CAPE BRETON UNIVERSITY

# Biosafety Manual

# 2016

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# Telephone Contact Information

## 1. Incidents/Accidents Involving Bio-Hazardous Materials

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## 2. Emergency Contact Information

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## Institutional Biosafety Committee Members

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## Chapter 1: Introduction

Cape Breton University (CBU) recognizes that ensuring the safety and security of students, faculty and staff is, above all else, the institution's most important mandate. In terms of its dealing with hazardous bio-materials, pathogens and toxins, CBU recognizes its fiduciary responsibilities of safety and security to the wider community and society as well. Ensuring an active and vibrant teaching and research atmosphere must include compliance with relevant regulatory frameworks and oversight procedures. To this end, CBU, here, presents its Biosafety Manual 2016, providing a framework of guidelines for the safe handling and disposal of bio-hazardous materials and waste within the university. Persons working or coming into contact with bio-hazardous materials and waste within CBU must read and acknowledge their commitment to comply with the policies and regulations herein.

Several governmental and institutional documents related to biosafety have been used as frameworks and references to construct this manual. They help to ensure the Biosafety Manual adheres to the requisite standards set out in relevant legislation and provide a robust framework for safe laboratory practices within the university. All workers are encouraged to reference governmental and institutional source materials beyond mandatory acknowledgment of the *CBU Biosafety Manual* and the *Canadian Biosafety Standards and Guidelines*. The relevant referenced documents are found below:

*Canadian Biosafety Standards and Guidelines, 2nd Edition, 2015(CBSG)*
*World Health Organization Biosafety Manual*
*PHAC Pathogen Safety Data Sheets*
*Dalhousie University Biosafety Manual, 2015*
*Cape Breton University Health and Safety Policy*
*Cape Breton University Needlestick and Blood Borne Pathogen Policy*
*Cape Breton University Escalation Procedure*

Additionally, those working or coming into contact with bio-hazardous materials and waste should be familiar with their responsibilities in relation to relevant municipal, provincial and federal legislation and regulation surrounding bio-hazardous materials.

Relevant governmental regulatory agencies include:

Public Health Agency of Canada (PHAC)
Canadian Food Inspection Agency (CFIA)

Relevant legislation, as per the *Canadian Biosafety Standards and Guidelines*, include:

Hazardous Products Act (HPA)
Human Pathogens and Toxins Act (HPTA)
Human Pathogens and Toxins Regulations (HPTR)
Health of Animals Act (HAA)
Health of Animals Regulations (HAR)

Legislation including guidelines and responsibilities for individuals include:

**Federal:**
Hazardous Products Act, 1985
Health of Animals Act, 1990
Transportation of Dangerous Goods Act, 1992
Controlled Drugs and Substances Act, 1996
Nuclear Safety and Control Act, 1997
Canadian Environmental Protection Act, 1999
Human Pathogens and Toxins Act, 2009

**Provincial (Nova Scotia):**
Environment Act, 1994-95
Occupational Health and Safety Act, 1996

Roles and Responsibilities: Routine and effective communication between the Biological Safety Officer (BSO), Research Supervisors (RS) and laboratory workers is critical to ensure a healthy and safe work environment for those interacting with bio-hazardous materials. A description of the roles and responsibilities of each of these parties is found below and follows guidelines set out in CBSG.

**Biological Safety Officer (BSO):**

- Knowledge of biosafety and biosecurity procedures and relevant municipal, provincial and federal legislation around the safe handling and disposal of bio-hazardous materials and waste.
- Development and oversight of standard operating procedures for all relevant laboratory equipment and sterilization protocols.
- Chair of the Institutional Biosafety Committee and related duties set out in the CBU Oversight Plan for Pathogens and Toxins, where appropriate.
- Routine communication with RS and laboratory personnel, ensuring that safety concerns and issues related to biosafety and security are addressed in a timely manner as to mitigate risk to students, faculty, staff and the wider community.
- Provide and/or coordinate training of students, faculty and staff where appropriate.
- Perform routine biosafety and biosecurity inspections of designated laboratory and teaching areas.
- Receive biosafety-related incident/accident reports and perform investigations where appropriate, reporting findings to relevant parties.

**Research Supervisor (RS):**

- Ensure all current and incoming students, visiting scholars and research assistants or technicians receive biosafety and laboratory orientation and training through CBU in addition to biosafety certificates for work in designated areas.
- Ensure the above mentioned persons read and acknowledge the guidelines and responsibilities included in this document.
- Enforce the university biosafety policies and relevant governmental regulations with respect to all students, faculty and staff working in the designated area.

- Perform routine safety inspections of designated areas under their supervision.
- Provide all workers with appropriate personal protective equipment for safe laboratory practices.
- Maintain an active inventory of all bio-hazardous materials and samples and update the BSO/IBC of changes.
- Ensure that all incidents/accidents are reported to the BSO and that required investigations and reports are completed in a timely manner.
- Maintain communication with BSO and relevant university safety and biosafety officials to ensure that potential risks are mitigated and that safety concerns involving the storage, handling and disposal of bio-hazardous materials are remedied in a timely manner.

**Laboratory Personnel:**

- Read and acknowledge the terms and conditions found within this manual. Ensure adequate understanding of all sections of the manual and follow up with RS or BSO to ameliorate confusion or misunderstanding.
- Follow directions from BSO and RS with respect to biosafety and biosecurity as well as policies and responsibilities outlined within the biosafety manual.
- Use appropriate personal protective equipment during all handling or transport of bio-hazardous materials and waste- without exception. If personal protective equipment are unavailable, cease laboratory or handling activity and contact the RS or BSO to receive proper protective equipment.
- Report all safety concerns and potential risks and hazards, including to yourself and colleagues, to the RS and/or BSO where appropriate.
- Report all incidents/accidents to RS and BSO as soon as possible.
- Participate in relevant laboratory and biosafety training sessions, including refresher courses.

## Chapter 2: Institutional Biosafety Committee and Administrative Oversight

Institutional Biosafety Committee:

- Mandatory members of the Institutional Biosafety Committee (IBC) include those instructors or research assistants participating in the handling of bio-material or in biosafety training of students or researchers within the designated areas.
- The IBC will be chaired by the Biological Safety Officer (BSO) (appointed by the VP, Academic & Provost) and may include a student representative (appointed by the BSO) trained in introductory microbiology. The Microbiology faculty at CBU are either active members of the Canadian Society for Medical Laboratory Science (CSMLS) or are Registered Microbiologists with the Canadian College of Microbiologists (CCM) or are trained in microbiology at the university level and are working towards certifications under the guidance of the BSO and other faculty. The BSO officer will at minimum meet one of either CSMLS or CCM registration. The present BSO has both. The number of members on the IBC may vary due to the fact there could be time periods where no active microbiology research is being done at CBU. At minimum the IBC will always include the microbiology faculty of which presently there are three.
- There are no term limits imposed on IBC mandatory or general membership.
- Tri-yearly IBC meetings will be called by the BSO in order to review biosafety practices within the university, address outstanding concerns/requests related to biosafety practices and modify practices and policies based on the changing needs of the institution and its students, faculty and staff. Due to the small size of CBU and the small number of people now or potentially involved in any microbiological work with risk group 2 pathogens/toxins three meetings per year will more than suffice. These will be in the fall, winter and spring/summer to coincide with the typical academic year terms.
- Unscheduled IBC meetings may be called by the BSO in special circumstances where appropriate. Members missing more than 2 consecutive meetings will have their IBC membership reviewed by the ABC and/or VP, Academic & Provost.

Reporting structure:

- The IBC through the BSO will report directly to the VP, Academic and Provost. The BSO may coordinate with the VP, Academic & Provost for routine updates and in the case of issues unresolved at the IBC level, as described elsewhere. The VP, Academic and Provost will report to the University Senate and President regularly or where appropriate.

## Chapter 3: Biosafety in the Laboratory

The following guidelines and policies follow those set out in the *Dalhousie University Biosafety Manual 2015*.

Safe laboratory practice is critical in preventing exposure when working with bio-hazardous materials. Anyone planning to work with bio-hazardous materials must be trained prior to beginning work. Supervisors are responsible for ensuring that all personnel in their laboratories are adequately trained.

### Biosafety Training

Prior to any work with bio-hazardous materials, students, faculty and staff must read both the *Cape Breton University Biosafety Manual* as well as the Public Health Agency of Canada's *Canadian Biosafety Standards and Guidelines*, and sign acknowledgment of having read and understood the responsibilities described therein. As well, Research Supervisors (including principal investigators, co-supervisors and visiting scholars where appropriate) are responsible for training those under their supervision in all laboratory specific procedures (including storage, handling and safe disposal of bio-hazardous materials) as defined in the relevant Standard Operating Procedures (SOP's).

Topics that may be covered in any Biosafety training session could include:

- Access/security controls
- Use of safety equipment
- Health hazards
- Safe work procedures
- Incident/Accident Reporting Mechanisms
- Emergency procedures

### Access/Security Controls

The *Canadian Biosafety Standards and Guidelines* requires the international biohazard warning symbol (Figure 1) to be displayed if bio-hazardous materials, including body fluids, unfixed cell, tissue or organ cultures, viral, bacterial, fungal or parasitic agents requiring **BSL 2** (see chapter 5 for Risk Groups/Containment Levels) or greater are present. The Containment Level of the laboratory must also be indicated (CL#). Laboratory doors should be locked when the laboratory is unoccupied and only authorized persons are permitted to enter laboratory working areas- there are no exceptions. Research Supervisors should also maintain a current approved worker list with the BSO to mitigate the risk of wrongful entry into the designated areas. Only with the permission and direct supervision of the BSO, or other members of the IBC, can children be admitted to research areas or the microbiology teaching lab. On occasion as part of community and school outreach the Microbiology faculty at CBU assist area school children, with very basic microbiology projects, microscopy, etc. in the Microbiology teaching lab only and always with direct supervision. Pets or animals are not to be permitted to enter laboratory working areas.


**Figure 1. Biohazard symbol to be posted in BSL2 or higher working/containment areas.**

**Use of Personal Protective Equipment (PPE)**

Using proper PPE is required in any lab area where research with Risk Group 2 pathogens/toxins are being used. Non-compliance may result in access privileges being revoked at the discretion of the BSO. A properly fastened laboratory coat, enclosed footwear with no or low heels, and gloves need to be worn in any lab area where research with Risk Group 2 pathogens/toxins are being used. Laboratory coats, enclosed footwear, and gloves prevent bio-hazardous materials from contact with the skin, including any areas where there might be cuts, abrasions, or dermatitis. The legs are a vulnerable area if uncovered, so it is inappropriate to wear short skirts or shorts. A properly fastened **laboratory coat** also protects street clothing from becoming contaminated and prevents possible cross contamination from any normal flora present on the skin. It is important that laboratory coats remain in the laboratory to prevent spread of contamination to non-laboratory areas or your home. Enclosed footwear with no or low heels protect the feet from spills as well as injuries from dropped sharps.

Appropriate gloves need to be worn for all procedures that may involve direct or accidental (unintended) contact with ANY BSL bio-hazardous materials. Latex or nitrile gloves offer a high level of dexterity and a higher level of sensitivity; however, they don't offer substantial protection from needle sticks, animal bites, or sharps. **Double gloving is recommended if working with the afore-mentioned.** Compatibility with chemicals being handled should also be considered by referring to relevant compatibility/manufacturer information. Glove fabrics differ in their resistance to permeation by different chemicals. **Open cuts or wounds must be covered before applying gloves.** Laboratory activity should be denied to persons with active skin infections due to the possibility of causing further accidental harm to them.

In addition to the minimal protective equipment described above, other protective equipment might be needed when working with infectious agents (also see chapter 5 for Risk Group and Containment Level information)

**Safety glasses** or **face shields** offer protection from splashes of bio-hazardous materials, impacting objects and ultraviolet light sources.

**Safe Work Procedures**

*In accordance with the Canadian Biosafety Standards and Guidelines:*

1. The laboratory should be kept neat, clean, and orderly.
2. Laboratory access should be limited to approved workers to avoid unnecessary exposure to ancillary personnel and to maintain the security of biological agents.
3. All laboratory personnel must become familiar with the biohazards that are likely to be encountered in their particular laboratories prior to the start of any work with bio-hazardous agents. A procedural manual should be available for all staff.
4. Personnel must receive training on the potential hazards associated with the work involved and the necessary precautions to prevent exposure to any uncontrolled release of the biological agent. Training must be documented.
5. Laboratory workers should be protected by immunization where appropriate.
6. Appropriate PPE must be available and worn. PPE is not be worn outside the laboratory areas. Spare lab coats should be made available to visitors to the laboratory. Appropriate PPE includes, but is not limited to – laboratory coats/gowns, disposable gloves, masks, and safety glasses.
7. Eating, drinking, storing of food, or other personal materials is not permitted in the laboratory.
8. The wearing of jewellery in the laboratory is discouraged and long hair is required to be tied back.
9. Applying cosmetics and handling contact lenses should not be permitted. In cases, when hazardous operations are performed safety glasses should be worn.
10. Hands must be washed after gloves have been removed using proper hand washing technique.
11. Avoid forcefully aspirating or expelling liquids.
12. When pipetting is involved in laboratory work, workers must use pipettors and pipette aids. ***__Mouth pipetting is strictly forbidden__.***
13. The use of needles, syringes, and other sharp objects should be strictly limited to avoid accidental inoculation. Contaminated needles should not be bent, sheared, recapped, or removed from the syringe. These must be promptly placed in an approved sharps container. **Procedures that require recapping of needles is to be discouraged**.
14. Work surfaces must be decontaminated with a suitable disinfectant at the end of the day and after any spill of potentially bio-hazardous material. Spills must be reported to the Environmental Health & Safety Office.

15. To avoid accidental spills or leaks, secondary containment should be incorporated when transporting biological agents. Shipping of biological agents to another facility should be overseen by an individual who has the appropriate Transportation of Dangerous Goods (TDG) training. If in doubt, contact the Environmental Health & Safety Office. No shipping from CBU of any Risk Group 2 pathogen/toxin can be done with consultation with the BSO.

**Glove Removal**

To avoid contaminating your hands when removing gloves, care must be taken to avoid touching the skin with the outside of the glove. The following technique for glove removal should be employed.

1. With both hands gloved, peel one glove off from the top to bottom and hold it with the gloved hand.
2. Grasp the inside of the cuff of the second glove with the exposed hand and peel it off from the top, tucking the first glove inside the second.
3. Dispose of the gloves promptly to an appropriate waste container.
4. ***__Never touch the outside of the glove with bare skin__.***
5. Wash hands as soon as possible using proper hand washing technique.

**Hand Washing**

Hands should be washed with soap under clean running water for a minimum of 15 seconds before leaving the working area. Using hot water and soap, the hands should be rubbed together to create a lather. The hands should be thoroughly scrubbed, including wrists, between fingers, under fingernails, and the backs of the hands. Soap should be rinsed off thoroughly. Using paper towel, hands should be dried completely. Paper towel should be used to turn off the faucet and open the door. Hand sanitizers may be used if they are effective against the pathogen or toxin used.

**Containment Level 2 Requirements (Cape Breton University will not work with pathogens above BSL2)**

In addition to the general requirements listed above, the following list describes minimum operational procedures for Containment Level 2 laboratories.

1. The universal biohazard symbol must be posted on laboratory doors. In addition to the universal biohazard symbol (Figure 1), the containment level of the laboratory must be posted at points of entry.
2. Non-university visitors to the Microbiology teaching laboratory should be approved and accompanied by the BSO or any member of the IBC.
3. Biosafety Cabinets (BSCs) must be used for procedures that may produce aerosols or that involve work with high concentrations and/or volumes of the agent. Animal handling and necropsy should be performed in the BSC as well.
4. Biological agents that appear on either The Australia Group's Human and Animal Pathogens and Toxins export control list or the Centers for Disease Control and

Prevention (CDC) Select Agents and Toxins list should be stored in a locked area when an authorized worker is not present in the laboratory.

**Potentially Hazardous Operations and Preventive Measures to Reduce Risks**
In the laboratory environment, there are many hazardous procedures that have the potential to cause accidental injection and/or create aerosols. Safe practices can prevent accidental injection, and limit the formation and dispersal of aerosols.

**Needles, Syringes, and Other Sharps**

In addition to the following, please refer to Cape Breton University's *Needlestick and Blood Borne Pathogen Policy.*

The greatest risk of using sharps is accidental injection and the creation of aerosols.

1. Needles and syringes should only be used when there is no reasonable alternative to handle contaminated liquids.
2. Sharps should never be bent, sheared, recapped, nor have needles removed from syringes. If a needle must be recapped do so by using forceps or using single-handed scooping (Figure 5).
3. Avoid the production of air bubbles when filling a syringe. Expel any trapped air into a pad moistened with disinfectant placed over the needle tip.
4. Maintain an approved sharps container in the immediate work area. Never over-fill these containers beyond ⅔ capacity.

***Preventing Needle Stick Injuries:***
Needle stick injuries are wounds caused by needles that accidentally puncture the skin. These injuries can occur at any time when needles are being used, disassembled, or being disposed of. Needle stick injuries can transmit infectious diseases, especially blood-borne viruses.

***Work practices that increase the risk of needle stick injury:***
- Recapping needles
- Transferring infectious material between containers
- Failure to dispose of used needles in puncture-resistant sharps containers

**How can needle stick injuries be prevented**?
***Employee training****:* Workers need to know how to safely use and dispose of sharps, as well as understand the risk associated with needle stick injuries, and know how to prevent them.

***Safety-engineered syringes****:* Safety-engineered syringes are recommended as they have safety features built into the product. Safety-engineered syringes include features such as protective shields, retractable needles, and blunt tips.

***Safe recapping procedures:***
In situations where recapping is necessary, two techniques may be employed.

1. *Single handed scooping* - The risks of recapping can be reduced if the cap is laid on a flat surface and scooped onto the tip of the syringe held in one hand. The free hand should be kept away from the sheath and well behind the exposed needle (Figure 5).
2. *Recapping Devices* - Several devices are available for recapping needles safely. Some devices permit single-handed recapping by parking a needle cap on a flat surface (Figure 6). Other devices are designed to protect the hand that holds the cap during two-handed recapping procedures.

***Disposal***

Disposal containers for sharps must be readily available. These sharps containers must be puncture proof. Disposal must follow university disposal procedures.

***Reporting***

Many incidents go unreported. Reporting of needle stick and other sharps injuries assists the Biosafety Office with determining the rate of needle stick injuries, investigating the cause of such injuries, ensuring that workers receive proper treatment, identifying areas where the prevention program needs improvement and providing practical strategies for dealing with the problem.

**Pipetting**

The greatest hazard of pipetting is the potential for the creation of aerosols and splashing.

1. Mouth pipetting is prohibited, mechanical pipetting aids must be used.
2. Where possible pipette biohazardous materials in a biosafety cabinet.
3. Never discharge biohazardous materials forcibly from pipettes. "To deliver" pipettes are recommended.
4. To avoid splashing, biohazardous material should be dispensed from the pipette by allowing it to run down the wall of the receiving container.
5. After use, re-usable pipettes should be placed in a proper container of germicidal liquid to completely cover them. The germicidal liquid must be effective against the pathogen in use.

**Centrifugation**

The greatest risk of centrifugation is the potential for the creation of aerosols.

1. Sealed tubes and safety buckets that seal with O-rings should be used. Prior to use O-rings should be inspected for damage to avoid the possibility of spills.
2. To avoid leaks, do not over fill centrifuge tubes. Wipe the outside of the tube with a disinfectant once filled and sealed prior to loading into the centrifuge.
3. Safety buckets should be removed from the centrifuge and loaded/unloaded in the BSC.
4. If "biosafety buckets" are not available, wait a minimum of 10 minutes after the spin is complete before opening the centrifuge.

**Blending, Grinding, Sonicating, Lyophilizing**

The greatest risk when performing any of these operations is the potential for aerosol production.

1. Perform these operations in a biosafety cabinet.
2. Use safety blenders which are designed to prevent leakage from the bottom of the blender jar and which can withstand autoclaving.
3. Avoid the use of glass blender jars.
4. Place a towel moistened with a disinfectant over the top of the blender while it is in operation. This practice can be adapted for sonicators and grinders as well.
5. Allow aerosols to settle for at least thirty minutes before opening blenders, grinders or sonicators.
6. Filter lyophilizer vacuum pump exhaust through HEPA (high efficiency particulate air) filters or vent to a biosafety cabinet.
7. Use polypropylene tubes in place of glass for storing biohazardous materials in liquid nitrogen.

## Chapter 4: Bio-hazardous Materials

Bio-hazardous materials are defined as those coming from living plants or animals that may pose risks to humans, non-human animals and plants. Generally, biological materials fit into one of the following categories:

***Microorganisms:*** bacteria, viruses, fungi, and protozoa.
***Parasites:*** organisms that require a host species in order to survive and propagate.
***Toxins:*** poisonous substances produced by living organisms.
***Prions:*** proteins held responsible for several fatal progressive neurological-based diseases. These proteins resist standard methods of sterilization and destruction and should be handled as Risk Group 2 or above, where appropriate.
***Recombinant DNA:*** where genomic DNA from a parent organism is transplanted into the genome of a subsequent organism. As per CBSG, risk mitigation protocols for handling and usage should bear the following in mind:

- Gene(s) being transferred
- Modification to genes already present in the organism
- Gene expression in the recombinant organism
- Biological containment offered by the host organism
- Interactions between the gene(s) transferred and the host vector systems
- The viability of the host vector systems

***Animals:*** by definition, animals being using in a research setting qualify as biological and bio-hazardous materials, considering their ability to contract and spread disease and illness to other organisms. Those working with animals in a research setting should be aware of potential physical risks including traumas associated with biting, hitting or kicking movements and to allergic reactions to hair or dander.
***Viral Vectors:*** Viral vectors are often designed to enter human cells and deliver genes of interest. Viral vectors are usually replication-deficient. There are several biosafety concerns that may arise, specifically:

1. Tropism (host range) – viral vectors that can enter human cells are often used.
2. Replication-deficient viral vectors can gain back the deleted genes required for replication through recombination.
3. Genes can be expressed in tissues and/or organisms where they are not normally expressed.

Work using viral vectors must be completed in a CL2 containment area.

***Bloodborne Pathogens:*** These pathogens are microorganisms borne from blood or blood products and can potentially pose serious health and safety concerns if not handled with care.

In research settings, particular pathogens raise concern: Hepatitis B Virus **(HBV),** Hepatitis C Virus **(HCV)** and Human Immunodeficiency virus **(HIV).**

****Hepatitis B vaccination has proven highly effective in preventing infection. It is recommended that all healthcare personnel, human health research faculty and housekeeping staff (or any others handling or removing waste from bathrooms) receive the Hepatitis B vaccine****

## Chapter 5: Risk Groups/Containment Levels

Risk assessments associated with bio-hazardous materials should begin with consultation of the relevant *Pathogen Safety Data Sheet*. In addition to reference to the relevant data sheet information, risk assessment should be mindful of the following:

***Pathogenicity:*** Can the pathogen successfully infect and cause disease in humans and non-human animals? How severe is the disease in humans and non-human animals.
***Virulence*:** How severe is the disease in humans and non-human animals.
***Epidemiology:*** What is the typical incidence level of the pathogen? Are certain groups of people or geographic areas assumed to have higher incidence levels?
***Host Range:*** What are the primary, intermediate, and dead-end hosts? Are a wide range of species affected, or is the host range more restricted?
***Infectious Dose****:* What amount of pathogen is required to cause an infection in the host (measured in number of organisms)?
***Mode of Transmission****:* How does the pathogen travel to the host (e.g., direct contact, indirect contact, causal contact, aerosolized droplet or airborne transmission, vectors, zoonosis, intermediate host)?
***Incubation Period****:* What is the period between the infection of an individual by a pathogen and the manifestation of the illness or disease it causes?
***Communicability****:* What is the pathogen's capability of being transmitted from person to person, animal to animal, animal to human, or human to animal?

<u>Risk Groups as defined by the CBSG and the World Health Organization:</u>

### Risk Group 1 Agents

Risk Group 1 agents include microorganisms, nucleic acids, or proteins which are not capable or unlikely to cause disease in healthy workers or animals. RG1 agents also pose a low risk to public health, livestock, or poultry. Most biohazardous materials at CBU fall into this risk group and would include many strains of *Escherichia coli* widely used in molecular biology studies.

### Risk Group 2 Agents

Risk group 2 agents are pathogens that pose a moderate risk to the health of individuals or animals, and a low risk to public health, livestock, or poultry. These pathogens can cause human and animal disease, but under normal circumstances are unlikely to do so. Laboratory exposures rarely cause infection leading to serious disease.

Examples of Risk Group 2 pathogens are:

- Bacteria such as *Salmonella enterica, Escherichia coli 0157:H7*
- Viruses such as Hepatitis A,B,C, influenza, measles, mumps, chickenpox

### Risk Group 3 Agents

Risk Group 3 agents pose a high risk to the health of individuals and animals, and low risk to public health. These pathogens are likely to cause serious disease in humans and animals. Treatment options and preventive measures are typically available and the risk of spreading the

disease caused by these pathogens is low. Depending on the pathogen and conditions, the risk of spread to livestock or poultry can range from low to high.

Examples of Risk Group 3 pathogens are:

- Bacteria such as *Bacillus anthracis* and *Mycobacterium tuberculosis*
- Viruses such as HIV and Yellow fever virus
- Unconventional agents such as Creutzfeldt-Jakob prion

**Risk group 4 Agents**
Risk group 4 agents pose a high risk to the health of individuals, animals, and public health. These pathogens are likely to cause serious disease which can often lead to death. Effective treatment and preventive measures are not typically available for these pathogens and they may be readily transmitted from one individual/animal to another. Depending on the pathogen and conditions, the risk of spread of disease to livestock or poultry can range from low to high.

Examples of Risk Group 4 pathogens:

- Lassa
- Ebola

*****Research and teaching work using microorganisms at Cape Breton University will not utilize bio-hazardous materials above BSL2 but are required to read and understand the information provided related to BSL3 and BSL4 bio-hazardous materials*****

Association between Risk Group and Biosafety Level:

The following table was constructed with reference to CBSG, WHO's Laboratory Biosafety Manual and Dalhousie Biosafety Manual 2015:

| Risk Group | Biosafety Level (BSL) | Lab Type | Lab Practices | Required safety equipment | Sample Organisms |
|---|---|---|---|---|---|
| **1** | 1 (Basic) | Basic teaching and research | *SMP | Open work bench | *E. coli* |
| **2** | 2 (Basic) | Primary health services, diagnostics, research | *SMP + protective clothing, biohazard sign | Open work bench + BSC for potential aerosols | Influenza, Hepatitis A,B and C |
| **3** | 3 (Containment) | Special diagnostics, research | BSL2 + special clothing, controlled access, directional airflow | BSC and/or other required primary devices for all activities | *Bacillus anthracis* and HIV |
| **4** | 4 (Maximum Containment) | Dangerous pathogens | BLS3 + airlock entry, shower exit, special waste disposal | Class III BSC, or positive pressure suits in conjunction with class II BSCs, double-ended autoclave, filtered air | Ebola virus |

***SMP: Standard Microbiology Procedures**

## Laboratory Acquired Infections

Laboratory acquired infections (LAIs) are defined as direct or indirect infections after exposure to an infectious biological agent in a laboratory or designated work area. The ensuing injection may be symptomatic or asymptomatic. Routes of entry include: ingestion, inhalation, absorption (including through skin) and puncture. Increased risks of LAI acquisition may exist for those with certain underlying health issues. Once infected, hosts can transmit the LAI to persons within or outside the designated work areas or laboratory.

Despite trends toward improved laboratory safety, LAI still take place and often go unreported. Risk mitigation and assessment, dawning of proper PPE, and safe work practices all work to minimize the risk of contracting a LAI. If an LAI is suspected or possible, proper incident/accident reports should be filed upon notification of the BSO. The BSO will investigate incidents/accidents or potential risks/harms in the workplace.

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## Chapter 6: Biosafety Cabinets

Biosafety cabinets (BSC) are ventilated workspaces that provide adequate protection from particulates, splashes and aerosols originating from (or in contact with) bio-hazardous materials. Biosafety cabinets differ from traditional chemical fume hoods by the integration of HEPA filtration and the existence of laminar-style air flow. HEPA filters trap high percentages of particulates, thereby trapping all known infectious microbes and materials and ensuring that clean, safe air is released from the cabinet. Filtered air is discharged over the cabinet workspace, protecting materials and surfaces from contamination.

Three classes of BSC exist and vary in the level of protection they offer, depending on parameters such as face velocity, air flow, and exhaust system.

### Effective Use of BSCs (as outlined in *Dalhousie Biosafety Manual 2015*)

1. Read the operator's manual and follow manufacturer's recommendations.
2. Locate the cabinet in an area where it will not be adversely affected by air currents, and is away from pedestrian traffic and other ventilation devices.
3. The use of UV light to decontaminate the BSC is not encouraged. If the UV light is in use, turn it off before commencing work in the BSC.
4. Turn on the fluorescent light and cabinet blower. Allow the BSC to run 15 minutes before using.
5. Wash hands thoroughly before proceeding and wear required personal protective equipment. If laboratory coats rather than gowns are worn, they must be buttoned and the gloves pulled over the wrist of the coat.
6. Workers should double glove when working in the BSC. The outer layer of gloves must be removed after completion of work ***PRIOR*** to removing their hands from the BSC.
7. Wipe down the interior surfaces with 70% ethanol (or other suitable disinfectant) and allow to dry.
8. Arm movement into and out of the cabinet should be kept to a minimum to avoid air turbulence. Place all materials needed for the procedure inside the cabinet prior to starting. Do not bring any unnecessary items into the cabinet. If it is necessary to move arms in and out of the cabinet, do so slowly. Arms should enter/exit the BSC perpendicular to the front opening.
9. Minimize air changes in the room by avoiding opening and closing laboratory doors and pedestrian traffic.
10. Work at least 10 – 15 cm from the opening of the cabinet. Objects should not be placed such that they obstruct the front or rear grilles.
11. Adjust stool so that the worker's face is above the front opening of the cabinet. The stool height should be such that the sash is level with the underarms of the worker.
12. Delay manipulation of materials for at least one minute after putting arms in the cabinet to allow the cabinet environment to stabilize.

13. Carry out work on a plastic-backed absorbent pad to contain small spills, making sure that this pad does not cover the front grille opening.
14. ***The use of open flames is prohibited in the BSC*** as they disrupt the air flow patterns and may damage the HEPA filter. To sterilize transfer loops, electronic loop incinerators, microincinerators, or disposable loops are an alternative.
15. The use of germicidal lamps is strongly discouraged. Their germicidal properties are not highly effective and there is the concern for inadvertent UV exposure to the eyes.
16. Clean up spills as soon as they occur.
17. Place contaminated items to the rear of the cabinet.
18. Materials should be discarded in a waste container located towards the rear of the BSC. Do not discard items in a container that is located outside of the BSC.
19. Disinfect the cabinet after use. A bottle of appropriate disinfectant should be kept in the BSC to avoid having to move hands out of the BSC.
20. ***NEVER*** **attempt to remove or change the HEPA filters.**
21. Leave the fan blower on in the cabinet for five minute after you have finished your procedure to allow the system to purge.

**BSC Certification**

New BSCs must be certified by an approved certification company upon installation and before use. BSCs are not to be used without certification. BSCs must be recertified annually, if repairs are conducted, or if they are moved to a new location. BSCs in the CBU Microbiology teaching lab are certified every December.

## Chapter 7: Sterilization and Disinfection

It is imperative that all bio-hazardous materials and consumables coming into contact with bio-hazardous materials be adequately inactivated subsequent to their use in research or teaching. Resistance to sterilization and disinfection varies with microorganisms species and strain. Differentiating between the terms below is important in understanding proper protocols:

***Sterilization:*** Removal of all living organisms from a material or consumable

***Disinfection:*** Killing of pathogenic materials via physical or chemical mechanisms- this does not necessarily imply sterilization.

***Decontamination:*** Process by which microorganism number is reduced to an acceptable level- can be achieved via either sterilization or disinfection.

Level of sterilization is dependent on: type/number of organisms, concentration, length of contact time, presence of particulate and organic matter and/or dirt, temperature and surface condition.

Sterilization agents may target the cell membrane structure of organisms or organism function including interfering with enzymatic function or protein denaturing, in the case of autoclaves, for example.

<u>Overview of Microorganisms and their susceptibility to decontamination:</u>

- Most bacteria, fungi, and lipid-containing viruses = relatively susceptible to chemical decontamination.
- Non-lipid containing viruses/bacteria with waxy coat = mid-level range of resistance
- Spores or spore-formers = most resistant to decontamination

Methods typically used to achieve sterilization and disinfection include: steam sterilization, dry heat, gas sterilization, UV lamps and chemical-based disinfectants.

### Autoclaves

Autoclaves are machines used to expose pathogens and bio-hazardous material (or materials coming into contact with such material) to high temperature and pressure steam as a means of sterilization. Depending on the size of the material load to be autoclaved, treatment times will vary. Volatile chemicals and radioactive waste are to be excluded from autoclaving. Following autoclaving, consumables and waste may be discarded ordinary unless otherwise specified. Volatile chemicals and radioactive waste are to be excluded from autoclaving. If in doubt about suitability for autoclaving, contact the BSO.

**Autoclave Safety Practices (as outlined in the Dalhousie University Biosafety Manual 2015):**

1. Do not exceed the manufacturer's recommended pressures and temperatures.
2. Arrange for regularly scheduled inspections/testing of autoclaves and ancillary equipment. CBU has one autoclave inspected by the manufacturer three times per year.
3. Report all malfunctions and tag the unit "Out of Service".
4. Ensure that the operational SOP is posted near the autoclave.
5. Do not use the autoclave unless you have specific training in safe operating procedures.
6. Ensure that your name is listed on the authorized user list, which must be posted in the vicinity of the unit.
7. Clean the drain strainer before loading the autoclave.
8. Load the autoclave as per the manufacturer's instructions.
9. Make the appropriate log entry.
10. Loosen caps on containers of liquids before loading to avoid having the bottles shatter during pressurization.
11. Use a tray with a solid bottom and walls as double containment to catch spills.
12. Add approximately 1 cm of water to the trays so that the bottles will heat evenly.
13. Do not load plastics that are incompatible with the autoclave.
14. Do not stack containers.
15. Do not overload the autoclave.
16. Firmly lock the autoclave doors prior to starting the run to prevent the sudden release of high pressure steam.
17. Ensure that the correct cycle is selected.
18. Before opening the autoclave door after the run and unloading the autoclave, wear a rubber apron, rubber sleeve protectors, heat resistant gloves, and a face shield.
19. Release the steam slowly, as bottle plugs may be ejected if the pressure is released too quickly.
20. Stand so the autoclave door shields your body from the contents of the unit and released steam while opening the autoclave.
21. Wait 5 minutes for loads containing dry glassware and 10 minutes for liquid loads before removing items. Vessels containing liquid volumes in excess of 20 litres should be allowed to cool in the autoclave before being unloaded as superheated liquids continue to boil for some time.
22. Allow glassware to cool completely before handling with ungloved hands.
23. Remove debris from the autoclave that could block drain valves and create a hazard for the next user.

Research supervisors must be aware that autoclaves are not to be used without certification. Autoclaves are to be recertified when a failure is indicated in efficacy testers. Research supervisors must ensure that students, faculty and staff using the specific autoclaves are

adequately trained to do so. With a few exceptions almost all autoclaving in carried by a member of the microbiology faculty. Access to the autoclave is strictly controlled.

**Ultraviolet Lamps**

Ultraviolet (UV) light is defined as electromagnetic radiation in the 200-400 nanometer (nm) region. Intense/prolonged exposure to UV light can result in painful eye injury (photokeratitis, retinl burns, skin burns, premature aging of skin and skin cancer. Proper shielding and PPE must be employed since regular glass does not protect one from UV light.

**UV Light Protective Measures**

1. Use required PPE, including gloves, laboratory coat, goggles, and face shields.
2. Clearly post a UV light symbol where UV light sources are operating at a wavelength capable of germicidal irradiation are present.
3. Limit exposure times.

**Symptoms of UV Light Overexposure**

- Skin: Sunburn-like symptoms.
- Eyes: Burning painful sensation, sensitivity to light, sensation of a foreign object (sand) in the eye, and tearing.

These symptoms usually develop several hours after overexposure to UV light has occurred. Medical attention should be sought immediately, especially if the eyes are involved.

**Incident reporting**

Incident/accident reporting should follow established University protocols where appropriate.