Biosafety Program Manual

Updated: 2017

Environmental Health and Safety

Ryerson University
Facilities Management & Development

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Abbreviations

CBS: Canadian Biosafety Safety Standard, 2nd edition
CFIA: Canadian Food Inspection Agency
HPTA: Human Pathogens and Toxins Act
HPTR: Human Pathogens and Toxins Regulation
PHAC: Public Health Agency of Canada

1. Emergency Procedures, Accident/Incident Reporting & Investigation

The location and proper use of emergency equipment (eyewash stations, first aid kits, etc.) should be known to all lab personnel prior to the start of any work in the laboratory.

1.1 Emergency Procedures

1.1.1 First Aid

Immediately after injury:

- Remove contaminated clothing & gloves
- Allow immediate bleeding or force bleeding of cut if possible

- Wash affected area with soap or antiseptic and water
- If eyes or mouth are involved, flush with large amounts of water
- If nose involved, blow into a tissue and then flush with large amounts of water
- Cover wound with dressing or band aid after cleaning
- Seek further medical attention if required – Contact Security (Dial 80 or activate blue pull station, 416-979-5040 from external phone)

1.1.2 Spills

In the event of any spill of a biological agent it is important to avoid unnecessary exposure and contamination. Since most commonly-used laboratory culture containers are small, it is anticipated that most spills within the laboratory will be minor. Although the specific response will depend on the type and nature of the incident, decontamination***** and clean-up procedures incorporating the steps outlined below are recommended. Wear appropriate personal protective equipment such as laboratory coat, eye protection and disposable gloves.

If spill occurs on bench top, evacuate the laboratory for a time sufficient for most aerosols to settle or be dispersed and removed by the ventilation system. Depending on the volume spilled, this could take up to 10 - 20 minutes. Prevent access to area by marking the area with warning signs and closing laboratory doors.

1. Report spill to laboratory supervisor *as soon as practical*, i.e., do not delay clean-up procedures simply because supervisor is not available.
2. Carefully place some absorbent material over the spill (trying to avoid generating more aerosols) and pour disinfectant solution over the absorbent material.
3. Allow sufficient contact time, normally 20 to 30 minutes; it is advised to leave the laboratory during this period (depending on magnitude of spill).
4. Remove absorbent material by wiping from the outside in towards the centre of the spill. Place all materials in biohazard waste container.
5. If there is still liquid left, repeat steps 3, 4 and 5. When surface is dry, apply disinfectant directly onto the surface and wipe to fully decontaminate***** surface.

***** Decontaminate all surfaces exposed to the spill with a disinfectant suitable for the microorganism(s) involved.

Appropriate, effective, disinfectants must be available in the laboratory at all times and for immediate use.

The BSO may develop specific emergency procedures applicable to the containment zone for: fire, power failure, failure of primary containment devices, puff-back from class II B2 BSCs (if present), natural disasters. Refer to section 4.5 for contingency planning requirements.

1.2 Incident Reporting and Investigation

It is important to report every exposure sustained in the workplace.

1.2.1 Internal Reporting

Report the exposure to the supervisor or the BSO without delay. The injured person should immediately contact Ryerson Security (Dial 80 from internal phone or activate blue pull station, 416-979-5040 from external phone) to obtain medical attention.

Personnel potentially exposed to bloodborne pathogens should seek medical advice. The physician will determine if the risk is significant, and the type of medical follow up required. Any incident/accident involving pathogens, toxins, other regulated infectious material, infected animals, or failure of containment systems or control systems should be investigated and documented on the:

- Ryerson's Internal Incident Form and send the completed report to the Environmental Health and Safety office within 24 hours of exposure; and;
- Workplace Safety and Insurance Board (WSIB) Form 7: Employer's Report of Injury/Disease by the supervisor, if the injured/exposed individual is an employee, and return to Human Resources within 24 hours.

Copies of all reports should also be made for the originating department. Further investigation follow-up (e.g., interviewing witnesses, contacting equipment supplier etc.) may be required to determine the root cause(s) and to implement measures to mitigate future risks.

1.2.2 External reporting

Incidents (including spills, exposure, inadvertent release/production, disease etc.) involving human pathogens and toxins in which it has reason to believe that the substance has, or may have, caused an exposure or disease in an individual, must be reported immediately to **the BSO, who will report to the Public Health Agency of Canada** (PHAC) **without delay**.
The information that is to be provided to PHAC includes:

- A description of the incident;
- Name of the human pathogen or toxin;
- The quantity released or produced (if applicable);
- The place and time of the release or production; and
- Any other information that may be required by PHAC.

An exposure follow-up report documenting the completed investigation is to be submitted to the PHAC within:

- 15 days of the notification report involving a **security sensitive biological agent (SSBA)**
- 30 days of the notification report involving a human pathogen or toxin other than an SSBA.

Emergency telephone numbers	
Biological Safety Officer	ext. 554212
After hours	Dial 80 (Campus Security) 24 hours

2. Administration and Responsibility

2.1 Introduction and Scope of the Biosafety Program

Biological material includes pathogenic and non-pathogenic microorganisms, proteins, and nucleic acids, as well as any biological matter that may contain microorganisms, proteins, nucleic acids. Examples include, but are not limited to, bacteria, viruses, fungi, prions, toxins, genetically modified organisms, nucleic acids, tissue samples, diagnostic specimens, live vaccines, and isolates of a pathogen (e.g., pure culture, suspension, purified spores).

At Ryerson University, biological agents are used in teaching and research activities. Supervisors and users of biological agents have a responsibility to protect themselves and other persons from the hazards arising from their use of these materials. The Biological Safety Manual was developed to assist faculty, staff and students to meet the requirements outlined in the Biological Safety Program at Ryerson University. The standard microbiological protection principles and internal safety practices described in this manual apply to all laboratory research and teaching activities conducted within the University and its affiliated institutions where such activities involve the use of known or suspected biological agents.

This Biosafety Program is based on the handling and storing of human pathogens or toxins (containment level 1 and 2 mainly) and the handling of blood and body fluids. For persons working with agents in large scale work (10 litres or greater), risk groups 3 or 4 or with prions, refer to the Canadian Biosafety Standard (CBS) – 2nd Edition for details.

For working with animals (terrestrial or non-terrestrial animals, small or large), animal pathogens or toxins, refer to the related legislation (Health of Animals Act) and the specific sections in the CBS – 2nd Edition for details.

2.2 Legislation and Guidance Documents

Human and animal pathogens and toxins are regulated by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) in accordance with the Human Pathogens and Toxins Act (HPTA) and the Human Pathogens and Toxins Regulation. The primary guidance document is the Canadian Biosafety Standard (CBS) – 2nd Edition (2015) which is a harmonized national standard for the handling or storing of human, terrestrial, animal pathogens and toxins in Canada.

PHAC is the national authority on biosafety and biosecurity for human pathogens and toxins. The CFIA is the national authority on biosafety and biosecurity for foreign animal diseases and emerging animal diseases.

The CBS will be used by the PHAC and the CFIA to verify the ongoing compliance of regulated facilities with the applicable legislation. The Biological Safety Program outlines the regulatory requirements.

2.3 Biological Safety Policy and Program

The University established a policy from Academic Council entitled Policy No. 58 Research Using Biohazardous Materials (1988, Rev 2002) which is available from the Academic Council website.

Ryerson University's Biological Safety Program is designed to take every reasonable precaution for the health and safety of the public, its employees and, students working with biological agents, through training and implementation of standard operating procedures and protocols to control the use, storage and disposal of biological agents. The Biological Safety Program is established in accordance with PHAC's and CFIA standards, guidelines and regulatory documents and applies to all teaching and research activities.

2.4 Authority and Responsibility

Responsibility for controlling all activities related to biological safety at Ryerson University rests with the offices of the Provost and Vice President Academic, as well as the Vice President Administration and Finance. Their authority in this regard is received from the President.

Under the HPTA, a licence must be obtained by the University from the Minister of Health that authorizes any person conducting the following activities:

A. possessing, handling or using a human pathogen or toxin;
B. producing a human pathogen or toxin;
C. storing a human pathogen or toxin;
D. permitting any person access to a human pathogen or toxin;
E. transferring a human pathogen or toxin;
F. importing or exporting a human pathogen or toxin;
G. releasing or otherwise abandoning a human pathogen or toxin; or
H. disposing of a human pathogen or toxin

2.4.1 Biological Safety Officer (BSO)

The University BSO is responsible for coordinating all activities related to biological safety and to oversee the biosafety and biosecurity practices.at the university. The BSO reports to the Director, Environmental Health and Safety & Risk Management within the broader Facilities Management and Development department.

The qualifications and functions of the BSO are outlined in the Human Pathogens and Toxins Regulations (HPTR).

The functions of the BSO are to:

1. verify the accuracy and completeness of licence applications;
2. communicate with the Minister of Health on behalf of Ryerson as the licence holder;
3. promote and monitor compliance with the provisions of the Act and the regulations, with the licence and with the applicable biosafety and biosecurity standards by, among other things,
    a. arranging for and documenting appropriate training

   b. informing the Minister, without delay, of all occurrences of inadvertent possession of a human pathogen or toxin that is not authorized by the licence,
   c. informing the Minister, without delay, of every situation that they are informed of regarding a human pathogen or toxin not received within 24 hours after the expected date and time
   d. conducting periodic inspections and biosafety audits and reporting the findings to the licence holder, and
   e. informing the licence holder in writing of any non-compliance by a person conducting controlled activities authorized by the licence that is not being corrected by that person after they have been made aware of it;
4. assist in the development and maintenance of the licence holder's biosafety manual and standard operating procedures related to biosafety and biosecurity; and
5. assist with internal investigations of incidents including inadvertent release, inadvertent production, in which there is a reason to believe that a human pathogen or toxin caused disease in an individual or a missing human pathogen or toxin or of any incident that results in a failure of or compromise to biocontainment.

2.4.2 Departmental Chairs

For areas using biological material, each departmental chair is responsible for providing adequate facilities, equipment, instruments, supervision to control biohazards and to comply with the University's biological safety requirements.

2.4.3 Biosafety Permit Holders

Each individual who directs the use of biological agents is required to obtain an internal Biosafety Permit. Typically, this individual may be a principal investigator or course instructor in a teaching laboratory using biological materials and will be deemed to be a Permit Holder. The Permit Holder is responsible to comply with the protocols and procedures outlined in the University's Biological Safety Program and any additional requirements prescribed by either PHAC, CFIA or other relevant agency.

Any activity with the human pathogens or toxins identified in the HPTA - Prohibited Human Pathogens and Toxins list (Schedule 5 of HPTA) is strictly prohibited.

Each Permit Holder is responsible for:

A. following the conditions as stated in the permit and that safe laboratory practices as stated in the standard operating procedures as prescribed by the Biological Safety Program;
B. ensuring all staff using biological agents have been trained regarding the policies, procedures and programs on the safe use of biological agents at Ryerson University and are authorized to use biological agents;
C. ensuring that students using biological agents are properly supervised and ensuring they receive instruction in the safety procedures and University protocols on the safe handling of biological agents;

D. ensuring that designating work and storage areas for biological agents are maintained in proper working order, kept clean, and properly labeled;
E. ensuring that any containment and sterilization equipment used by the laboratory staff is adequate to the task and functioning properly;
F. maintaining a list of authorized users and allowing only authorized persons to enter rooms that are specified as restricted areas for reason of biohazard protection;
G. coordinating all purchases, acquisitions, exports, imports, transfers and disposal of biological agents, with the Biological Safety Officer, prior to any arrivals or movement off campus;
H. maintaining an up to date list of biological agents on the permit. This is to include a listing of the rooms in which biological agents are located or used;
I. ensuring all biological agents are properly stored and secured;
J. reporting to the Biological Safety Officer any incidents involving abnormal activities such as loss of materials, suspected exposures;
K. notifying the Biological Safety Officer whenever the permit holder will be unavailable to supervise (e.g. sabbatical or leave of absence) and identifying another permit holder who has accepted the responsibility as the temporary supervisor.
L. not obstructing the BSO when the officer is exercising their powers or carrying out their functions under the licence or the legislation and providing any records as required by the BSO in carrying out his/her functions.

Permit Holders who fail to comply with the requirements of the conditions of their Permit and the University's Biological Safety Program may have their permits suspended or revoked, if non-compliance issues are not corrected.

2.4.4 Authorized Users - Individuals working with biological agents

All individuals working with biological agents have responsibilities which include:

A. working in compliance with all related legislation, policies, procedures and requirements of the University;
B. using equipment required for the safe manipulations of biological agents;
C. reporting to the Permit Holder any defective equipment, or accidental releases or potential exposures to biological agents;
D. not creating or participating in any activity which may endanger themselves, any other worker or create the potential for unauthorized release of biological agents to the environment.
E. not obstructing the BSO when the officer is exercising their powers or carrying out their functions under the licence or the legislation and providing any records as required by the BSO in carrying out his/her functions.

2.5 Record Retention

Documents that are required under the HPTA must be maintained for 5 years after the day on which they are prepared and must be provided to the Ministry of Health upon request.
Animal pathogen import permit requirements for animal pathogens, toxins, and other regulated infectious material to be kept on file for a minimum of 2 years following the date of disposal,

complete transfer, or inactivation of the imported material.

The retention period is 10 years for documents that relates to:

A. An incident that is related to an inadvertent release, inadvertent production or a lost or stolen human pathogens and toxins or a human pathogen or toxin, that is believed, has or may have caused disease in an individual; and
B. Any incident that results in a failure of or compromise to biocontainment.

3. Biological Hazards and Risk Assessment

3.1 Pathogens and Risk Groups

A pathogen is a microorganism, nucleic acid, or protein capable of causing disease in humans or terrestrial animals. Human pathogens are listed in the HPTA, which is reproduced in Appendix B and toxins are listed in Appendix A.

PHAC has defined four levels of risk in classification of organisms. The classification system is based on the relative hazards of the potential risk of causing disease in humans or in animals and the risk to the community.

Risk Group 1 (low community and low individual risk of disease)

Any biological agent that is not capable of or unlikely to cause disease in healthy workers or animals. Agents that pose little or no risk are assigned to Risk Group 1.
The legislation administered by PHAC and CFIA does not apply to Risk Group 1 material; however, due care should be exercised and safe work practices should be followed when handling these materials.
Examples: *Lactobacillus spp., Bacillus subtilis, Naegleria gruberi, Micrococcus spp., E. coli K12*

Risk Group 2 (low community risk and moderate individual risk to disease)

A category of human pathogens that pose a moderate risk to the health of individuals and a low risk to public health and includes the human pathogens listed in Appendix B (Schedule 2 of the HPTA). They are able to cause serious disease in a human but are unlikely to do so. Effective treatment and preventive measures are available and the risk of spread of disease caused by those pathogens is low.
Examples: *Hepatitis B virus, E. coli, measles virus, aspergillus fumigatus*

Risk Group 3 (low community risk and high individual risk to disease)

A category of human pathogens that pose a high risk to the health of individuals and a low risk to public health and includes the human pathogens listed in Schedule 3 of the HPTA (http://laws.justice.gc.ca/eng/acts/H-5.67/page-10.html#h-24). They are likely to cause serious disease in a human. Effective treatment and preventive measures are usually available and the risk of spread of disease caused by those pathogens is low.
Examples: *Hantaan virus, Yersinia pestis, Bacillus anthracis, HIV*

Risk Group 4 (agents with extremely high community and individual risk

Agents that pose the greatest risk are assigned to Risk Group 4. A category of human pathogens that pose a high risk to the health of individuals and a high risk to public health and includes the human pathogens listed in Schedule 4 of the Act (http://laws.justice.gc.ca/eng/acts/H-5.67/page-10.html#h-24). They are likely to cause serious disease in a human. Effective treatment and preventive measures are not usually available and the risk of spread of disease caused by those pathogens is high.
Examples: *Marburg virus, Ebola virus, Crimean-Congo hemorrhagic fever virus*

3.2 Containment Levels

The risk group system does not take into account the procedures that are to be employed during the manipulation of a particular organism. Thus classification of organisms according to risk groups is not meant to establish the actual handling protocols for biological hazards in a laboratory setting. **Containment levels** are selected to provide the end-user with a description of the minimum physical containment and operational practices required for handling infectious material or toxins safely in the laboratory. PHAC and the CFIA outlines four containment levels ranging from a basic lab (level 1) to the highest level of containment (level 4).

Containment Level 1 (CL1)

A basic level laboratory that handles agents requiring containment level 1 requires no special design features beyond a well-designed functional laboratory. Work may be done on an open bench top and containment is usually achieved through the use of good work practices in a basic microbiology laboratory. Biological safety cabinets are not required.

Containment Level 2 (CL2)

The primary exposure hazards associated with organisms requiring CL2 are through the ingestion, inoculation and mucous membrane route. Agents requiring CL2 facilities are not generally transmitted by airborne routes but care must be taken to avoid the generation of aerosols. Primary containment devices used in these types of laboratories includes such as biological safety cabinets or centrifuges in addition to proper personal protective equipment such as laboratory coats, gloves, eye protection are required. Minimization of contamination includes proper hand washing facilities and decontamination facilities such as autoclaves. **Appendix C** outlines PHAC's **physical** containment requirements for Containment Level 2 laboratories. Refer to section 5.4 of this manual for operational practice requirements.

Containment Level 3 (CL3)

These agents used in a CL3 laboratory may be transmitted by airborne routes, often have a low infectious dose to produce effect and can cause serious or life-threatening disease. At this level of containment, primary and secondary barriers are required to minimize the release of infectious organism into the immediate laboratory area and the environment. Additional features may be required to prevent transmission of CL3 organisms such as HEPA filtration of exhausted laboratory air and controlled access. Note: Depending on the organism being used, both PHAC and the CFIA are required to certify the laboratory prior to use of these biological agents.

Containment Level 4 (CL4)

The agents used in this type of containment level are highly pathogenic, low infectious dose and have the potential for aerosol transmission and produce very serious and often fatal disease.

This containment level offers maximum containment and a complete sealing of the perimeter of the laboratory facility. CL4 laboratories are very rare in Canada.

3.3 Risk Assessment

An overarching or broad risk assessment must be conducted and documented prior to commencing work with any biological agent. This pathogen risk assessment will determine the risk group and containment level of the biological agent based on the following characteristics:

Type and properties of the material, such as:

- Human/animal pathogens or toxins (risk group and containment level classification)
- Pathogen risk assessment by PHAC (refer to pathogen safety data sheets published by PHAC)
- infectious dose
- route of infection
- pathogenicity and virulence
- host range
- vectors
- disease incidence and severity
- prevention & treatment
- whether the pathogen is indigenous to Canada
- effect on animals, plants, fish

Due to the unknown characteristics, emerging pathogens and novel agents may require more stringent specialized practices and procedures for their safe handling. The Biological Safety Officer should be consulted prior to the start of a procedure.

How the material is being used

For example, work involving release of microbial aerosols, increase the hazard to laboratory staff. Large volumes (>10 litres) and high concentrations of a biological agent in growth media may pose greater risks than smears of the same agent on a microscope slide. Non standard manipulations may also increase the hazard. As a general precaution, agents should be elevated to the next risk group when manipulation may result in the production of infectious droplets and aerosols.

The classifications of biological agents reflect the judgements made on their inherent risks. This overarching risk assessment is facilitated by completing the Permit Application Form.

A local or site-specific risk assessment should be conducted to examine each task involving infectious material or toxins so that the risks are identified and safe work practices are developed. For example, the movement of infectious material or toxins within the containment zone or between zones within a building; the process involves the generation of aerosols thus requiring a respiratory protection program.
Safe work practices should be documented in standard operating procedures (SOPs) and should be enforced by the supervisor or P.I.

Pathogen safety data sheets are available for several hundred biohazardous materials from PHAC's website: www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php.

3.4 Cell Lines and Tissue Cultures

Cell cultures derived from humans or animals suspected or known to be infected with a pathogen should be assigned to the risk group appropriate for the suspected or known pathogen and handled using the relevant containment level and work practices.

In addition, cell cultures may carry unsuspected oncogenic or infectious particles. It is impractical to screen such cultures for all potentially harmful microorganisms; even well characterized lines with a history of safe use can become contaminated with infectious microorganisms. For this reason, it is prudent to treat all eukaryotic cultures as moderate risk agents (Risk Group 2) and to use CL2 facilities and work practices whenever working with them.

3.5 Recombinant DNA

In vitro incorporation of segments of genetic material from one cell into another is termed "recombinant DNA" has resulted in altered organisms that can manufacture products such as vaccines, enzymes, etc. Genetically engineered organisms are used for treatment of waste and spills and plants can be made resistant to disease or adverse weather conditions. A genetically altered organism may be directly pathogenic or toxic and if released into the environment crowd out beneficial organisms or transfer undesirable genetic traits to wild species or mutate to pathogenic form. The risk assessment for recombinant DNA should include:

- source of the DNA to be transferred
- ability of vector to survive outside the laboratory
- interaction between transferred gene and host

When assessing the risk group and containment level for a genetic engineered protocol, the components of a genetic manipulation are not hazardous then the altered organism is unlikely to present a risk and no restrictions are required. However, if one of the components is potentially hazardous, a risk level appropriate to the known hazard is assigned.

3.6 Toxins

Biological toxins are poisonous substances that are a natural product of the metabolic activities of certain microorganisms, plants, and animal species. Unlike pathogens, toxins are non-infectious and unable to propagate when isolated from the parental organism. An exhaustive list of toxins regulated under the HPTA is listed in **Appendix A** (extracted from Schedules 1 and 5 of the HPTA); whereas any imported microbial toxin derived from an animal pathogen is also regulated under the Health Animals Act. In general, toxins capable of producing human or animal disease are safely handled in CL2 zones, at a minimum. Additional physical containment or operational practice requirements may be necessary, based on risk.

3.7 Blood and Body Fluids

The inherent risk associated with blood and bodily fluids is the possible exposure to bloodborne pathogens which may be present in contaminated blood and bodily fluids and are capable of causing disease in exposed individuals. The pathogens of greatest concern are the hepatitis B virus (HBV), the hepatitis C virus (HCV) and the Human Immunodeficiency Virus (HIV).

Hepatitis B is caused by a potentially fatal virus that destroys liver cells and may permanently damage the liver. It can be transmitted not only by percutaneous exposures, but also via mucous membranes. The incubation period for hepatitis B is 45 to 160 days. Of the people infected with hepatitis B, 10% become chronic carriers and may develop cirrhosis and an increased susceptibility to liver cancer. Immunization is a very effective method of preventing hepatitis B. Personnel in high risk groups, must show confirmation of vaccination against hepatitis B.

Hepatitis C is caused by a virus and the interval between exposure and seroconversion is approximately 8 to 10 weeks. At least 85% of people infected with the virus will become chronically infected. An increased risk of liver cancer does exist, especially in individuals who develop cirrhosis.

Human Immunodeficiency Virus is a retrovirus that causes Acquired Immunodeficiency Syndrome (AIDS). The mean incubation period is 10 years. It is difficult to become infected with HIV through a needle stick injury or other exposure to blood or other body fluids. The risk depends on the amount of virus to which one is exposed and the titre of HIV viral RNA. There is no vaccine for HIV, but drugs are available which reduce the risk of becoming infected with the virus. To be effective, the drugs must be started within 1 to 2 hours after the exposure.
The types of body fluids capable of transmitting HIV, HBV, and HCV from an infected samples include:

- blood, serum, plasma and all biologic fluids visibly contaminated with blood
- laboratory specimens, samples or cultures that contain concentrated HIV, HBV, HCV
- organ and tissue transplants
- pleural, amniotic, pericardial, peritoneal, synovial and cerebrospinal fluids
- uterine/vaginal secretions or semen (unlikely able to transmit HCV)
- saliva (for HCV, HBV, and HIV if a bite is contaminated with blood and for HBV if a bite is not contaminated with blood).

Feces, nasal secretions, sputum, tears and urine are not indicated in the transmission of HIV, HBV or HCV unless visibly contaminated with blood.

To minimize the risk of exposure, **universal precautions** must be used when working with samples containing confirmed or suspect blood-borne pathogens (see Section 5.6). A **medical surveillance program** may be required based on the local risk assessment (assessing the duration of the task, the materials or equipment being used, and the potential for exposure). The physician will determine whether the employee is fit to perform the required task or if e.g. Hepatitis B immunization is required. Contact the BSO for information.

Personnel who have had an exposure are advised to promptly seek medical attention.

3.8 Animal Pathogens

The Canadian Food Inspection Agency (CFIA) establishes the biocontainment levels, procedures and protocols that are needed to work safely with animal and zoonotic pathogens, chemical hazards, and plant pests of quarantine significance, and to protect laboratory staff, the Canadian public, and the environment. As of April 1, 2013, the PHAC is now the single window for stakeholders who require an import permit for both human and terrestrial animal (includes avian and amphibian animals but does not include aquatic animals, bees and invertebrates) pathogens. The CFIA continues to issue permits for animal pathogens that are not indigenous to Canada (pathogens causing foreign animal and emerging animal diseases), aquatic and plant pathogens as well as for animals, animal products and by-products, tissue, sera and blood that are infected with animal pathogens.

The Canadian Biosafety Standards (CBS) replaces the *Laboratory Biosafety Guidelines*, the *Containment Standards for Veterinary Facilities* and *Containment Standards for Laboratories*, *Animal Facilities and Post Mortem Rooms Handling Prion Disease Agents*.

For persons that import animal pathogens, or toxins, or animals, animal products or by-products or other substances that may carry an animal pathogen or part of one that retains its pathogenicity have specific requirements under the **Health of Animals Act (HAA)** and the **Health of Animals Regulations (HAR)** in addition to the requirements described in the CBS. An importation permit must be applied for from PHAC under the authority of HAR.

3.9 Plant Pests

The Canadian Food Inspection Agency is proposing a draft for containment standards for facilities housing plant pests. The risk to laboratory personnel from plant pests is relatively low risks, since plant pests rarely infect healthy people. However, some plant pests, pose a significant threat to agricultural production, and natural environments. As a result, it is important that personnel working with plant pests take steps to prevent the accidental escape of potentially damaging pests into the environment. The level of containment required to prevent escapes will depend on specific pest biology and the impact that an escape might have on the Canadian environment.

3.10 Prions and Unconventional Pathogens

Some progressive neurological diseases are caused by unconventional or slow viruses, e.g. prions (small, proteinaceous infectious particles) have been associated with transmissible degenerative disease of the central nervous system in humans (e.g., Creutzfeldt-Jacob) and in animals (such as encephalopathy). The most likely route of transmission of infectious prions is through inoculation or ingestion. Prions are resistant to decontamination procedures and processes commonly effective against other pathogens. The following precautions should be observed when handling neurological material from suspected infected humans or animals:

A. handle as a minimum as Risk Group 2 or higher, depending on nature of the work and amount of agent being manipulated;

B. handle tissue as if still infectious even if tissue is fixed with formalin or embedded in wax;
C. maintain up-to-date knowledge of the latest disinfection protocols.

Refer to the Canadian Biosafety Standards for specific requirements for activities with prions.

3.11 Security Sensitive Biological Agents (SSBAs)

Security sensitive biological agents (SSBAs) are human pathogens and toxins that have been determined to pose an increased **biosecurity** risk due to their inherent dual-use potential for bioterrorism. They are identified as “prescribed human pathogens and toxins” in the HPTA and regulation. Prescribed human pathogens are all RG3 and RG4 human pathogens and all toxins listed in the HPTA Schedule 1 or Appendix A (1) of this manual.

SSBAs that require security clearance (or requirements in section 33 of the HPTA) are:

- All RG3 and RG4 human pathogens except for Duvenhage virus, Rabies virus and all other members of the Lyssavirus genus, Vesicular stomatitis virus, and Lymphocytic choriomeningitis virus; and
- All toxins listed in Appendix A(1) except for those listed in Appendix A(2) that is present in a quantity less than or equal to the specified trigger quantity.

Note: Check the PHAC website (www.phac-aspc.gc.ca/lab-bio/regul/ssba-abcse-eng.php) for an exhaustive list maintained by PHAC as amended from time to time of all SSBAs, including toxin trigger quantities.

A toxin present in a facility in a quantity below the trigger quantity is not an SSBA; however, it remains a regulated toxin, and subject to the requirements in the Canadian Biosafety Standards on the minimum containment level.

3.12 Laboratory Acquired Infection

It cannot be stressed enough that even with controls in place, the possibility of Laboratory Acquired Infection (LAI) still exists. PHAC reports that laboratory-acquired infections are not uncommon and there have been over 5,000 reported cases and 190 deaths up to 1999 worldwide. It is also estimated that only 20% of infections can be attributed to any known, single exposure event. In fact, 80% of laboratory acquired infections (LAI's) go undetected due to long incubation periods, mild symptoms, or symptoms common to everyday illnesses (i.e. flu-like symptoms).

There are a number of ways in which infectious substances can enter the body and cause infection, including ingestion, inhalation or contact with mucous membranes, including transfer of microorganisms to the eyes by contaminated hands or with non intact skin. Infections are caused from exposure to infectious aerosols, spills, splashes, needle stick injuries, cuts, and centrifuge accidents.

Exposure to aerosols is estimated to be the single largest cause of laboratory infections. Operational practices and techniques must be used to minimize the creation of aerosols associated with common laboratory procedures. In addition, where chemical disinfection procedures are employed, effective concentrations and contact times must be used. Chemical disinfectants used to decontaminate materials to be removed from the laboratory must be replaced regularly.

Every incident (no matter how small) must be investigated to determine if the risk of exposure exists, and what could be done to prevent the possibility of reoccurrence. Individual health status can greatly determine if one's immune system is able to combat infection.

4. Setting Up a Laboratory for Biological Agents

Before any work is performed with any biological agent, advance preparation is required to set up equipment, and implement regulatory and administration protocols. Included below are the major requirements that must be completed before any biological agent is purchased.

4.1 Approval and Designation of Laboratory Containment Levels

All areas intended to be used for the handling, storage or disposal of a biological agent must conform to the requirements set out in the Canadian Biosafety Standards (CBS) – 2nd edition. A copy of the standard may be obtained from the Biological Safety Officer.

The laboratory and associated areas will be classified by containment levels (see section 3.1) based on the characteristics of the biological agent, physical and operational requirements. Approvals for Containment Level 1 and Level 2 laboratories are made by the BSO in consultation with the Biosafety Committee. This will involve a review of the information submitted in the biosafety permit application (which includes the types of biological agents used, experimental protocol, etc.) and an inspection of the facilities before any use of biological agents is permitted in the laboratory. If multiple agents are proposed to be used in the same location, then the proposed laboratory would be classified according to the highest containment level. For example, if a researcher intends to use Containment Level 1 and Containment Level 2 agents, the laboratory would be required to be classified as a Containment Level 2 laboratory. A summary of the physical and operational requirements for Containment Level 2 (laboratory area) can be found in Appendix C.

Researchers intending to work with biological agents requiring Containment Level 3 or higher will require further approval and certification of the facilities from the Public Health Agency of Canada (PHAC). If the proposed agent(s) is a non-terrestrial animal pathogen or zoonotic, additional requirements may be necessary from the Canadian Food Inspection Agency (CFIA).

4.2 Biosafety Permit (Permit)

A Ryerson Biosafety Permit issued by the Environmental Health and Safety Office is required for any purchase, possession, production, storage, transfer or use of biohazardous agents. Principal investigators or teaching instructors involved with potentially hazardous

biological agents such as viruses, bacteria, fungi, parasites, recombinant DNA, tissue (human or animal), cells, blood and body fluids, etc. will be required to obtain a Biosafety Permit before any agents are either purchased or brought onto campus. This requirement applies to all acquisitions of biohazardous material, whether purchased, transferred, or donated.

Applications for a biosafety permit are made online through the EHS Biological Safety program page. Applications are reviewed and approved by the BSO in consultation with the Biosafety Committee. An internal permit does not normally cover off-campus use of biological agents. A separate approval may be required in those conditions. Biohazardous agents may not be purchased, handled, or in a location not listed on the permit. Any changes to the location, type of inventory,or significant changes to experimental protocols may not occur without prior approval from the BSO.

4.3 Equipment

Essential biosafety equipment is key to ensuring effective containment of pathogens, toxins, and other regulated infectious material. This includes all primary containment devices (e.g., biological safety cabinets [BSCs], isolators, centrifuges with sealable cups, process equipment, fermenters, microisolator cages, ventilated cage racks, and sealed biological waste containers).

Process equipment, closed systems, and other primary containment devices should be designed to prevent the release of infectious material. This may include the use of HEPA filters on ports and vents, gaseous decontamination, etc.

Decontamination technologies should be provided with monitoring and recording devices that capture operational parameters. An autoclave, where present, should be capable of operating at the appropriate temperature for decontamination, as determined by validation.

Vacuum systems should be equipped with a mechanism that prevents internal contamination.

Depending on the biological agent being used and the potential for aerosol generation, biological safety cabinets may be required in Containment Level 2 facilities. Refer to section 5.5 for details.

4.4 Training

All persons working with biohazardous agents must receive training in the safe handling of biohazardous materials **prior to beginning work with these materials.**

It is the responsibility of the Biosafety Permit Holder to ensure that all personnel working with biohazardous agents under the permit holder’s control receive the appropriate training and be familiar with the University policies and procedures for the use of biological agents before beginning work. Summer students or temporary employees are also required to be trained before beginning work with biological agents. They may not work with biological agents without direct supervision by someone who has successfully completed the regular training requirements.

Training can be obtained in-class or through self-study. Classroom training can be organized with the BSO. Self-study materials are available from the Ryerson's Environmental Health and Safety website. A certificate will be provided upon successful completion of the training course.

Visitors, maintenance and janitorial staff, contractors, and others who require temporary access to the containment zone should be trained and/or accompanied in accordance with their anticipated activities in the containment zone.

The BSO can assist in conducting a training needs assessment to determine the relevant training requirements for different workplace parties.

4.4.1 Content of Training

The BSO develops, implements and maintains the training program. The training program encompasses both education (i.e. theoretical) and training (i.e., practical). A part of the practical component may be provided by the Permit Holder based on the specific infectious material or toxins in use.

The training program, as outlined in the CBS section 4.3, shall include:

- Potential hazards associated with the work involved and the necessary precautions to prevent exposure to, or release of, pathogens or toxins
- Relevant physical design and operation of the containment zone and system
- Relevant components of the Biosafety Manual and SOPs (e.g., personal protective equipment, operation of laboratory equipment, use of autoclaves, waste disposal etc.)
- Relevant legislation and enforcement agency
- Emergency response
- Incident reporting

Administrative requirements such as purchasing practices, inventory control and security issues are also required to be reviewed. Trainees shall demonstrate knowledge of and proficiency in the SOPs on which they were trained. The Biological Safety Manual may be downloaded from the Biosafety webpage on the Environmental Health and Safety at Ryerson website.

4.4.2 Frequency of Training

Refresher training on emergency response procedures is to be provided annually. Ryerson University requires refresher training on the biosafety program once every 3 years by reviewing the on-line training module along with the successful completion of a quiz. Additional or more frequent refresher training will be determined by the BSO based on a training needs assessment or when users have demonstrated unfamiliarity with standard operating procedures (SOPs). Retraining may also be required when incidents of non-compliance are found during lab inspections or a significant change is made to the biosafety program.

4.4.3 Contingency Planning

In the event of a utility failure (e.g., power failure) that may affect sample storage or an experiment, the principal investigator is required to provide Facilities Management and Development with information and guidance for contingency planning for the biological agents stored under the Permit Holder's control. Pre-planning should include plans of required backup systems (e.g. backup power) or prearranged movement of samples into other unaffected secure areas.

5. Standard Operating Procedures for Laboratory Work

5.1 Standard Microbiological Practices and Infection Control

Good microbiological practice is a basic code of practice that should be applied to all types of work involving microorganisms irrespective of containment level to reduce the risk of exposure. The objectives of good microbiological practice are to:

- **prevent contamination of laboratory workers and the environment**
- **prevent contamination of the experimental samples**

Good work practices can significantly reduce the risk of aerosol production, and contamination of experimental equipment and work surfaces; thus containing the biological agent and reducing the risk of infection. Personnel must be trained and proficient in the practices and techniques such as proper disinfection and housekeeping required to safely manipulate biological agents.

Good microbiological practices include the following:

A. no food or drink items are allowed to be stored and eaten in the laboratory;
B. washing hands at any time contamination is suspected, prior to and following manipulations of organisms even if gloves have been worn and upon exiting laboratory;
C. all work with infectious material should be carried out in a specific area, and the material should not be carried throughout or out of the lab unless in a closed container;
D. disinfection of: work surfaces with a suitable disinfectant before and after experiments/ manipulations of organisms, and materials that have come in contact with your organism
E. clean spills immediately according to established protocols and disinfect the area thoroughly;
F. keep benchtop uncluttered;
G. minimize traffic and unnecessary movements around the work area (movement can stir up air currents which can carry contaminants into the work area);
H. minimize aerosol generation or conduct work in a biological safety cabinet;
I. use proper aseptic technique for the transfer and handling or microorganisms and instruments;
J. keep sterile and non-sterile objects separate;
K. minimize exposure to outside air (i.e. keep lids off sterile containers for as little time as possible);

L. discharge from pipettes should never be dropped from a height. Contents should be allowed to run down the wall of the tube or bottle or be discharged as close as possible to the fluid or agar level;
M. gently manipulate lab equipment and specimens, avoid sudden movements;
N. use disposable inoculating loops;
O. when aerosols are created within a closed container, wait a few minutes before opening

Frequent hand washing has proven to be the single most effective means of avoiding infection.

Hand Washing:

1. Wet hands with warm running water.
2. Add any type of soap and then rub your hands together and wash the front, back, between fingers and under nails, making a soapy lather for at least 10 seconds.
3. Rinse hands well under warm running water and dry thoroughly with clean paper towel for at least 5 seconds.

5.2 Containment of Aerosols

Aerosols are gaseous suspensions of fine solid or liquid particles ranging in sizes from 0.01 to 100 μm and can remain suspended in air for extended periods of time. Pathogens such as viruses and bacteria are so small that they can travel within one aerosol droplet and be dispersed by building ventilation. Aerosols can settle on many surfaces where personnel may unwittingly be exposed to a potentially infectious material through the risk of direct contact.

The generation and dispersal of aerosols must be minimized and controlled. Therefore, it is important to be aware not only of the characteristics of the agent in the laboratory but also procedures and devices that create aerosols. There are numerous procedures and devices which can result in the generation of aerosols and may include, but are not limited to pouring liquids, using centrifuges/shakers/blenders, opening pressurized vessels, inserting a hot loop into a culture, pipetting, etc.

To eliminate or minimize the generation of aerosols, standard microbiological practices (as outlined in Section 5.1) should always be employed. When appropriate, other primary barriers such as splash shields, face protection or gowns should also be used. When conducting procedures that create considerable aerosols or when using agents classified at Containment Level 2, work should be conducted in a Biological Safety Cabinet.

5.3 Safety Precautions for Containment Level 1 Area

Containment level 1 requires no special design features beyond a well-designed functional laboratory. Biological safety cabinets are not required. Work may be done on an open bench top and containment is usually achieved through the use of good work practices in a basic microbiology laboratory. Follow the standard microbiological practices and infection control procedures in section 5.1 of this manual.

5.4 Safety Precautions for Containment Level 2 Areas

The physical containment requirements are outlined in Appendix C.
Below are the operational practice requirements for containment level 2 as outlined in the CBS 2nd Edition:

CBS Ref. #	Operational Practice Requirements for CL2 (no animal work)
4.1.7	A biosecurity risk assessment to be conducted and documented.
4.1.8	A local risk assessment (LRA) to be conducted to examine each task involving infectious material or toxins so that the risks are identified and safe work practices developed and documented.
4.1.9	A training needs assessment to be conducted.
4.1.10	A biological safety program manual to be accessible to all personnel.
4.1.11	A biosecurity plan (as outlined in section 6 of this manual) to be implemented and followed.
4.1.13	A respiratory protection program to be in place when respirators are in use.
4.1.14	A training program, based on a training needs assessment, to be implemented, evaluated and improved as necessary, and kept up to date.
4.1.15	SOPs specific to the nature of the work being conducted in the containment zone to be developed and documented, including:
	● personal protective equipment(PPE) requirements; ● entry/exit procedures for personnel, animals, and materials; ● use of primary containment devices; ● decontamination and waste management; ● the safe and secure movement and transportation of infectious material and toxins, ● any procedure or task involving infectious material, toxins, and/or infected animals, as determined by an LRA.
4.1.16	An ERP, based on an overarching risk assessment and LRAs, to be developed, implemented, and kept up to date.
4.2.2	Containment zone personnel to immediately inform appropriate internal personnel (e.g., supervisor, BSO) of any incident or exposure or disease.
4.3.1	Personnel to be trained on the relevant components of the Biosafety Manual and standard operating procedures (SOPs), as determined by the training needs assessment.
4.3.2	Personnel to be trained on the potential hazards associated with the work involved or release of pathogens or toxins.

4.3.6	Visitors, maintenance and janitorial staff, contractors, and others who require temporary access to the containment zone to be trained and/or accompanied in accordance with their anticipated activities in the containment zone. Contact BSO for information.
4.3.7	Personnel to demonstrate knowledge of and proficiency in the SOPs on which they were trained.
4.3.8	Trainees to be supervised by authorized personnel when engaging in activities with infectious material and toxins until they have fulfilled the training requirements.
4.3.9	Review of training needs assessment to be conducted, at minimum, annually. Additional or refresher training to be provided as determined by the review process or when warranted by a change in the biosafety program.
4.3.10	Refresher training on emergency response procedures to be provided annually. Contact BSO.
4.4.2-4.4.4	Follow PPE requirements in section 5.9 of Ryerson's Biological Safety Manual
4.5.1	Containment zone doors to be kept closed.
4.5.2	Access to containment zone to be limited to authorized personnel and authorized visitors.
4.5.5	Access to supporting mechanical and electrical services for the containment zone to be limited.

Entry Procedures	
4.5.8	Current entry requirements to be posted at point(s) of entry to the containment zone. Refer to signage in Figure 2 of Ryerson's Biological Safety Manual.
4.5.10	Personal clothing to be stored separately from dedicated PPE.
4.5.11	Personal belongings to be kept separate from areas where infectious material or toxins are handled or stored.

Exit Procedures	
4.5.14	Personnel to doff dedicated PPE, in a manner that minimizes contamination of the skin and hair, when exiting the containment zone.

4.5.15	Personnel to remove gloves and wash hands when exiting the containment zone, animal room, animal cubicle, or PM room.
4.6.1	Contact of the face or mucous membranes with items contaminated or potentially contaminated with pathogens or toxins to be prohibited.
4.6.2	Hair that may become contaminated when working in the containment zone to be restrained or covered.
4.6.3	Type of footwear worn to be selected to prevent injuries and incidents, in accordance with containment zone function.
4.6.5	Oral pipetting of any substance to be prohibited.
4.6.6	Open wounds, cuts, scratches, and grazes to be covered with waterproof dressings.
4.6.7	Traffic flow patterns from areas of lower contamination (i.e., clean) to areas of higher contamination (i.e., dirty) to be established and followed, as determined by a local risk assessment (LRA).
4.6.8	Dedicated paper/computer work areas to be utilized for paperwork and report writing.
4.6.9	Use of needles, syringes, and other sharp objects to be strictly limited and avoided when suitable alternatives are available.
4.6.10	Bending, shearing, recapping, or removing needles from syringes to be avoided, and, when necessary, performed only as specified in SOPs.
4.6.11	Work surfaces to be cleaned and decontaminated with a disinfectant effective against the pathogen(s) in use, or a neutralizing chemical effective against the toxin(s) in use, at a frequency to minimize the potential of exposure to infectious material or toxins.
4.6.14	Verification of the integrity of primary containment devices to be performed routinely, as described in SOPs.
4.6.15	BSCs, when present, to be certified upon initial installation, annually, and after any repairs, modification, or relocation.

Handling Infectious Material and Toxins	
4.6.18	Good microbiological laboratory practices to be employed.
4.6.19	Samples of pathogens, toxins, or other regulated infectious material to be opened only in containment zones that meet the containment level requirements to which that infectious material or toxin has been assigned.

4.6.20	Containers of pathogens, toxins, or other regulated infectious material stored outside the containment zone to be labelled, leak proof, impact resistant, and kept either in locked storage equipment or within an area with limited access.
4.6.24	A certified BSC to be used for procedures involving open vessels of infectious material or toxins that: ● may produce infectious aerosols or aerosolized toxins, when aerosol generation cannot be contained through other methods; ● involve high concentrations of infectious material or toxins; or ● involve large volumes of infectious material or toxins. [Not required when collecting samples from or inoculating animals housed in an animal cubicle.]
4.6.26	Procedures to be followed to prevent the inadvertent spread of contamination from items removed from the BSC after handling infectious material or toxins.
4.6.27	Personnel to wash hands after completing tasks that involve the handling of infectious material or toxins and before undertaking other tasks in the containment zone.
4.6.28	Centrifugation of infectious material where inhalation is the primary route of infection to be carried out in sealed safety cups (or rotors) that are unloaded in a BSC.
4.6.30	Use of on-demand open flames in a BSC to be strictly limited and avoided when suitable alternatives are available; sustained open flames to be prohibited in a BSC.
4.6.31	Procedures, as determined by an LRA, to be in place to prevent a leak, drop, spill, or similar event during the movement of infectious material or toxins within the containment zone or between containment zones within a building.
4.6.33	Collecting samples, adding materials, or transferring culture fluids from one closed system to another to be performed in a manner that prevents the release of aerosols or the contamination of exposed surfaces.
4.6.34	Experimentally infecting cells or other specimens derived from the person conducting the experiment to be prohibited.

Housekeeping and General Maintenance	
4.6.35	Containment zone (including floors) to be kept clean, free from obstructions, and free from materials that are in excess, not required, or that cannot be easily decontaminated.

4.6.37	An effective rodent and insect control program to be maintained.
4.6.39	An acceptable mechanism to be utilized for the safe removal of high efficiency particulate air (HEPA)filters. Consult BSO.

Decontamination and Waste Management	
4.8.1	Gross contamination to be removed prior to decontamination of surfaces and equipment, and disposed of accordingly.
4.8.2	Disinfectants effective against the pathogen(s) in use and neutralizing chemicals effective against the toxin(s) in use to be available and used in the containment zone.
4.8.3-4.8.15	Refer to requirements outlined in section 5.12 of Ryerson's Biological Safety Manual.

Emergency Response Procedures	
4.9.1	The ERP is to describe emergency procedures applicable to the containment zone for: ● accidents/incidents; ● medical emergencies; ● fires; ● chemical/biological spills (small/large; inside/outside BSC and centrifuge); ● power failure; ● failure of primary containment zone; ● puff-back from class II B2 BSCs, where present; loss of containment; emergency egress; ● external and internal notification; natural disasters; and ● incident follow-up and recommendations to mitigate future risks.
4.9.2	ERP to include procedures for any infectious material or toxins stored outside the containment zone.

Incident Investigation and Reporting	
4.9.7-4.9.10	Refer to procedure in section 1 of Ryerson's Biological Safety Manual.

Records and Documentation	
4.10.1	Training and refresher training to be documented; records to be kept on file.

4.10.2	Inventory of pathogens, toxins, and other regulated infectious material in long-term storage to be maintained, including location and risk group(s) and infectious material stored outside of the containment zone.
4.10.5	Records of regular inspections of the containment zone and corrective actions to be kept on file.
4.10.6	Records of building and equipment maintenance, repair, inspection, testing, or certification, including performance verification and testing records, in accordance with containment zone function, to be kept on file.
4.10.7	Equipment used for performance verification and testing of containment systems and essential biosafety equipment to have a valid calibration certificate at the time of testing; calibration certificates to be kept on file.
4.10.9	Records of validation and routine verification of decontamination technologies and processes to be kept on file.
4.10.10 - 4.10.11	Record retention: refer to section 2.5 of Ryerson's Biological Safety Manual for record retention duration.

Note: Appropriate signage (Figure 2) must be posted outside each laboratory. Refer to section 5.8 of this manual. If infectious agents used in the laboratory require special provisions for entry, the relevant information must be included on the sign; contact information of the laboratory supervisor or other responsible person(s) must also be listed.

5.5 Biological Safety Cabinets and Laminar Flow Hoods

Biological Safety Cabinets (BSC) provide an effective means of physical containment for procedures involving open vessels of infectious material or toxins that:

- May produce infectious **aerosols** or aerosolized toxins, when aerosol generation cannot be contained through other methods;
- Involve **high concentrations** of infectious material or toxins; or
- Involve **large volumes** of infectious material or toxins.

Their main role is to provide protection to personnel and the environment; and in most cases the product. Protection is achieved through the control of air movement within and prior to leaving the cabinet, and through the use of HEPA (high-efficiency particulate air) filtration. HEPA filters are designed to remove particles with a minimum size of 0.3 microns with an efficiency of 99.97%.

Laminar Flow Cabinets (LFC) have a much different role, as they only protect the product. These cabinets are sometimes referred to as Clean Benches. Though similar in appearance, laminar flow cabinets are not biological safety cabinets. These cabinets intake room air which is passed through a pre-filter and a HEPA filter to remove contaminants, dust and other particles. The purified air then enters the work surface in a laminar flow (non-turbulent) which is directed out of the cabinet or down into intakes. Therefore, these cabinets provide product protection

only and must not be used when working with any form of biohazard.

The effectiveness of a BSC / LFC is dependent upon:

A. the integrity of the cabinet – (if the integrity of the cabinet is jeopardized, the risk of exposure to a biohazardous agent increases);
B. proper practices within the cabinet (do not result in turbulence, dead air, etc.);
C. placement of the cabinet in a room (to avoid the air curtain being jeopardized by traffic flow or air currents due to room ventilation);
D. proper microbiological technique and work practices that do not disturb established; airflow velocity and cause reverse currents that can re-introduce contaminants into the work area;
E. continued maintenance and certification.

5.5.1 Cabinet Certification

Biological safety cabinets (BSCs) must be certified upon initial installation, annually, and after any repairs, modification, or relocation. Certification is to be certified in accordance with NSF/ANSI 49, where possible or following manufacturer's specifications. Certification ensures the HEPA filter has not be damaged, leaked or plugged. If a cabinet has been moved, it must be re-certified before it can be used again. It is very easy to compromise the containment system. Under most circumstances the cabinet is decontaminated prior to any certification activity. This is necessary to protect not only the individual undertaking the certification but also laboratory staff. In addition, prior to disposal, HEPA filters & cabinets must be decontaminated.

Laminar flow hoods should be certified every second year. Contact the BSO for information on cabinet certification.

5.5.2 Flames in Biological Safety Cabinets

In accordance to the Canadian Biosafety Standards (section 4.6.30), the use of on-demand open flames (e.g., touch-plate microburner) in a BSC is to be strictly limited and avoided when suitable alternatives are available; sustained open flames (e.g., Bunsen burner) are to be prohibited in a BSC.

An open flame in a BSC creates two major problems. First, a flame creates turbulence, which disrupts the pattern of air supplied to the work surface, therefore reducing maximum efficiency. Secondly, the use of flame causes heat to build-up inside the BSC. Not only can the excess heat damage the HEPA filters, but it also creates a fire hazard. Viable alternatives to flames include using disposable sterile inoculating loops and needles, or microincinerators.

If a flame is deemed necessary and suitable alternatives cannot be used, a touch-plate micro-burner equipped with a pilot light to provide a flame on demand may be used. This device will minimize internal cabinet air disturbances and heat build-up. During use, the heat source should be placed to the rear of the workspace where resulting air turbulence will have minimal effect. An emergency shut off valve should be placed just outside the BSC the gas supply line and during the use of any burner, all combustible materials and solvents must be removed.

5.5.3 Ultraviolet Lamps

Ultraviolet (UV) lamps in BSCs are intended to destroy microorganisms in the air or on exposed surfaces. However, these lamps have limited penetrating power and are only effective when the lamps are properly cleaned, maintained, and checked to ensure that the appropriate intensity is being emitted. Dust is attracted to the lamps which reduces the transmission of the germicidal effect. The lamps have a limited life span – even if the blue-violet glows, the lamps are not effective if the terminal ends are blackened even slightly. The UV lamps themselves are potential hazards since UV light can be harmful to the eyes and skin and should therefore be turned off when work is not being conducted in the cabinet.

5.6 Human Blood and Body Fluids

Exposure to human blood and bodily fluids increases the risk to exposure to bloodborne pathogens. Blood borne pathogens are, microorganisms that are present in blood and bodily fluids and are capable of causing disease in exposed individuals. The pathogens of greatest concern are the hepatitis B virus, the hepatitis C virus and the Human Immunodeficiency Virus (HIV).

In a laboratory setting, bloodborne pathogens are transmitted via;

- Direct contact with infected blood e.g., through open sores, mucous membranes (e.g., eyes, mouth,) or animal bite.
- Injection/inoculation, e.g., accidental puncture by a needle, broken glass or other “sharps”, contaminated with the pathogen.

To minimize the risk of exposure all individuals should practice universal precautions when the potential for exposure to bloodborne pathogens exists during the course of their work. Universal precautions may be defined as the minimal standard of work performance to prevent transmission of bloodborne pathogens. The components include the exposure to pathogenic agents; education, personal protective equipment, hand washing, and employing safe work practices. **It requires the individual to assume the material to be infectious.**

These **Universal Precautions** must always be used when handling blood or body fluids. When contact with human blood and body fluids is anticipated, control measures should be routinely utilized to prevent skin and mucous membrane exposure.

1. Gloves should be worn when handling blood or bodily fluids or potentially contaminated surfaces.
2. Avoid touching items that are NOT contaminated when gloves are being worn.
3. Masks, eye protection or face shields and lab coats should be worn during procedures that are likely to generate droplets of blood or bodily fluids.
4. Contaminated clothing and gloves should be removed immediately after procedure is completed.
5. Hand washing is the most important preventative tool for preventing transmission of bloodborne pathogens. Hands should be washed immediately after gloves are removed and before leaving a work area.

6. Caution should be used when handling contaminated needles, blades. Do not recap syringes/needles or break off syringe tips. Syringes should be placed in approved puncture resistant containers for disposal.
7. Individuals with contact dermatitis, cuts etc. should refrain from procedures that will involve handling blood or body fluids.

A **medical surveillance program** may be required based on the local risk assessment (assessing the duration of the task, the materials or equipment being used, and the potential for exposure). The physician will determine whether the employee is fit to perform the required task or if e.g., Hepatitis B immunization is required. Contact the BSO for information.

5.7 Needle Stick Injury Prevention

Sharps such as needles, blades or syringes can cause injury due to inadvertent penetration of skin into tissue or bloodstream. Needle stick injuries may result from lack of training on proper work practices, crowded work conditions, incorrect recapping of non retracting needles or poor disposal practices (discarding spent sharps in regular garbage, non approved sharps containers or overfilling disposal containers). The risk of injury exists to the user and support staff such as caretaking or hazardous waste disposal personnel. **To avoid needle stick injury, needle-less techniques or safety-engineered needles or retractable sharps should be used wherever possible.** Bending, shearing, recapping or removing needles from syringes should be avoided, and, when necessary, performed only as specified in SOPs.

Designated puncture-proof sharps containers should be used for disposal that will not allow penetration or direct access to sharps.

Report needle stick injuries immediately to the Permit Holder and/or the BSO and seek a medical assessment. Such injuries must not be ignored - potential laboratory associated infections could develop without appropriate treatment.

5.8 Signs and Postings

Internal Permits are required to be posted in a visible location inside all locations listed on a Permit. The current staff list must be posted with the Permit in the main laboratory of the permit holder. In addition, active work, storage and waste areas must be identified with biohazard symbol labels. Table 1 provides a summary of postings required in biosafety locations.

Entrances to areas where Containment Level 2 agents or greater are being used or stored must be marked with **biohazard warning signage** on the door (shown in Figure 2) that has a biological warning symbol, containment level, name and telephone number(s) of contact person, entry requirements and emergency contact information.

All new Biosafety Permit holders of CL2 laboratories will receive a door sign with their approved Biosafety Permit. Signs may also be obtained from the Environmental Health and Safety Biological Safety Program website.

Table 1: Requirements for Biosafety Laboratory Postings

Location	Information and Type of Posting
Entrance (laboratory, storage area, or other permitted area	• Biosafety Door Sign for CL2 with Permit Holder name and office phone #, 24 hour contact (see Fig 2)
Inside the lab, in a prominent location	• Copy of the Internal Biosafety Permit, • Current list of authorized users (inside main lab only)
Work Area and Storage & Waste Areas	• Warning sign/tape identifying biohazardous area

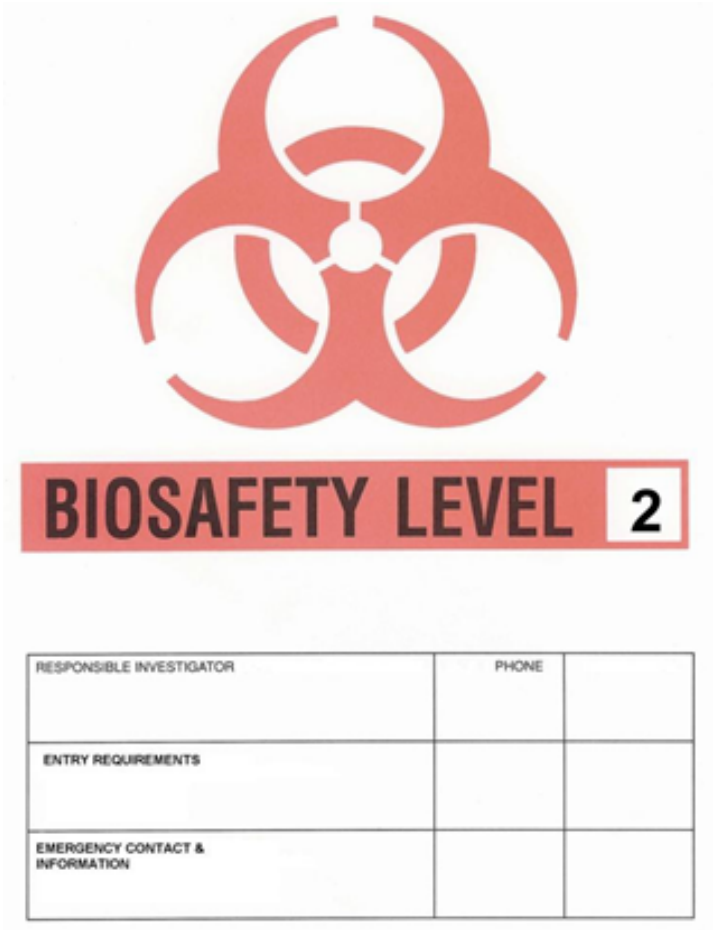

Figure 2: Door sign for Level 2 Containment Laboratory

5.9 Personal Protective Equipment

The use of personal protective equipment is the last line of defense against exposure in the event of unplanned exposures and complements existing control measures such as engineering and administrative controls, good work practices and training. As a minimum requirement, personal protective equipment in any biosafety containment laboratory shall consist of:

5.9.1 Laboratory coat

Laboratory coats will be worn fully buttoned. If the wrist opening of the lab coat has not been restricted, care must be taken to prevent exposing the skin; this may be achieved by tucking the sleeve into the glove. Dangling sleeves may also result in contamination as result of the edge of the sleeve dragging over multiple surfaces. Should the lab coat be known to be contaminated or after operations engaging a high risk of aerosol generation (spill), the coat should first be autoclaved. Non disposable laboratory coats will require periodic cleaning. This involves bagging of the lab coat to a cleaning facility, use of a bleach and laundry soap using a dedicated wash cycle.

Lab coats shall not be worn into non laboratory areas such as any eating areas.
Where possible, coat hooks should be installed near the exit door to encourage laboratory personnel to remove such clothing before leaving the laboratory.

5.9.2 Eye or Face protection

Safety glasses/goggles are required for any work involving biological agents. It is recommended that contact lenses not be worn in a laboratory. The choice of eye or face protection (e.g., goggles or face shield) will depend on the risks from work being conducted in the laboratory such as: splashes due to spills, or aerosols generation.

5.9.3 Gloves

Disposable, gloves used for laboratory work must be removed before leaving the laboratory or after use to prevent the spread of contamination. Choice of glove material should be based on effectiveness of prevention of penetration of biological agents/chemicals, length of cuff, thickness of glove material, potential for latex or other allergic conditions. Gloves should be changed frequently. Double gloving may help address the potential weakness associated with the above mentioned conditions.

5.9.4 Footwear

Open-toed shoes are not permitted when handling biological agents.
Dedicated storage space (e.g., hooks, lockers, shelves etc.) is necessary to store the PPE (e.g., lab coat, face shields, respirators) used in the containment zone and to separate these items from personal clothing in order to prevent contamination.

5.10 Acquisition (Purchasing, Importing, Transferring) and Exportation of Pathogens or Toxins

5.10.1 Acquisition of Human Pathogens or toxin

Under the HPTA, a licence from PHAC **must** be obtained for importing, exporting, transferring or disposing of human pathogen or toxin.

Under the HPTR (section 4(1)), the Biological Safety Officer (BSO) must be notified **before** making arrangement to:

I. import a human pathogen or toxin,
II. possess a human pathogen or toxin as a result of receiving from another licence holder or from a person who is authorized by another licence (e.g., transfers from other institutions, loans, gifts)

Only Biosafety Permit Holders or designated Authorized Users may acquire biological agents after approval of a Ryerson Biosafety Permit has been issued. Advance notice of incoming pathogens allows the BSO to provide a copy of the licence to the international supplier, and to ensure appropriate containment is available in the laboratory to properly work and store the biological agents. Biological agents not listed on the Biosafety Permit cannot be ordered, prior to notification to the BSO.

Any Containment Level 2 biological agents arriving at the University for which there is no prior approval may be confiscated.

5.10.2 Transfer of Biological Agents and Inventory Control

5.10.2.1 Inventory Control

A Biosafety Permit Holder will maintain an accurate inventory of human pathogens and toxins in his/her possession. The biological agents should be current with the list in the Biosafety Permit. The records must be kept up to date and available to the Biological Safety Officer for inspection.

5.10.2.2 Transfer within the University

“Lending” or “borrowing” of human pathogens and toxins between laboratories is not permitted without prior approval from the BSO. The BSO must ensure that the appropriate containment is available. The “borrower” is required to record the date, type, quantity, the Permit Holder Name and the Biosafety Permit Number of the originating laboratory.

5.10.2.3 From/to Outside Institutions

Human pathogens and toxins may not be transferred to or from another institution or person outside the University, without prior written notice and approval from the BSO.
The person who intends to transfer a human pathogen or toxin must, before the transfer, take reasonable care to be satisfied of the following:

I. that the intended recipient is exempt from the requirement to hold a licence, or

II. that the intended recipient will conduct activities under a licence

Materials must be properly transported in accordance with Transport Canada Regulations. The transportation of infectious substances in Canada is regulated by the federal Transportation of Dangerous Goods Act and Regulations. The regulations outline the requirements for packaging, labeling, and documentation, training and emergency response. For specific information on shipping infectious biological agents, please contact the BSO. Several companies exist that provide appropriate packaging and labeling for such materials.

If the materials are not received within a reasonable time (for security sensitive biological agents, within 24 hours) after it was expected to be received, the intended recipient must make reasonable efforts to locate it, inform the BSO of the situation without delay and provide the BSO with any other information that is relevant to prevent undue risk to the public.

Once the material transfer has occurred, Permit Holders must ensure their Biosafety Permit is updated to include the new inventory of biological agents.

5.10.2.4 Research Ethics Board Approval for Transferred Human Cells and Tissue

If the transferred biological agents involve human cells or tissue, then a valid Research Ethics Board approval is required prior to the transfer of material occurs. If the transfer is occurring from another institution to Ryerson University, then a copy of the other institutions Research Ethics Board approval will also be required. Please contact the Office of Research Services or review the online application for Research Ethics approvals at www.ryerson.ca/ors/reb.

5.10.3 Importation of Animal Pathogens and Toxins

For the import of animal pathogens and toxins, and an animal, animal product, animal by-product, or other organism carrying an animal pathogen or toxin, an importation permit must be obtained from PHAC or the Canadian Food Inspection Agency (CFIA) under the authority of the Health of Animals Act (HAA) and the Health of Animals Regulations (HAR).
An import permit issued under the HAR shall contain conditions as the PHAC and/or the CFIA consider advisable to prevent the introduction of communicable disease into Canada and the spread of communicable disease within Canada.

Consult the BSO prior to importing an animal pathogen or toxin. **Applications for permits to import animal pathogens can be obtained from:**

http://www.inspection.gc.ca/animals/biohazard-containment-and-safety/pathogen-imports/eng/1300215299626/1320599995275

In the case of pathogens which affect both humans **and** animals, importation permits are required from both PHAC and CFIA.

A copy of the import permit into Canada must be provided to the BSO immediately upon receipt of permit and prior to ordering the animal pathogen.

5.10.4 Importation of Plant Pests

The Canadian Food and Inspection Agency (CFIA) controls the importation and use plant pathogens into Canada. The Plant Import Unit of CFIA, issues permits, is responsible for the enforcement for issued dealing with plant pests, and establishes the conditions under which these pathogens will be distributed, maintained and work carried out.

Applications for permits to import plant pathogens can be obtained from:

http://www.inspection.gc.ca/plants/imports/eng/1299168480001/1299168593866

5.11 Exportation of Biological Agents

The Public Health Agency of Canada issues Licenses that control the export of certain biological agents. Persons exporting human pathogens and toxins outside of Canada must take reasonable care to satisfy themselves that the intended recipient will follow applicable biosafety and biosecurity standards and policies in the foreign jurisdiction.

5.12 Decontamination and Waste Disposal

Materials, such as laboratory bench tops and other surfaces, equipment, etc., contaminated with biohazards must be decontaminated prior to reuse, serving, transfer or disposal. The means of treatment for decontamination may involve chemical agents (bleach, ethanol, etc), or physical means (autoclaving, incineration, etc.). Laboratory procedures must be in place to ensure the effectiveness of the methods used to decontaminate contaminated items.

The CBS specifically outlines the following decontamination and waste management requirements for CL2 labs (with no animal usage):

- Sharps be discarded in containers that are leak proof, puncture-resistant, and fitted with lids, or specially constructed for the disposal of sharps waste.
- Primary containment devices (e.g., BSCs, glove box etc.) to be decontaminated prior to maintenance.
    - Note: Decontamination of **HEPA** filters through in situ fumigation with formaldehyde or vaporized hydrogen peroxide allows for the decontamination of HEPA filters prior to their removal. If prions are used, a suitable alternative for safe removal of HEPA is required.
- All clothing and personal protective equipment (PPE) to be decontaminated when a known or suspected exposure has occurred.
- Contaminated liquids to be decontaminated prior to release to sanitary sewers.
- Contaminated equipment, materials and waste to be decontaminated and labelled as decontaminated prior to cleaning, disposal or removal from containment zone or placed in closed, labelled, and leak proof containers that have been surface decontaminated prior to removal from the containment zone for the transportation to waste storage area.
- Decontamination technologies and processes to be validated prior to initial use and when significant changes to the process (e.g., new protocol or concentration) are

implemented or new pathogens are introduced to confirm that the equipment is functioning properly and that the process of decontamination is effective.
- Verification of decontamination equipment (through the use of biological indicators, chemical integrators etc.) is performed upon initial use, and routinely thereafter to confirm that the process is effective for the decontamination of materials prior to their removal. Frequency of verification to be determined by a local risk assessment.

5.12.1 Autoclaves

Sterilization is a process which results in the total destruction of all living and viable organisms (with a probability of 1 in 1 million that 1 organism survived), and therefore is the most preferred treatment.

Sterilization is achieved through autoclaves. Autoclaves utilize both pressure and high temperatures and provide an effective and efficient means of sterilizing and decontaminating items that may have become exposed to biohazardous or potentially biohazardous material. Laboratory items such as pipettes, culture tubes, petri dishes, glassware can be effectively decontaminated in an autoclave. The effectiveness of decontamination from a steam autoclave depends on appropriately loading items into the autoclave. Load distribution, and the size of containers will influence inside temperatures and thus the effectiveness of the sterilization.

Departments are required to develop procedures and provide training to users for in-house autoclaves, which include, loading procedures, storage of materials awaiting autoclaving, unloading autoclave, separation of sterilized materials, appropriate labeling of sterilized materials awaiting disposal and the use of performance indicators to verify effectiveness of sterilization. Contact the BSO for assistance.

5.12.2 Disinfection

Disinfection refers to the destruction of the most resistant vegetative microbes or viruses, but it does not destroy spores. Chemical disinfection methods are used to decontaminate surfaces and equipment that cannot be autoclaved and in the cleanup of spills. Chemical disinfection includes hypochlorite solution, 10% formalin, glutaraldehyde, 70% ethanol. The choice of disinfectant will be determined by the resistance of the organism being used in the laboratory.

5.12.3 Waste Disposal

Biohazardous waste must be stored away from regular work space. Ryerson coordinates the disposal of biohazardous waste with an external disposal service provider. Containers should be properly packaged when they are almost full and transferred to the waste holding room located in the Kerr Hall 2nd floor. Regular waste pick-up service is weekly or as scheduled. Detailed information on how to properly handle biohazardous waste is available in Appendix D.

5.13 Large Scale or High Concentration Work

If working with high concentrations or shifting to large scale work (i.e. >10 litres are being manipulated in a single volume or multiple vessels with a total volume of 10 litres or greater), than an increase in containment may be required.

A risk assessment should be carried out to by assessing the quantity, the process and the type of infectious materials being used. It is determined in consultation with the PHAC and/or CFIA on a case-by-case basis, whether or not particular activities conducted in a containment zone are required to follow the increased or unique requirements for large scale production areas.

Contact the BSO who will assist with the risk assessment and liaising with the relevant government agency(ies).

6. Biosecurity

Biosecurity includes security measures designed to prevent the loss, theft, misuse, diversion, or intentional release of pathogens, toxins, and other related assets (e.g., personnel, equipment, non-infectious material, and animals).

It is the Permit Holders' responsibility to ensure that all biological agents are kept secure at all times.

A biosecurity plan should begin with a biosecurity risk assessment, based on type of biological agent, the containment level required, location and proposed used. The assessment identifies and prioritizes assets, defines threats, and determines risk and mitigation strategies. Appropriate controls must be in place at the laboratory as well as departmental level. A plan to secure access to unauthorized persons may include:

- Physical security (see Appendix C for physical containment requirements and section 6.2), e.g.,
    - controlled access through key control programs or key card access;
    - self-locking doors;
    - physical barriers;
    - design to reflect the transition from general public to laboratory zones.
- Personnel suitability and reliability (see section 6.1) e.g.,
    - Identify authorized personnel and questioning strangers and restricting access to only authorized personnel;
    - Only for a person that holds a security clearance can enter a facility where controlled activities with SSBAs are authorized
- Accountability for pathogens, toxins, and other regulated infectious material, e.g.,
    - Issuing internal permit to track users, location and types of biological agents being used or stored
    - tracking of inventory (documenting acquisition and disposal of biological agents)

    - minimizing inventory stored in a biosafety containment laboratory;
    - reporting any loss of infectious materials or toxins.
- Inventory, e.g.,
    - inventory of regulated pathogens and toxins in long-term storage to be maintained, including location and risk group(s).
- Incident and emergency response, e.g.,
    - Developing a procedure to report a loss, theft or release of regulated materials or toxins (see section 6.3)
- Information management, e.g.,
    - Managing new pathogen, facility renovation

The biosecurity plan must be maintained through regular review and kept up to date.

6.1 Authorized Access

Only Authorized Users and the BSO may have access to biological agents. All biological materials must be secured at all times from unauthorized personnel. Persons unknown to the occupants of an area where agents are used or stored should not be permitted into the area without proper identification and a legitimate reason for entry.

No person shall enter the part of a facility in which SSBAs are stored and handled unless:

A. they hold a security clearance for that part of the facility; or
B. they are accompanied and supervised by a person who holds a security clearance to that part of the facility.

6.2 Maintaining Security

When an Authorized User is not present in a room containing biological agents, that material must be locked within a storage unit (refrigerator, freezer, etc.). When the biosafety laboratory is unattended, the area must be secured by locking the laboratory door. To ensure that the security of these materials is maintained, the Biological Safety Officer or Ryerson Security will lock doors if the area is found to be unoccupied and biological agents not secured.

Note: Restricted access into the part of the facility where SSBAs are stored and handled to be provided through a controlled access system. When key-locks are used as the controlled access system, non-reproducible keys must be used.

6.3 Reporting Missing Biological Agents

Any suspicion of missing materials due to loss, theft or misuse must be reported immediately to:

- Biological Safety Officer (BSO) at extension 554212.
- After-hours: Ryerson Security at 416-979-5040 who will contact the BSO

The BSO will work with the permit holder to take reasonable measures to locate the missing human pathogen or toxin and to inform PHAC.

7. References

Canadian references

1. **Public Health Agency of Canada**
   Office of Laboratory Security
   613-957-1779
   http://www.phac-aspc.gc.ca/ols-bsl/

   - **Canadian Biosafety Standards, 2nd Edition**
   - **Pathogen Safety Data Sheets**
   - **Import Permit for Human Pathogens**

2. **Canadian Food Inspection Agency Biohazard and Containment Unit**
   613-221-7069
   www.inspection.gc.ca/english/sci/bio/bioe.shtml

   - **Import Permit for Animal Pathogens**
   - **Import Permit for Plant Pathogens**
   - **Containment Standards for Facilities Handling Plant Pests**

International references

1. **Centre for Disease Control and Prevention– Biosafety**
   - **Biosafety in Microbiological and Biomedical Laboratories 4th ed**
2. **World Health Organization**

Professional biological safety organizations

1. **American Biological Safety Association**

Appendices

Appendix A (1) - Toxins, prescribed names

(from Human Pathogens and Toxins Act, Schedule 1)

- Aerolysin
    - *Aerolysine*
- Alpha toxin
    - *Toxine Alpha*
- Anthrax toxins: Lethal Toxin and Oedema Toxin
    - *Toxines du charbon : toxine létale et toxine d'oedème*
- Bordetella pertussis Adenylate cyclase toxin
    - *Toxine pertussique d'adénylate cyclase*
- Botulinum neurotoxin
    - *Toxine botulique*
- Cholera toxin
    - *Toxine du choléra*
- Clostridium botulinum C2 and C3 toxins
    - *Toxines C2 et C3 de Clostridium botulinum*
- Clostridium difficile toxins A and B
    - *Toxines A et B de Clostridium difficile*
- Clostridium perfringens Epsilon toxin
    - *Toxine Epsilon de Clostridium perfringens*
- Dermonecrotic toxin
    - *Toxine dermonecrotique*
- Diphtheria toxin
    - *Toxine diphthérique*
- Escherichia coli toxins: E. coli Cytotoxic Necrotizing Factor (CNF), Heat-labile E. coli enterotoxin (LT), Heat-stable E. coli enterotoxin (ST), Cytolethal distending toxin (CLDT) and Enteroaggregative Shiga-like toxin 1 (EAST)
    - *Toxines Escherichia coli : facteur cytotoxique nécrosant (CNF), entérotoxine labile à la chaleur (LT), entérotoxine stable à la chaleur (ST), entérotoxine cytolétale et distendante (CLDT) et toxine entéroagrégative Shiga-like 1 (EAST)*
- Exfoliative toxin (also called Exfoliatin)
    - *Toxine exfoliative*

- Exotoxin A
    - *Exotoxine A*
- Hemolysin
    - *Hemolysine*
- Listeriolysin O
    - *Listeriolysine O*
- Pasteurella multocida toxin
    - *Toxine de Pasteurella multocida*
- Perfringolysin O
    - *Perfringolysine O*
- Pertussis toxin
    - *Toxine pertussique*
- Pneumolysin
    - *Pneumolysine*
- Pyrogenic exotoxin
    - *Exotoxine pyrogène*
- Shiga-like toxin (verotoxin)
    - *Toxine Shiga-like (vérotoxine)*
- Shigatoxin
    - *Shigatoxine*
- Staphylococcal enterotoxins
    - *Entérotoxine de staphylocoques*
- Staphylococcus aureus Toxic shock syndrome toxin
    - *Toxine du syndrome du choc toxique de Staphylococcus aureus*
- Streptolysin O
    - *Streptolysine O*
- Tetanolysin
    - *Tetanolysine*
- Tetanospasmin (Tetanus toxin)
    - *Tetanospasmine (toxine tétanique)*

Appendix A (2) - Trigger quantities

A toxin that is set out in column 1 of the table to this section is not a prescribed toxin if it is present in a part of a facility in a quantity that is less than or equal to the quantity set out in column 2.

Column 1 Toxin	Column 2 Quantity (mg)
Alpha toxin Toxine Alpha	5
Botulinum neurotoxin Toxine botulique	0.5
Cholera toxin Toxine du choléra	20
Clostridium botulinum C2 and C3 toxins Toxines C2 et C3 de Clostridium botulinum	5
Clostridium perfringens Epsilon toxin Toxine Epsilon de Clostridium perfringens	5
Hemolysin Hemolysine	10
Shiga-like toxin (verotoxin) Toxine Shiga-like (vérotoxine)	1
Shigatoxin Shigatoxine	1
Staphylococcal enterotoxins, Type B Entérotoxine de staphylocoques, type B	1
Staphylococcal enterotoxins, types other than Type B Entérotoxine de staphylocoques, types autres que le B	10
Staphylococcus aureus Toxic shock syndrome toxin Toxine du syndrome du choc toxique de Staphylococcus aureus	5

Appendix B - Risk Group 2 Human Pathogens

(from Human Pathogens and Toxins Act, Schedule 2)

Risk Group 2 Human Pathogens Bacteria

- Actinobacillus pleuropneumoniae
- Actinobacillus ureae
- Actinomyces israelii
- Aerococcus ureinae
- Aeromonas hydrophila
- Aggregatibacter actinomycetemcomitans
- Arcanobacterium bernardiae
- Bordetella bronchiseptica
- Bordetella parapertussis
- Bordetella pertussis
- Borrelia burgdorferi
- Campylobacter jejuni
- Chlamydia trachomatis
- Chlamydophila pneumoniae
- Citrobacter freundii
- Clostridium botulinum
- Clostridium difficile
- Clostridium perfringens
- Clostridium tetani
- Corynebacterium diphtheriae
- Enterococcus faecium
- Escherichia coli
- Francisella novicida
- Haemophilus influenzae
- Haemophilus parainfluenzae
- Helicobacter pylori
- Klebsiella pneumoniae
- Legionella pneumophila
- Leptospira interrogans
- Listeria monocytogenes
- Moraxella catarrhalis
- Mycobacterium avium
- Mycobacterium leprae
- Mycobacterium smegmatis
- Mycoplasma genitalium
- Mycoplasma pneumoniae
- Neisseria gonorrhoeae
- Neisseria meningitidis
- Pasteurella multocida
- Porphyromonas gingivalis

- Proteus mirabilis
- Proteus vulgaris
- Pseudomonas aeruginosa
- Salmonella
- Serratia marcescens
- Shigella dysenteriae
- Shigella flexneri
- Shigella sonnei
- Sphingobacterium faecium
- Staphylococcus aureus
- Staphylococcus saprophyticus
- Streptococcus agalactiae
- Streptococcus pyogenes
- Streptococcus salivarius
- Treponema pallidum
- Ureaplasma urealyticum
- Vibrio cholerae
- Yersinia pseudotuberculosis

Viruses

- Adenovirus
- Avian influenza virus (excluding highly pathogenic strains)
- Colorado tick fever viruses
- Cowpox virus
- Coxsackievirus
- Epstein Barr virus
- Hepatitis A virus
- Hepatitis B virus
- Hepatitis C virus
- Hepatitis D virus
- Hepatitis E virus
- Herpes simplex viruses
- Human coronavirus (excluding SARS-CoV)
- Human herpesvirus 5 (cytomegalovirus)
- Human herpesvirus 6 (roseolovirus)
- Human herpesvirus 8 (Kaposi’s sarcoma-associated herpesvirus)
- Human parvovirus
- Human rotavirus
- Influenza virus, types A-C (excluding Type A 1918 Spanish Flu and H2N2 strains)
- Measles virus

- Molluscum contagiosum virus
- Mumps virus
- Newcastle disease virus
- Norwalk virus
- Papillomaviruses
- Parainfluenza virus (types 1-4)
- Reoviruses
- Respiratory syncytial virus
- Rhinovirus
- Semliki Forest virus
- Sendai virus
- Simian virus 40
- Vaccinia virus

Fungi

- Aspergillus fumigatus
- Aspergillus niger
- Aspergillus oryzae
- Candida albicans
- Cryptococcus neoformans
- Microsporum audouinii
- Microsporum ferrugineum
- Sporothrix schenkii
- Trichophyton concentricum
- Trichophyton rubrum
- Trichophyton schoenleinii
- Trichophyton tonsurans

Protozoa

- Acanthamoeba castellanii
- Leishmania aethiopica
- Leishmania braziliensis
- Leishmania chagasi
- Leishmania donovani
- Leishmania guyanensis
- Leishmania infantum
- Leishmania panamensis
- Plasmodium falciparum
- Trypanosoma brucei gambiense
- Trypanosoma brucei rhodiense
- Trypanosoma cruzi

Prions

- Chronic wasting disease agent

Appendix C - Physical Containment Requirements for Containment Level 2 Laboratories (no animal work)

Structure and Location

CBS reference	Structure and Location	Comments
3.1.1	Containment zones to be separated from public and administrative areas by a door.	A door is a physical barrier that protects against the release of infectious material or toxins by separating the containment zone (i.e., “dirty” or contaminated area) from public and administrative areas (i.e., “clean” or uncontaminated areas) while also providing a security barrier to limit access to the zone.

3.1.2	Dedicated paper/computer work stations within the containment zone to be segregated from laboratory work stations.	Minimizing the risk of contamination of materials which may be difficult to decontaminate (e.g., paper, notebooks) or may become damaged by decontamination (e.g., electronic devices). This can be achieved by locating these stations in a dedicated room within the containment zone, by installing a physical partition (e.g., splash shield) between a paperwork station adjacent to a laboratory bench, or by locating paper/computer workstations in a space inside the containment zone but at a distance from benches.

Containment Barrier

CBS reference	Containment Barrier	Comments
3.2.1	Openable windows positioned on the containment barrier are to include effective pest control and security.	Openable windows that have basic pest control and security measures protect against the entry of small-sized animals and insects and prevent the release of infectious material out of the containment zone, especially where the window opens directly to the outdoors. This can be achieved by fitting windows with properly installed screens that are in good repair and by closing and locking windows to prevent unauthorized entry, specifically when the containment zone is unoccupied.

Access

CBS reference	Access	Comments
3.3.1	Doors to the containment zone to be lockable.	Lockable doors provide a basic security barrier to prevent unauthorized access to the containment zone and to safeguard the infectious material and toxins stored inside.
3.3.2	Biohazard warning signage (including the international biohazard warning symbol, containment level, name and telephone number(s) of contact person, and entry requirements) to be posted at the containment zone point(s) of entry.	Biohazard warning signage is designed to 1) advise that infectious material or toxins are present in the containment zone, 2) indicate any special entry requirements, and 3) provide contact information in case of an emergency.
3.3.3	Where unique hazards exist, project-specific signage to be posted at the room point(s) of entry.	Project-specific signage posted at the room point(s) of entry informs of any special entry requirements for a particular experiment or study where hazards unique to a room exist (i.e., the same hazard is not present in the other adjoining rooms within the containment zone).

3.3.9	Space to be provided for the storage of personal protective equipment (PPE) in use.	Dedicated storage space is necessary to store the PPE used in the containment zone (e.g., lab coat, coveralls, face shields, respirators) and to separate these items from personal clothing (e.g., coats, hats, boots) in order to prevent contamination. Hooks, lockers, or shelves are examples of dedicated storage space for PPE (kept separate from such space for street clothes).

Surface Finishes and Casework

CBS reference	Surfaces Finishes and Casework	Comments
3.4.1	Surfaces and interior coatings, including but not limited to floors, ceilings, walls, doors, frames, casework, benchtops, and furniture to be cleanable, non-absorbent, and resistant to scratches, stains, moisture, chemicals, heat, impact, repeated decontamination, and high pressure washing, in accordance with function.	Cleanable and resistant surface materials and finishes (e.g., paint, epoxy, and other protective finishes) provide protection against the stresses associated with activities performed inside the containment zone. Non-absorbent materials may include stainless steel, epoxy resin surfaces or chemical resistant plastic laminate for benchtops, and urethane or vinyl for stools and chairs.
3.4.5	Floors to be slip-resistant in accordance with function.	Slip-resistant floors (e.g., textured surfaces) help prevent slips and falls and the associated risk of exposure to infectious material or toxins (e.g., via a splash, spill, inoculation or scratch). Different rooms or spaces may require a different degree of slip resistance (i.e., coefficient of friction), as determined by the activities and function of the different spaces inside the containment zone.

Facility Services

CB, reference	Facility Services	Comments
3.6.4	Sinks to be provided and located to facilitate hand washing upon exit from the containment zone.	Handwashing prevents the spread of many types of pathogens and toxins outside of the containment zone. Locating a dedicated sink near the point(s) of exit from the containment zone reduces the risk of recontaminating hands after washing but before exit.
3.6.6	Emergency eyewash and shower equipment to be provided in accordance with containment zone activities.	Emergency eyewash and shower equipment provide on-the-spot treatment to flush out, dilute, and remove any hazardous materials, including infectious materials and toxins, that have contaminated the eyes, face, or body before serious injury can occur.

Essential Biosafety Equipment

CB, reference	Essential Biosafety Equipment	Comments
3.7.1	Certified BSCs and other primary containment devices to be provided, based on work activities.	Examples of primary containment devices include BSCs, fermenters, and centrifuges with sealable cups or rotors. When properly maintained and used in conjunction with good laboratory techniques, these devices provide effective personnel and environmental protection when working with open vessels of infectious or toxic material.

3.7.3	Class II B2 BSCs to be installed and set-up in a manner to eliminate reversal of airflow from the face of the BSC (i.e., puff-back) during the failure of an HVAC system, or the BSC exhaust fan. Where elimination of puff-back cannot be achieved, the risk associated with puff-back should be mitigated through physical and operational means.	In the event of an exhaust fan failure, Class II B2 BSCs can produce a reversal of airflow from the face of the BSC (i.e., puff-back) as a result of a delayed reaction to shutdown. Every effort should be made to eliminate puff-backs mechanically (e.g., supply blower brake, isolation damper for BSC supply intake). Where puff-backs cannot be eliminated, it can be physically minimized (i.e., duration and airflow velocity as low as achievable) and additional operational mechanisms can be implemented to address the risks associated with the puff-back, based on the pathogen(s) and procedure(s) in use. Examples of operational mechanisms include the use of additional PPE, such as respirators and face protection, by all personnel in the immediate work area, and posting emergency protocols to be followed in the event puff-back occurs.
3.7.4	Process equipment, closed systems, and other primary containment devices to be designed to prevent the release of infectious material or toxins.	Preventing the release of infectious material and toxins from process equipment, closed systems, and other primary containment devices prevents contamination and protects personnel. This may include the use of HEPA filters on ports and vents, incineration, gaseous decontamination, or fully enclosing the primary vessels in ventilated housings that are exhausted through HEPA filters (e.g., walk-in containment enclosure).
3.7.6	BSCs, where present, to be located as far as possible from high traffic areas, doors, openable windows, and air supply/exhaust diffusers.	The protective air curtain created at the front of the BSC is quite fragile and can be easily disrupted by air currents or drafts created by traffic or HVAC systems in close proximity. Locating a BSC away from high traffic areas, doors, open windows, and air supply/exhaust diffusers protects the BSC air curtain and personnel from exposure to the release of pathogens and toxins.

3.7.11	Decontamination technologies for the decontamination of materials to be provided within the containment zone, or standard operating procedures (SOPs) to be in place to safely and securely move or transport waste out of the containment zone to a designated decontamination area.	The decontamination of waste and other contaminated material inside the containment zone or their safe and secure transport to a decontamination area (e.g., centralized facility decontamination location or certified off-site waste disposal service) prevent the release of pathogens from the containment zone. Examples of decontamination technologies include autoclaves and incinerators.
3.7.14	Decontamination technologies to be provided with monitoring and recording devices to capture operational parameters.	Monitoring operational parameters such as date, cycle number, time, temperature, chemical concentration, and pressure confirms that the decontamination technology is working properly
3.7.15	An autoclave, when present, to be capable of operating at the appropriate temperature for decontamination, as determined by **validation**.	The appropriate temperature and treatment time to effectively decontaminate waste materials are determined based on pathogen(s) in use. For example, an autoclave capable of operating at 134°C is critical for the effective decontamination of all waste materials coming from a containment zone where prions are handled and autoclaving is used as a single-step decontamination process of this material.
3.7.17	Vacuum systems to be equipped with a mechanism that prevents internal **contamination**.	Vacuum systems are used to aspirate liquid from a tube or to create a void in filtration units (e.g., centralized vacuum system). Vacuum pumps may cause aerosolization of infectious material or toxins and lead to contamination of vacuum lines and pumps. A device such as a HEPA filter, a small in-line filter (e.g., 0.2 µm filter), or a disinfectant trap can be used to protect vacuum systems from internal contamination with infectious material or toxins.

3.7.18	Two-way communication system(s) to be provided inside the containment barrier that allows communication between inside the containment barrier to outside the containment zone, in accordance with function.	A communication system increases personnel safety in the event of an emergency in the containment zone, and can also be used to minimize the movement of notebooks/papers and personnel into and out of the containment zone. Examples of communication systems include telephones, fax machines, intercom systems, networked computers, pagers, etc.

CL2 Lab spaces using prions and/or SSBAs

The following are requirements IN ADDITION to those outlined for Basic CL2 Lab spaces.

Structure and Location

CBS reference	Structure and Location	Comments
-	No additional requirements	

Containment Barrier

CB reference	Containment Barrier	Comments
3.2.2	Windows on the containment barrier to be closed and secured at all times.	Keeping windows on the containment barrier that can open locked in a closed position protects against unauthorized entry to the containment zone.
3.2.10	All penetrations of the containment barrier at or below the work surface and any other surface that may become contaminated, including all conduits and wiring, to be sealed with a non-shrinking sealant that is compatible with the disinfectant(s) in use.	Penetrations (e.g., from conduits, plumbing, and wiring) could allow the inadvertent release of infectious material. Sealing these gaps with sealants that are non-shrinking and compatible with the chemical disinfectants in use (e.g., silicone, polyurethane, or polyether caulking) allows for proper spill cleanup and surface decontamination, and maintains the integrity of the containment barrier.

Access

CBS reference	Access	Comments
3.3.4	**Restricted access** into the part of the **facility** where **security sensitive biological agents** (SSBAs) are stored and handled to be provided through a **controlled access system**.	Examples of controlled access systems include biometric readers, electronic access card systems, keypads, key code systems, key-locks with non-reproducible keys, or an equivalent system. SSBAs can only be accessed by those who have received a security clearance from the government.
3.3.5	Restricted access into the containment zone to be provided through a controlled access system.	As above, includes biometric readers, electronic access card systems, keypads, key code systems, key-locks with non-reproducible keys, or an equivalent system.
3.3.7	Non-reproducible keys to be used when key-locks are used as the controlled access system.	Keys that cannot be reproduced without the authorization of the containment zone director, supervisor, manager, or other delegated individual protects against unauthorized entry and restricts access to the containment zone to only authorized personnel to whom keys have been issued.
3.3.10	Dedicated change area to be provided at personnel entry to the containment zone to allow for separation of personal clothing from dedicated containment zone clothing (i.e., **“clean” change area** separated from **“dirty” change area**).	Dedicated clothing change areas at the entry and exit to the containment zone provide the space necessary to don and doff dedicated PPE used inside the containment zone and protect against the contamination of personal clothing. The change areas may be an anteroom, part of an anteroom, or, in some cases, a designated area at the entry and exit of the containment zone.

Surface Finishes and Casework

CBS reference	Surfaces Finishes and Casework	Comments
3.4.2	Surfaces to be continuous with adjacent and overlapping materials.	The continuity of adjacent surfaces (e.g., walls and floors, benchtops and other work surfaces) and overlapping material (e.g., flooring, baseboards, coving, backsplashes) provides a continuous barrier designed to prevent contaminated liquids from reaching surfaces that are hard to access and decontaminate.
3.4.4	Backsplashes, when installed tight to the wall, to be sealed at the wall-bench junction and continuous with work surfaces.	Backsplashes that are continuous or sealed at the junction between the wall and bench provide a continuous barrier to prevent contaminated liquids from reaching surfaces that are hard to access and decontaminate.
3.4.7	Continuity of seal to be maintained between the floor and wall.	When the continuity of the seal between the floor and wall allows liquids on the floor to be contained, it facilitates decontamination after a spill in a laboratory work area and routine cleaning.

Air Handling

CBS reference	Air Handling	Comments
-	No additional requirements	

Facility Services

CBS reference	Facility Services	Comments

3.6.18	Services and equipment critical to maintaining containment and **biosecurity** to be supported by emergency power.	The continued operation of equipment critical for the containment and security of infectious material and toxins during power outages is crucial to maintain containment integrity and to safeguard the security of the zone. In high containment zones, this includes HVAC systems and controls, as well as equipment essential for personnel safety (e.g., air supply).

Essential Biosafety Equipment

CBS reference	Essential Biosafety Equipment	Comments
-	No additional requirements	

Appendix D - Waste Disposal Procedures for the Department of Chemistry and Biology

Biohazard Waste Procedures

All materials that that come into contact with biohazards must be properly disposed of as biohazardous waste. Cytotoxic waste is defined as any material that may have come into contact with any agent that can kill cells, such as chemotherapeutic drugs.

The biohazardous and cytotoxic waste will be picked up every Tuesday morning. Be sure to drop-off your biohazardous waste by Monday in KHN-202-D (located in Kerr Hall North).

Cardboard boxes

First assemble the bottom side of box. Make sure you tape it shut. Place a liner inside the box (yellow). All waste must be inside the liner. Smaller autoclave bags may be placed inside the liner. When the liner is approximately 75% full, the box must be taped up

securely. Write your LAB NAME & the DATE on the side of the box or on the pail lid.

What to place in a Cardboard Box

Solid waste that cannot puncture a plastic bag (e.g., contaminated gloves, paper towels, unbreakable plastic bottles, plastic petri dishes, full sharps containers, etc.).

What NOT to place in a Cardboard Box

Waste that can puncture through a plastic bag (e.g., serological pipettes, glass pipette tips, glass tubes, slides, syringes/needles, etc.) USE A PAIL/TOTE.

IF boxes contain cytotoxic waste, place the cytotoxic sticker beside lab info.

Pails / Totes

Objects that can puncture a bag should go into the biohazard pails/totes.

When the pail/tote is 75 % full, firmly push down on the lid; it should seal on the pail/tote. If necessary, use a mallet to ensure it is tightly sealed all around.



TIP: If possible, use one pail for all serological pipettes to maximize space use in pail.

New supplies which include flattened cardboard boxes, pails and liners can be picked up in KHN-202-D.