ruth Blanchette	Add the updated forms and links in "Appendix A" and "Appendix B". Reconcile document control number throughout the document.	ULSC	November 2020
Version 1.3	1Feb23	Ruth Blanchette	1. Canadian Biosafety Standard 3rd edition 2. Sections 1.2.2.1 & 2.1.1 - training 3. Section 1.3.2.2.2 - new BSC class II type C 4. Section 2.2.2 – Define a local risk assessment 5. Section 3.3.6 – Extended Laboratory Shut-down 6. Section 3.2.2 –Reference to the BSC SOP 7. Remove Sections 3.2.3, 3.2.4, 3.2.5 as the materials are covered in the BSC SOP 8. Add Section 3.4.9 – Liquid nitrogen storage	ULSC	March 29, 2023

Appendix A: Heat Decontamination Methods

METHOD PRINCIPLE / CONDITION	ADVANTAGES	DISADVANTAGES	USES
DRY HEAT THERMAL INACTIVATION: DESTROYS BY OXIDATION	Non-corrosive simple design and principle	Less effective than moist heat; requires longer times and/or higher temperatures	Materials that are damaged by, or are impenetrable to, moist heat
HOT AIR OVEN: 160-180^{O}C FOR 2-4 HOURS	Penetrates water-insoluble materials (e.g., grease and oil) less corrosive to metals and sharp instruments than steam	Slow diffusion, penetration; loading, packing critical to performance; not suitable for reusable plastics	Anhydrous materials, such as oils, greases and powders; laboratory glassware, instruments; closed containers
RED-HEAT FLAME: OXIDATION TO ASHES (BURNING)	Rapid	Initial contact with flame can produce a viable aerosol; possibility of accidental fire	Inoculating loops, needles
INCINERATION: OXIDATION TO ASHES (BURNING) 1- 60 MINS: TEMPERATURES MAY EXCEED 1000^{O}C	Reduces volume of waste by up to 95%	Improper use may lead to emission of pathogens in smoke; requires transport of infectious waste; excess plastic (>20%) content reduces combustibility	For decontamination of waste items prior to disposal in landfill
PASTEURIZATION: HEATING TO BELOW BOILING POINT (GENERALLY 77^{O}C) FOR UP TO 30 MINS	Can be used on heat sensitive liquids and medical devices; low cost	Not reliably sporicidal	Milk and dairy products; some heat-sensitive medical equipment
TYNDALLIZATION (FRACTIONAL STERILIZATION): HEATING TO 80-100OC FOR 30 MINS ON 3 SUCCESSIVE DAYS, WITH INCUBATION PERIODS IN BETWEEN	Resistant spores germinate and are killed on the second and third days	Time consuming; not reliably sporicidal	Heat sensitive materials such as bacteriologic media, solutions of chemicals, biological materials
BOILING: MAXIMUM TEMPERATURE OBTAINABLE IS APPROX. 100^{O}C 10-30 MINS	Minimal equipment required	Cumbersome; not practical for everyday lab use; not reliably sporicidal	Small instruments/ equipment
AUTOCLAVING: STEAM UNDER PRESSURE 121^{O}C/15 PSI FOR 15-90 MINS (GRAVITY DISPLACEMENT AUTOCLAVE); 132^{O}C/27 PSI FOR 4-20 MINS (PRE-VACUUM AUTOCLAVE)	Minimal time required; most reliable sterilization method in the laboratory	Loading and packing critical to performance; shielding dirt must first be removed; maintenance and quality control essential; damages heat-sensitive items	Preparation of sterile glassware, media and instruments; decontamination of reusable supplies and equipment; decontamination of infectious waste

Appendix B: Chemical Disinfection

Halogen-releasing chemical

germicides

CHLORINE COMPOUNDS:	
Sodium hypochlorite solution[1] (liquid bleach)	
Effective concentrations, contact times	100-10,000 ppm (.01-1%) free chlorine, 10-60 min (3,000 ppm for broad spectrum)
Advantages	Broad spectrum; inexpensive; widely available; bactericidal at low temperature
Disadvantages	Toxic, corrosive to skin and metals; efficacy decreases as pH increases; inactivated by organic matter; deteriorates under light and heat: shelf life of dilutions is less than 1 week
Some uses	General disinfectant; waste liquids; surface decontamination; emergency spill clean-up; instrument disinfection
Calcium hypochlorite[2] granules, powder, tablets	
Effective concentrations, contact times	As for liquid bleach
Advantages	As for liquid bleach, but more stable
Disadvantages	As for liquid bleach above, except shelf life is longer
Some uses	As for liquid bleach
NaDCC[3] (Sodium dichloroisocyanurate) powder, granules, tablets	
Effective concentrations, contact times	As for liquid bleach
Advantages	More stable than hypochlorites
Disadvantages	Toxic; corrosive; inactivated by organic matter
Some uses	As for liquid bleach
Chloramine-T[4] (Sodium tosylchloramide) powder or tablets	
Effective concentrations, contact times	As for liquid bleach
Advantages	More stable, less affected by organic matter than hypochlorites; longer activity than hypochlorites
Disadvantages	Deteriorates under humidity, light and heat
Some uses	As for liquid bleach
Chlorine dioxide[5]	
Effective concentrations, contact times	Demand-release of chlorine dioxide *in situ*
Advantages	Longer activity than other chlorine compounds; less corrosive, less toxic than other chlorine compounds; effective at pH 6-10
Disadvantages	Aqueous solutions decompose under light
Some uses	Instrument disinfection; gas sterilization of germ-free animal chambers
IODINE PREPARATIONS:	
Iodophors[6]	
Effective concentrations, contact times	30-1,000 ppm (.003-.1%) free iodine, 10-30 min
Advantages	Broad spectrum; germicidal over a wide pH range; generally nonstaining, less toxic and less irritating than aqueous or alcoholic iodine solutions
Disadvantages	Not consistently sporicidal; efficacy reduced by organic matter; some iodophor solutions support growth of *Pseudomonas* [7]
Some uses	Germicidal soaps and **antiseptics**; surface decontamination; work surface wipe down; instrument disinfection

[1] a 1/10 dilution of 5.25% bleach provides 5,250 ppm available chlorine

[2] "high tested" provides 70-72% available chlorine; chlorinated lime or bleaching powder provides approximately 35% available chlorine

[3] approximately 60% available chlorine

[4] approximately 25% available chlorine

[5] To avoid shipping of this extremely reactive product, reagents ("base" and "activator") from commercially available kits are mixed with water to generate chlorine dioxide immediately prior to use

[6] 10% povidone-iodine provides 1% available iodine

[7] Iodophor stock solutions can be less effective germicide than dilutions. For example, full-strength (10%) povidone-iodine provides approximately 10 times less free available iodine than a 1/100 dilution. Iodophors must be used at the manufacturer's recommended concentrations.

Summary of recommended concentrations, contact times, advantages and disadvantages of non-halogen chemical germicides. The wide ranges of effective concentrations and contact times cited reflect the interdependence of time and concentration as well as factors such as resistance of the particular class or strain of target microorganism(s) and desired effect.

Non-halogen chemical germicides

ALCOHOLS	
Effective concentrations, contact times	70-80% ethanol 60-95% isopropanol 10-30 min
Advantages	Low toxicity; rapid action; low residue; non-corrosive
Disadvantages	Rapid evaporation limits contact time; flammable, eye irritant; may damage rubber, plastic, shellac; ineffective against bacterial spores
Some uses	Skin disinfectant (antiseptic); surface decontamination; benchtop, cabinet wipe down
PHENOLIC COMPOUNDS	
Effective concentrations, contact times	400-50,000 ppm (.04-5%), 10-30 min
Advantages	Tolerant of organic load; "hard" dilution water leaves an active residue (may be desirable on some surfaces); biodegradable
Disadvantages	Pungent odour; corrosive; some forms toxic; not sporicidal; limited activity against viruses; leaves a residual film (undesirable in culture systems); may support growth of bacteria[1]
Some uses	Disinfection of instruments, equipment, floors and other surfaces; component of antiseptic soaps and lotions
QUATERNARY AMMONIUM COMPOUNDS	
Effective concentrations, contact times	500-15,000 ppm (.05-1.5%), 10-30min
Advantages	Combined detergent and germicidal activity; stable; working dilutions have low toxicity
Disadvantages	not sporicidal; limited activity against viruses, mycobacteria; most formulations not readily biodegradable; may support growth of bacteria[2]
Some uses	Surface decontamination; equipment wipe down; antiseptic formulations available; floors and walls
HYDROGEN PEROXIDE	
Effective concentrations, contact times	Aqueous solution 3-30% for 10-60 min 6% for 30 min may kill spores
Advantages	Rapid action; no residue; low toxicity; environmentally safe
Disadvantages	Limited sporicidal activity; corrosive to some metals; potentially explosive at high concentrations; stock solutions irritating to skin and eyes
Some uses	Surface decontamination; instruments and equipment
PERACETIC ACID (PAA)	
Effective concentrations, contact times	Aqueous solution: .001-.3% gas phase: 2-4%, 5-120 min
Advantages	Broad spectrum; sporicidal at low temperature; can tolerate organic load; rapid action; nontoxic decomposition products; leaves no residue
Disadvantages	Pungent odour; corrosive to some metals; shelf life of dilutions is less than 1 week; stock solutions irritating to skin and eyes; stock must be protected from heat & light; gas phase: respiratory irritant; fire hazard above 55°C
Some uses	Instruments and equipment; gas phase sterilization of chambers for germ-free animals

[1] especially bis-phenols, which have reportedly been contaminated with gram-negative bacteria such as *Pseudomonas spp*. and *Serratia marcescens*

[2] especially benzalkonium chloride, reported to be contaminated with gram-negative bacteria

Non-halogen chemical germicides

ALDEHYDES:	
Glutaraldehyde	
Effective concentrations, contact times	0.5-2.5% alkalinized aqueous solution, 2-30 mins; up to 12 hours to kill all spores
Advantages	Broad spectrum; does not corrode metal; can tolerate organic load
Disadvantages	Expensive; pH, temperature dependent; pungent odour; toxic: skin, eye, respiratory tract irritant; activated solutions have less than 2-week shelf life
Some uses	Cold sterilization and fixative; surface decontamination; instruments, equipment, glassware
Formalin (37% aqueous formaldehyde)	
Effective concentrations, contact times	3-27% formalin (1-10% formaldehyde) in 70-90% alcohol 10-30 min
Advantages	Broad spectrum; inexpensive; does not corrode metal; can tolerate organic load
Disadvantages	Pungent odour; skin, eye and respiratory tract irritant; potential carcinogen (animal studies); may require 24 hrs or more to kill all spores
Some uses	Cold sterilization and fixative; surface decontamination; instruments and equipment
Formaldehyde (gas)	
Effective concentrations, contact times	1-3 hours
Advantages	As for formalin; effective penetration
Disadvantages	As for formalin; flammable; poor penetration of covered surfaces
Some uses	On site decontamination of biological safety cabinet HEPA filters; enclosed areas
ETHYLENE OXIDE GAS	
Effective concentrations, contact times	50-1200 mg/L, 1-12 hours
Advantages	Broad spectrum; no heat or moisture evolved; penetrates packaging materials
Disadvantages	Flammable, reactive; toxic: potential carcinogen and mutagen; some sterilized items may need more than 24 hours for outgassing
Some uses	Heat or moisture sensitive supplies, instruments and equipment