Queen's University Biosafety Manual 2021

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INTRODUCTION to the Queen's Biosafety Program and Manual

The intent of the Biosafety Program at Queen's University, and the purpose of the Queen's Biosafety Manual, is to guide personnel in how to work safely with biological material that can be hazardous to people and/or animals. The goal is to prevent laboratory acquired infections (LAIs) in laboratory workers and to protect those outside the laboratory from the harmful effects that a release of the biological material might have.

The Manual will guide you in how to fulfill the requirements of legislation that regulates the use of biohazardous material, as set out in The Canadian Biosafety Standards (CBS) and other regulations and guidelines. The Canadian Biosafety Handbook (CBH) is another key document that provides further information and recommendations on how to comply with the CBS and work safety with biohazards. Some of the text in the Queen's Biosafety Manual is copied directly from the CBS or the CBH and presented here for your convenience. Nevertheless, it is recommended that you review the CBS and CBH to become familiar with it as a resource document after you read the Queen's Biosafety Manual. Read the Table of Contents in these documents so that you have an overview of the valuable information that they contain.

There is also legislation in Canada regarding work with Aquatic Animal Pathogens and Plant Pests, however that material is beyond the scope of this Biosafety Manual. The titles in the previous sentence provide links to the relevant standards. For further information on such material, contact the Biosafety Officer.

Queen's University has a Biosafety Program in place to:

- identify biohazards on campus
- reduce the risk of adverse effects from these materials for those working with them
- protect the community, the environment, and animal resources from harm by preventing the release of infectious material or toxins.
- promote and reinforce safe work practices, improve safety performance, and increase regulatory compliance through a combination of training, documentation, inspections, evaluation, review, and communications.

The Queen's University Biohazards Committee reviews applications for research or teaching with microorganisms that infect humans or both humans and animals (zoonotic pathogens), or materials that are likely to contain such agents. In addition, the University Biosafety Officer will assist labs in meeting the legislative requirements for microorganisms that are strict animal, aquatic animal, or plant pathogens.

Good microbiological laboratory practices are the foundation for all safe work practices involving biological material.

All personnel who handle potentially infectious material or toxins must be able to demonstrate proficiency in the Standard Operating Procedures and the lab-specific procedures in which they have been trained,

- And their training must be documented.

The training required for an individual to be authorized to work independently with biohazardous material is related to the containment level in which the individual is working and to the equipment and procedures that they will employ.

Risk Groups - How hazardous is it?

Laboratory-acquired infections can happen in laboratory personnel who work with human pathogens, zoonotic agents (animal pathogens that are also pathogenic to humans), cell cultures, or animal/human specimens. The outcome of accidental infections ranges from complete recovery, to permanent disability, to death depending on the organism, the infected individual and the medical treatment available.

The Risk Group, which is the degree of hazard associated with a biological material is assessed qualitatively by:

how likely it is to cause disease in people,
how serious that disease is likely to be,
whether or not effective treatment is available.
microorganisms are classified into one of four Risk Groups based on how much potential

for

containment facilities in Canada, in both academic and private institutions. Level 3 laboratories are extremely expensive to construct and maintain. Level 3 lab construction and its ongoing operation must be certified by the Public Health Agency of Canada. There is only one containment level 4 facility in Canada, at the National Microbiology Laboratories in Winnipeg.

Risk Group 3 *high individual risk, low public health risk*
A pathogen that:

poses a high risk to the health of individuals and/912 . p-4(r)9(ida)13(imc)-2(n)-4(d)13(/91ighl-3(iw ril)5

If a researcher disagrees with the PHAC risk group assignment, then they may perform a risk assessment citing evidence from the literature and submit it to PHAC for evaluation. PHAC provides standard forms for such a risk assessment.

- The form is available from the University Biosafety Officer.
- The appeal to PHAC would be made through the University Biosafety Officer.

If a commonly accepted risk group has not been assigned then when applying for a biohazard permit, the investigator must assign a risk group and justify the assignment to the Queen's University Biohazard Committee. A form to structure the risk assessment is available from the University Biosafety Officer.

Certain criteria will be more important, and the consideration of some factors may be eliminated in some cases.

For example, if there is strong evidence that an agent is not pathogenic then it really doesn't matter whether it is stable in the environment.

Operational containment refers to the practices used when working with biological materials to minimize exposure, including the type of equipment, where and how it is used, and the personal protective equipment to be worn. The operational procedures required for each level of containment are described in detail in Chapter 4 of the Canadian Biosafety Standards (CBS), and later in this manual.

Biosafety level 1 containment involves good general laboratory practices that include the use of appropriate personal protective equipment.

Biosafety level 2 containment involves enhanced practices to avoid splashes, the generation of aerosols and environmental contamination.

Biosafety level 2+ employs the level 3 operational practices that can be employed in a level 2 physical facility.

Aerosols are fine droplets of liquid that can carry infectious organisms and stay suspended in the air for various periods of time depending on the size of the droplet. Aerosols are produced

The program may include: a medical examination; serum screening; immunizations; testing and/or storage; and possibly other tests as determined by the risk assessment process.

In practice at Queen's med

Occupational Health services for personnel working in and around Queen's research laboratories is available through Walsh and Associates Occupational Health Services.

- Details and a map are located at https://www.safety.queensu.ca/biosafety/occupational-health-services-

How is the use of biohazards regulated at Queen's?

The Queen's University Biohazard Committee regulates the use of biohazards on campus to ensure both our safety and our compliance with federal and provincial regulations. It is a Principal's Committee that comprises at least six faculty members with differing expertise, one member of Queen's University staff who work

laboratory.

RESPONSIBILITIES

Human Pathogens and Toxins Act (HPTA) conditions that apply to every licence

1. Licence holders and persons conducting controlled activities authorized under the licence must not obstruct a Biosafety Officer (BSO) when the BSO is exercising their powers or carrying out their functions.
2. The BSO must be notified before arrangements are made to do the following:
    - import a human pathogen or toxin.
    - receive a human pathogen or toxin from another facility.
    - transfer a human pathogen or toxin to another facility.
3. Persons transferring human pathogens or toxins within Canada must take reasonable care to satisfy themselves that the intended recipient is licensed to work with the agent or otherwise exempted from the requirement to hold a licence.
4. 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. Intended recipients of human pathogens and toxins must make reasonable efforts to locate human pathogens and toxins that are not received within a reasonable time of when they are expected to be received, and the BSO must be notified of the situation without delay; and persons who discover that they are inadvertently in possession of a human pathogen or toxin that they are not authorized to possess and that is not a Schedule 5 agent must immediately notify the BSO, ensure that the human pathogen or toxin is handled and stored appropriately, and dispose of it or transfer it to a licence holder authorized to conduct controlled activities with that pathogen or toxin within 30 days.

Licence Holder

The Queen's V. P. Finance and Administration is named as the Licence Holder for Queen's University's Pathogens and Toxin's Licence from the Public Health Agency of Canada.

Requirements under the HPTA and CBS:

HPTA 18(4) & HPTA 18(7) - Licences are subject to conditions, and a licence holder and all persons conducting controlled activities under a licence must comply with those conditions.

- informing the PHAC of every situation where a shipment of a security sensitive biological agent (SSBA) has not been received within 24 hours of when it was expected;
- conducting periodic inspections and biosafety audits and reporting the findings to the licence holder and the animal pathogen import permit holder, as applicable;
- informing the licence holder and animal pathogen import permit holder, as applicable, in writing of any non-compliance by a person working with human or animal pathogens, toxins, or other regulated infectious material that is not being corrected by that person after they have been made aware of it;

assisting in the development and maintenance of the Biosafety Manual and SOPs;
assisting with internal investigations of incidents.

Department of Environmental Health and Safety

Create

- I have reviewed the current biohazard permit application and risk assessment for Dr. xxx, that has been approved by the Queen's Biohazard Committee with a version date of yyyy.
-

Ensure that all people working in the lab who may come into contact with hazardous chemicals attend WHMIS training offered by the Department of Environmental Health and Safety.

Adequately supervise personnel and correct work errors or deficiencies in conditions that could pose a risk to employees, students and/or the environment or result in noncompliance with the regulations and guidelines pertaining to your research.

Ensure that Emergency Procedur.584 Tm0.122 06C39ff0.286 0.49 RG[( )] TJETQq0.00000912 0 612 792 reV

Report any spills, release from containment, or stolen or missing Risk Group 2 material to the Biosafety Officer in the Department of Environmental Health and Safety within 24 hours of the incident.

Report to their supervisor and the BSO when a human pathogen or toxin that

BSCs have commonly been called laminar flow hoods/cabinets but this is not strictly correct because:

- Not all laminar flow cabinets are BSCs. Some are designed for product protection only and these are not BSCs.

Not all BSCs are the same, although all protect personnel and the environment from contamination.

- Class I BSCs do not provide an aseptic environment to protect the product from contamination (e.g. a cell culture would not be protected from contamination)
- Class II BSCs do provide an aseptic environment to protect the product
- Different types of Class II BSCs function differently, primarily in the proportion of air that is recirculated in the cabinet and in where the exhaust air goes (into the lab or directly exhausted from the building)

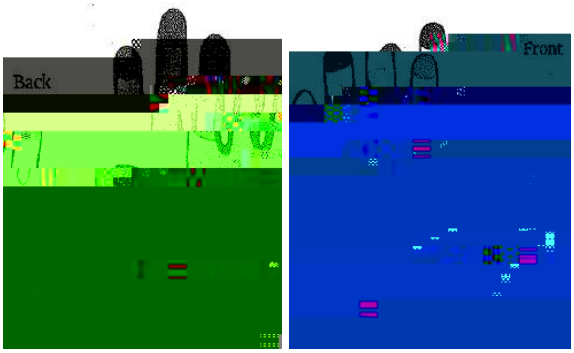
All BSCs and their use must be in compliance with the Queen's University Biological Safety Cabinet Standard Operating Procedure (SOP-Biosafety-

2. Personnel must receive training on the potential hazards associated with the work involved and the necessary precautions to prevent exposure to infectious agents and release of contained material; personnel must show evidence that they understood the training provided; training must be documented and signed by both the employee and supervisor; retraining programs should also be implemented.
3. Eating, drinking, smoking, storing of food, personal belongings, or utensils, applying cosmetics, and inserting or removing contact lenses are not permitted in any laboratory; the wearing of contact lenses is permitted only when other forms of corrective eyewear are not suitable; wearing jewelry is not recommended in the laboratory.
4.

d. metal mesh gloves can be worn underneath the glove.

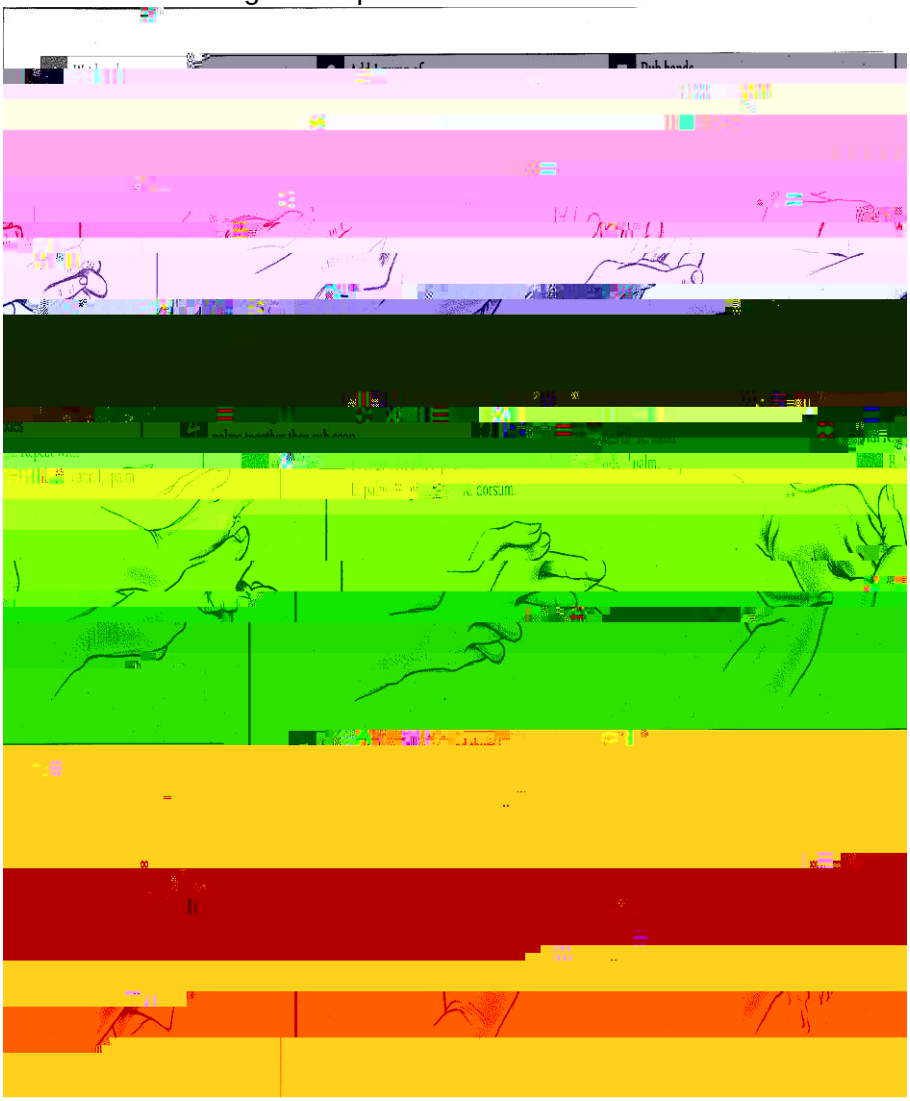
13. Protective laboratory clothing must not be worn in non-laboratory areas; laboratory clothing must not be stored in contact with street clothing. Personal belongings (e.g.

Hand washing



Areas commonly missed in hand washing are shown with darker shading. When washing, take care to clean these areas.

Good handwashing technique is illustrated below.



If taps are not hands-free, remember to turn them off with paper towel (post a reminder sign).

Containment Level 2 Operational Practices

Containment level 2 laboratories that are regulated under the HPTA must meet the operational practice requirements delineated in the matrix in the CBS Chapter 4.

At level 2, the major addition to the good microbiological practices described above, is that a Biological Safety Cabinet (BSC) must be used for procedures that may produce significant infectious aerosols, or that involve high concentrations or large volumes of biohazardous material.

- standard operating procedures must be written and approved by the Biohazard Committee
- standard operating procedures must be used for personnel training

Some practices that are more stringently controlled at level 2+ are:

1. All activities with infectious materials are conducted in a BSC; if this is not possible, other primary containment devices in combination with personal protective clothing and equ ts-612 792 reW*nBT/F9 11.04 Tf1 0 0 1 98.304 619.3 Tm0 g0 G[eiuu3(;s i)-4(s)1 w4(e mk(als wth)

Infectious RNA

Purified positive-sense viral RNA is capable of causing infection and subsequent generation of complete, functional viruses in host cells. Consequently, it is necessary to exercise additional care when manipulating genomic material of positive-sense RNA viruses. Examples of RNA viruses that produce infectious positive-sense RNA capable of causing disease in humans include poliovirus, hepatitis C virus, and SARS-CoV-2. Examples in animals include foot-and-mouth disease virus and classical swine fever virus. West Nile virus is an example of a zoonotic virus with a positive-sense single stranded RNA genome. A Local Risk Assessment should include the following considerations before handling infectious positive-sense viral RNA:

- the efficiency of infection with positive-sense viral RNA is lower when compared to infection with whole virus particles;
- RNA can withstand significantly higher temperatures than proteins, which means that infectious positive-sense RNA can be extracted from heat-inactivated viruses;
- the DNA copy of certain RNA viruses is also infectious (e.g., poliovirus, retroviruses);
- the infectivity of positive-sense viral RNA is unaffected by virus-specific antibodies;
- infectious single-stranded positive-sense viral RNA may have increased tropism (i.e., cell type and host range) when compared to whole virus particles.

Viral Vectors

Viral vectors are vehicles derived from viruses that are used to deliver genetic material into host cells for subsequent gene expression. These systems have been used for both research and gene therapy applications. Viral vector systems used for recombinant gene transfer are usually based on viruses present in the human population such as adenoviruses, herpesviruses and retroviruses. Genetic modifications are typically made to these vectors to improve gene delivery efficiency and to enhance the safety of the system.

Retroviral vector systems, including lentiviral vectors derived from HIV-1, are competent gene transfer vehicles which are widely used for their stable integration into the chromosome of non-dividing and dividing cells and for their long-term transgene expression.

The risks associated with viral vectors depend on the type of virus from which that the vector was derived, and how it has been engineered. Therefore, a risk assessment for each type of viral vector in use is required. In particular, viral vectors that can infect human cells need to be described in detail, including:

how the engineering and production methods attempt to ensure that replication competent virus is not produced (e.g. via recombination)
the transgene and any deleterious outcome that might be associated with its accidental expression in lab personnel
- Containment requirements may be increased depending on the nature of the transgene to be expressed
- all new transgene use must be reviewed by the Biohazard Committee as an amendment application to the biohazard permit

if used *in vivo*, how long is the viral vector expected to be shed and if a replication competent virus was transferred how would it be shed?

Lab specific operational protocols, training and testing must be developed for viral vector systems in consultation with the Biosafety Officer. To obtain an example SOP contact the Biosafety Officer.

Prions

When working with any neurological tissue, the possibility that prion proteins could be present should be considered and good laboratory practices followed.

If you intend to work with neurological material that is likely to contain prions, you are cautioned that work with prions will likely require facility renovations and will require an amendment to the University's Pathogen and Toxin licence, so allow plenty of lead time. Consult the CBS and the Biosafety Officer about additional mitigation measures that will be required and include these in the local risk assessment associated with your Biohazard Permit application.

Prions are small, proteinaceous infectious particles that are generally accepted to be the cause of a group of progressive neurodegenerative diseases in humans and animals known as Transmissible Spongiform Encephalopathies (TSEs). When an infectious prion enters a healthy host, it induces the normally folded prion protein to convert to the disease-associated, misfolded prion isoforms. The pathogenic isoform acts as a template that guides the misfolding of more prion proteins, which eventually leads to an accumulation of large amounts of the extremely stable, misfolded protein in infected tissue, causing tissue damage and cell death. Examples of TSE agents that infect animals include bovine spongiform encephalopathy (BSE), scrapie, and chronic wasting disease (CWD). Examples of TSE agents that infect humans include Creutzfeldt-Jakob disease (CJD), variant Creutzfeldt-Jakob disease (vCJD), Gerstmann–Straussler–Scheinker

The most likely route of transmission to personnel handling infectious prions is through accidental inoculation or ingestion of infected tissues. There is currently no intentional work with prions at Queen's, so the details of the containment requirements are not presented in this manual.

Human Blood, Tissues and Bodily Fluids

Human blood, tissues, and bodily fluids can contain pathogens that are a risk for infection by the mucosal or parenteral route. When obtaining human samples, it is important to ascertain the health status of the donor as an indication of the probability that these pathogens are

Culturing continuous cell lines without the routine use of antibiotics and fungicides is recommended. These agents can mask poor tissue culture technique and result in a higher probability that the culture will be contaminated with the more difficult to detect mycoplasma and non-lytic viral pathogens.
Cell lines are commonly misidentified so you might not be working with what you think you are and thus the hazard of the cell line could be higher than you think. Published reports have estimated that 20 to 30% of cell lines were misidentified when deposited with cell banks and that less than 50% of researchers regularly verify the identities of their cell lines using any of the standard techniques such as DNA fingerprinting.

For these reasons, it is prudent to treat all eukaryotic cultures as moderate risk agents (Level 2), even if they have been classified as Risk Group 1 (eg. by the supplier) because it has not been demonstrated that they contain a pathogen (often because no one looked). It is Queen's policy to use containment level 2 facilities and work practices when working with all mammalian cell lines. This is usually relatively easy to do since continuous cell culture is done under aseptic conditions in a biological safety cabinet (BSC).

Consider what you are doing with the cells after you harvest them, if you would like to work outside of the BSC. Remember that aerosols carrying pathogens will settle on surfaces that then become sources of infection when touched, especially by ungloved hands.8.584 Tm0.122 0.286 0.49 rgsi8q0.0000

embedded in the outer layer of the cell wall of certain Gram-negative bacteria, such as *Escherichia coli* and *Shigella dysenteriae*.

When compared to microbiological pathogens, it is fairly easy to control the spread of toxins. Toxins do not replicate, are not infectious, and are not transmitted from person to person. The most likely route of transmission to personnel handling toxins is through accidental inoculation or by the exposure of mucous membranes to aerosols.

Regulation of Toxin Use:
Only certain microbial toxins, whether naturally derived from a microorganism or synthetically produced, are regulated by the PHAC and the CFIA under the HPTA, HPTR, Health of Animals Act (HAA) and Health of Animals Regulations (HAR). If you are working with a toxin produced by a microorganism, you will require a Queen's Biohazard Permit.

Although not listed in the HPTA, other biological toxins, such as those produced by plants, coral, etc., may be just as hazardous or more so, but they are not regulated through the Biohazards Committee. Nevertheless, an SOP for their safe handling and disposal should be developed and Queen's EH&S will assist upon request.

A complete list of the toxins regulated by the PHAC under the HPTA is included in the Queen's Biohazard Permit application form.

The importation of toxins derived from non-indigenous animal pathogens is regulated solely by the CFIA (i.e. is not covered by the PHAC/CFIA licence and a separate import permit will be required).
Factors to consider when doing a risk assessment for a biological toxin are described in the CBH. Read the CBH section 4.3.1 before writing your risk assessment and SOP.

- An SOP for the handling of the toxin will be required as part of a Biohazard Permit application or amendment.
- If the toxin will be introduced into animals, a specific SOP for ACS staff is required.
- An SOP might already have been written by another investigator, so contact the Biosafety Officer for assistance.
- Toxins can be resistant to the chemical disinfectants commonly used to effectively decontaminate microorganisms. When working with toxins, a neutralizing chemical capable of denaturing and inactivating the toxin is needed for effective decontamination in the containment zone.

  Decontamination/inactivation of toxins by thermal or chemical means is described in the CBH section 15.11

Further information, including details about the inactivation of specific biological toxins, can be found in Appendix I of the U.S. Centers for Disease Control and NIH document, Biosafety in Microbiological and Biomedical Laboratories, 6th Edition, 2020 (BMBL)

Prescribed Toxins:

Certain regulated toxins are identified in the HPTR as "prescribed toxins" due to their dual-use potential when present in a quantity greater than the identified trigger quantity, as described by Section 10(2) of the HPTR (summarized in the table below). "Prescribed toxins" identified by the HPTR are also referred to as security sensitive biological agents (SSBAs), as they have additional biosecurity considerations.

- A toxin present in a part of a facility in a quantity greater than the trigger quantity is considered an SSBA and requires enhanced security measures (e.g., Human Pathogens and Toxins Act Security Clearances for all personnel who have access to the room where the toxin is used).
- A toxin in a total quantity at or below the trigger quantity is not considered an SSBA; however, it remains a toxin, and is subject to the CBS (i.e., the minimum containment level for handling a regulated toxin is CL2).
- SSBAs are discussed in further detail in CBH Section 4.3.3 and CBH Chapter 6.

Queen's PHAC/HPTA Licence is only for Prescribed (SSBA) toxins below the trigger quantity so the amount of toxin in areas accessible to one individual must be kept below the trigger quantity.

A quantitative real-time inventory of SSBA toxins must be maintained.

The BSO must be told how much of a previous order remains before an SSBA toxin is ordered because the BSO maintains a central inventory of all laboratories that use SSBA toxins to ensure that we remain below the trigger quantity.

Summary of Prescribed Toxins and Associated Trigger Quantities
Reproduced from the HPTR 10(2).

Toxin	Trigger Quantity
Alpha toxin	5 mg
Botulinum neurotoxin	0.5 mg
Cholera toxin	20 mg
Clostridium botulinum C2 and C3 toxins	5 mg
Clostridium perfringens Epsilon toxin	5 mg
Hemolysin	10 mg
Shiga-like toxin (verotoxin)	1 mg
Shigatoxin	1 mg
Staphylococcal enterotoxins, Type B	1 mg
Staphylococcal enterotoxins, types other than Type B	10 mg
Staphylococcus aureus Toxic shock syndrome toxin	5 mg

It is important to select the

Donning Order (Descending)	Single Gloves and Lab Coat	Double Gloves and Lab Coat	Doffing Order (Ascending)
	Lab coat (properly fastened) Gloves (fitted over cuffs of lab coat)	Inner gloves	

Foot

Closed-toe and closed-heel shoes must be worn in all laboratories at all times.

Compatibility of Glove materials with Common Chemical Disinfectants (CBH Table 9.1.1)

See the table below for susceptibility of different types of microorganisms/pathogens and disinfectant recommendations.

Susceptibility	Microorganism	Disinfectants reported to be effective
Extremely resistant	Prions (non-microorganism; pathogenic protein)	Unusually resistant to chemical disinfectants. High concentrations of sodium hypochlorite (NaOCl) or heated strong solutions of sodium hydroxide (NaOH).
Highly resistant	Protozoal oocysts	Ammonium hydroxide, halogens (high concentrations), halogenated phenols.
	Bacterial endospores	Some acids, aldehydes, halogens (high concentrations), peroxygen compounds.
Resistant	Mycobacteria	Alcohols, aldehydes, some alkalis, halogens, some peroxygen compounds, some phenols.
	Non-enveloped viruses	Aldehydes, halogens, peroxygen compounds.
Susceptible	Fungal spores	Some alcohols, aldehydes, biguanides, halogens, peroxygen compounds, some phenols.
	Gram-negative bacteria	Alcohols, aldehydes, alkalis, biguanides, halogens, peroxygen compounds, some phenols, some quaternary ammonium compounds (QACs).
	Gram-positive bacteria	
	Enveloped viruses	
Highly susceptible	Mycoplasma	Acids, alcohols, aldehydes, alkalis, biguanides, halogens, peroxygen compounds, phenols, QACs.

Ethanol

Note that 70% ethanol has limitations. It is commonly used as a surface disinfectant spray in laboratories. It is effective against vegetative bacteria and enveloped viruses. However, its efficacy is limited by the fact that it evaporates quickly from surfaces, reducing the contact time. Also note that8.76(o)13(rat)-5(e)13e that8.76(o)13(rQT/F1 9.48 Tf1 0 0 1 166.22 5144.26 324.53 0.72 0.72 ref

Less corrosive alternatives are commercially available for use on metal surfaces.
The concentration of NaClO degrades quite rapidly in diluted bleach, so a 1:10 dilution of bleach should be made up fresh (and not kept for more than a week).
Cultures should be decontaminated by the addition of fresh stock commercial bleach to a final 1:10 dilution (or cultures should be autoclaved, not both – do not autoclave bleach).

Organic Load

Organic matter (e.g., serum, cells, tissue, blood, bedding, feces) protects microorganisms and toxins from contact with disinfectants and can neutralize many germicides (e.g., bleach). Pre-cleaning with a detergent to remove bedding, litter, and/or feed prior to disinfection reduces organic load and achieves proper disinfection.

Pre-cleaning should be carried out in a manner to avoid exposure and all cleaning materials must be decontaminated prior to disposal (R4.8.8, R4.8.9).
Pre-cleaning prior to disinfection may not always be appropriate and, in these cases, disinfectants that remain active in the presence of considerable amounts of organic material should be selected (e.g., phenolic disinfectants).
It may be appropriate to saturate the contaminated material with a disinfectant, allowing it to remain wet for a long contact time (e.g., 30 minutes), then dispose of gross contamination and thoroughly clean surfaces before reapplying the disinfectant.

Additional Information

For more information about considerations in selecting and using disinfectants, refer to Chapter 15 of the CBH. It contains tables with information about the characteristics of different types of disinfectants, their activity against different types of microorganisms, and the disadvantages of different disinfectants.

A table of common disinfectants and their typical effective concentrations, is in Appendix B, Table 1 in the BMBL 6th Edition.

Appendix I of the BMBL, tables 1 and 2, have information about the physical and chemical inactivation of some biological toxins.

Irradiation

Ultraviolet irradiation (UV) should not be relied upon as the sole method of decontamination for materials to be removed from containment equipment (biological safety cabinets) or

facilities. UV has limited penetrating power and is primarily effective against unprotected microbes on exposed surfaces or in the air.

Biohazard Waste Treatment

Many types of biohazardous material can simply be decontaminated or disinfected and subsequently handled as normal waste for disposal through the municipal waste stream or down the sewer.

For biohazard material from laboratories:

> Level 1 must be disinfected prior to disposal.
> Level 2 must be disinfected before removal from lab or double bagged for transport to an autoclave or incinerator.

However, some material must be sent for incineration through the Department of Environmental Health and Safety as described below.

Procedures for Disposing of Specific Waste Types

For more detailed information on handling, treating, and disposing of different types of waste, including chemical and radioactive waste, see the Standard Operating Procedure for Hazardous Waste Disposal, SOP-CHEM-01,.

For disposal forms refer to the Waste Disposal page on the Environmental Health and Safety Website.

Sharps and Glassware

Anyone disposing of sharps and/or glassware, whether contaminated or not, must comply with the Queen's University Sharps Disposal Standard Operating Procedure (SOP-Safety-12).

Biohazardous sharps must be packaged in an approved sharps container or other approved container as described in SOP-Safety-12 and sent for incineration.

Solid Biohazardous Waste

Solid biohazardous waste (e.g. contaminated plastic flasks, tubes, etc.) should either be surface decontaminated by chemical means

contaminated plastic bottles may be decontaminated by soaking in bleach prior to disposal as non-biohazardous waste).

Solid tissues must be incinerated. Human cadaver material has special provisions to be buried with the cadaver.

Liquid Biohazardous Waste

Most aqueous liquid biohazardous waste generated from biological research activities (after disinfection) are suitable for sewer disposal.

All wastes to be disposed of by sewer must be decontaminated and registered and approved by the Department of Environmental Health and Safety to ensure compliance with the City of Kingston sewer use bylaw.

If you have any questions about what liquids are suitable for sewer disposal after disinfection contact the Department of Environmental Health and Safety at ext. 32999.

Most liquid biohazardous waste can be decontaminated in one of two ways:

1. In a 10% dilution (final) of household bleach for 30 minutes, after which waste can be disposed of down the drain.

OR

2. By autoclaving, BUT such waste must not contain chemical hazards incompatible with autoclaving – do not autoclave bleach.

    a. and it must be covered securely during transport,

    b. labelled clearly with a contact name and identification of contents.

    c. When liquid waste is transported in the hallways, it should be covered and placed in an unbreakable container on a cart with sides to prevent contamination of hallways.

    d. Autoclave procedures must comply with SOP-Biosafety-09.

EMERGENCY PROCEDURES

Emergency response procedures must be in place for any incidents that might occur in the laboratory. These are more specific to the laboratory than the general Queen's book, "Your Guide to Responding to Emergencies" which should also be present in the laboratory.

A template word document for Emergency Laboratory Proced/4.3e0 0 1 0e0 0 1 0e0 0 1 0e0 0 1 0e0 0 1 0e09 RG[(

Biosecurity breeches, e.g. the intentional misuse or theft of biohazardous materials or toxins, can lead to serious undesirable consequences and a plan is in place at Queen's to prevent such

Loss or theft of biohazardous material (or other material from laboratories) is to be reported immediately to Principal Investigator and the Biosafety Officer.

DUAL USE

PHAC requires that an assessment of Dual-use potential be part of a biohazard risk assessment. Consider the qualities of a pathogen or toxin that allow it to be either used for legitimate scientific applications (e.g., commercial, medical, or research purposes), or intentionally misused as a biological weapon to cause disease (e.g., bioterrorism).

The following chart depicts the process to determine Dual-use potential. At Queen's the Biohazard Committee makes an assessment of dual use potential during its review of a biohazard permit application. However, it is also the responsibility of the researcher to consider the potential dual use of their research methods or results. If the dual use potential is significant then risk mitigation measures must be considered.

An import permit will

Anyone transporting dangerous goods, as they are defined under the Transportation of Dangerous Goods Regulation (TDG) , within Canada (even within Kingston if leaving the Queen's/KGH campus e.g. to Hotel Dieu hospital), or internationally must be in compliance with the Queen's University Transportation of Dangerous Goods Standard Operating Procedure, SOP-CHEM-02 .

Individuals packaging hazardous materials for shipment or receiving hazardous materials must be trained and certified. TDG training and certification is available on-line through the Department of Environmental Health and Safety (in the training section of the safety website).

TDG regulations do not use the Risk Group classification of an infectious substance. TDG uses a two-category system in which Category A is for high risk substances and Category B is for other substances. Unlike many chemicals, there is no small volume exemption for biological hazards. There are "exemptions" i.e. changes in packaging and labeling requirements, for some biological/clinical samples being sent for testing. Check the definition carefully to determine whether your sample qualifies. Information can be found in the Biohazard Module of the TDG training slides.

BIOHAZARD ADMINISTRATION at QUEENS - LABORATORY PERMITS, COMMISSIONING and DECOMMISSIONING

Biohazard Permits

A Queen's University Biohazard Permit is required for all research and teaching activities which involve the use, manipulation and storage of biohazardous material, even risk group 1 material (including, but not limited to, viruses, bacteria, fungi, parasites, recombinant DNA, biological toxins, prions and other micro-organisms/genetic systems, and human and animal tissues, cells, blood and body fluids). When such activities are:

1. supervised or conducted by employees or members of the University, or
2. conducted on University premises, or in a building or location administered by or under the control of the University, or
3. supported by funds provided by or administered through the University.

The types of material requiring a Biohazard permit are described in

The content of the biohazard permit application is the responsibility of the Principal Investigator and must be submitted under their electronic signature. However, they may delegate the work of preparing the form and attached documents (risk assessment(s), biohazard inventory, training statement, SOPs) to a member or members of their team.

Awarding of a Queen's Biohazard Permit is a two-stage process:

1. Review and approval of the application, by the Biohazards Committee, of the research or teaching activity as described in a Biohazard Permit Application. The Committee meets monthly, 9 times per year. Submission and meeting dates are posted at https://www.safety.queensu.ca/biosafety/biohazard-committee
2.

All laboratory personnel will be required to read the approved biohazard permit documents and submit an attestation form indicating that they understand the risks and will follow the risk mitigation measures described.

Biohazard Renewal

Biohazard permits must be renewed annually by the anniversary date of approval. Renewal event forms must be submitted by the Principal Investigator for review and approval by the Biosafety Officer.

Instructions will be provided when the Principal Investigator and Secondary Biohazard Contact are reminded that the renewal date is approaching.

The renewal form also serves as a reminder of annual retraining requirements.

Biohazard Amendment

Changes to an approved biohazard permit may be made with a biohazard amendment event form.

If the amendment is for a similar type of material and procedure to that already approved, with no increase in risk group, then the Biosafety Officer may review and approve it.

If the amendment application requests a change in the type or risk group of biohazardous material, or if it requests in a change in procedures that alter the risk (e.g. beginning *in vivo* work with biohazardous material that was previously only used *in vitro*) then it will be reviewed by the Biohazard Committee, otherwise the Biosafety Officer may review it.

Laboratory Commissioning and Ongoing Monitoring

Before a laboratory is used for work with biohazardous materials it must be inspected by members of the University Biohazards Committee. This commissioning inspection will ensure that the physical requirements and operational practices are in place for the level of containment approved on the Biohazard Permit.

Amendments to the biohazard permit that result in changes in the containment requirements will necessitate a re-inspection.

The continued use of approved containment practices and the maintenance of the facility are monitored during routine inspections by members of the Biohazard Committee. The frequency

APPENDIX I

Operational Requirements for Specific Equipment and Supplies

Ampoules of lyophilized cultures

Avoid snapping the neck of an ampoule which can lead to the sudden inrush of air and dispersal of contents. Instead, make a file mark near the middle of the cotton plug and apply a hot glass rod to crack the glass; allow time for air to seep into the ampoule and gently remove the top and plug; add liquid for resuspension slowly to avoid frothing.

Centrifuges

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

Materials for centrifugation must be placed in screw-capped tubes (or sealed tubes if appropriate for ultracentrifugation), which must not be overfilled or leak. Disinfect and clean up any leaks immediately.

To avoid the creation of aerosols after centrifugation, decant supernatants carefully. Avoid vigorous shaking and blowing bubbles with your pipette when resuspending packed cells/bacteria. Work in a biological safety cabinet to contain aerosols generated when resuspending risk group 2 material.

For low speed centrifugation sealed centrifuge buckets (safety cups) are recommended for level 2 material and for all cell lines. Safety cups are strongly recommended for known infectious level 2 material (eg. virus, viral vectors, and bacteria). For level 2+ safety cups are required and must be opened only in a biological safety cabinet.

Microcentrifuges should not be placed in the BSC for operation, as air currents during operation compromises the integrity of the BSC. Safety cups for microcentrifuges are now available.

In the event of a centrifuge equipment malfunction, follow instructions outlined in the section on Equipment Associated Emergencies in the Emergency Response Procedures posted in your lab.

- If storing highly pathogenic material in liquid nitrogen use commercially available tubing designed to seal the vials.
- It is also wise, if feasible, to store known infectious pathogenic material in a different tank from material that will be treated only as a level 1 biohazard.

Note that DMSO, commonly used when freezing cell lines, can solubilize organic material and carry it through rubber (latex) and the skin, into the circulation. Take care to avoid contact with DMSO and check the permeation time of the disposable glove material that you use.

Flow Cytometers and Cell Sorters

Fume Hoods

Fume hoods are for containing and exhausting potentially harmful chemical gasses, vapours, mists, aerosols and particulates generated during the manipulation of chemical substances. Fume hoods are NOT to be used for biohazard containment. A biological safety cabinet must be used to contain biohazards.

> Some work may require a biological safety cabinet with fume hood abilities (Type II B2 cabinaet; e.g. using biohazards with chemicals that produce toxic fumes or volatile compounds labell

Gloves used to handle contaminated specimens should be removed before using the microscope.

Mixing Apparatus

Homogenizers, shakers, blenders, grinders and sonicators can release significant amounts of aerosols during their operation.

When using any mixing equipment, remember to:

Use a biological safety cabinet if possible
Check the condition of gaskets, caps and bottles before using.
Disinfect all exposed surfaces after use.
Be aware of the hazards associated with moving parts of equipment; wear protective eyewear and hearing protection if appropriate

Blenders:

- Use laboratory blender with a tight-fitting gasket lid and leak-proof bearings (domestic kitchen blenders leak and release aerosols)
- Wait as long as possible before opening the lid after mixing (at least 5 minutes)
- Consider covering the tops of blenders with a disinfectant-soaked paper towel during operation.

Sonicators:

- Immerse sonicator tip into solution to a depth sufficient to avoid creation of aerosols.
- After sonication in a bath, open tubes in a biological safety cabinet, or if that is not possible then allow aerosols to settle for 5 to 10 minutes after use before opening containers

Tissue grinders:

- Wrap glass grinders in a wad of absorbent paper and wear gloves. Polytetrafluoroethylene (PTFE, "Teflon") grinders are safer, as they will not br") tu:

Keep pipettes upright while in use and between steps of a procedure to prevent contamination of the mechanical aid. Consider the use of easier-to-handle shorter pipettes when working inside a biological safety cabinet.
Avoid loss of m

APPENDIX II

CBS Requirements for Containment Level 2 and

The tables below present the containment level 2 requirements as stated in the Canadian Biosafety Standards (CBS) 2nd Edition, 2015. These requirements form the basis of the Queen's Biosafety Program, and the Level 2 inspection checklists from the Biohazard Committee.

Information is presented in these tables for all PHAC (HPTA) or CFIA designated:

CL2 laboratories - for work *in vitro*

CL2-SA - rooms with small animals in primary containment caging e.g. Micro isolator lids, or HEPA filtered vented racks under negative pressure

CL2-Ag - large animal rooms where the room provides the primary means of containment i.e. animals are not in primary containment caging

It is recommended that when first reading an item in the Standards, or if you have any doubt about its intention, you also read the Explanatory Notes of each item in the Appendix of the CBS. If still in doubt, consult the University Biosafety Officer and the Canadian Biosafety Handbook.

Table 1. CL2 FACILITY and OPERATIONAL PRACTICE REQUIREMENTS

CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
3.1.1 - Containment zones to be separated from public and **administrative areas** by a door.
3.1.2 - Dedicated paper/computer work stations within the containment zone to be segregated from **laboratory** work stations, **animal rooms**, **animal cubicles**, and **post mortem rooms** (PM rooms).
3.2.1 - Openable windows positioned on the containment barrier to include effective pest control and security. [***Only applies to CL2 laboratory work areas***.]

3.3.1 -

3.6.6 - Emergency eyewash and shower equipment to be provided in accordance with containment zone activities.

[***Not required for CL4 zones where positive-pressure suits are worn.***]

3.7.1 - Certified BSCs and other primary containment devices to be provided, based on work activities.

3.7.3 - Class II B2 BSCs, where present, 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 a failure of the heating, ventilation, and air conditioning (HVAC) system or the BSC exhaust fan; where

- conducting periodic inspections and biosafety audits and reporting the findings to the licence holder and the animal pathogen import permit holder, as applicable;
- informing the licence holder and animal pathogen import permit holder, as applicable, in writing of any non-compliance by a person working with human or animal pathogens, toxins, or other regulated **infectious material** that is not being corrected by that person after they have been made aware of it;

assisting in the development and maintenance of the Biosafety Manual and SOPs;

assisting with internal investigations of **incidents**.

4.1.3 - Contact information provided to the PHAC and the CFIA, as applicable, to be kept up to date.

4.1.4 - **Program intent** to be documented and kept up to date.

4.1.6 - An **overarching risk assessment** to be conducted and documented to identify the hazards and appropriate mitigation strategies for the proposed activities involving infectious material or toxins.

4.1.7 - A **biosecurity risk assessment** to be conducted and documented.

4.1.8 - A **local risk assessment**

a brief description of the physical design and operation of the containment zone and systems;

a description of the:

- biosafety program;
- biosecurity plan;
- **medical surveillance program**;
- training program;
- **emergency response plan** (ERP) and incident reporting procedures;
- housekeeping program;
- facility and equipment maintenance program for components of the containment zone, including integrity testing of **primary containment devices**; and
-

4.1.12 -

4.4.2 - Face protection to be used where there is a risk of exposure to splashes or flying objects.

4.4.4 - Gloves to be worn when handling **infectious material**, toxins, or infected animals.

4.5.1 - Containment zone, **animal room**, **animal cubicle**, and **post mortem room** (PM room) doors to be kept closed.

4.5.2 - Access to containment zone to be limited to **authorized personnel** and authorized visitors.

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.

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 -

placed in closed, labelled, and leakproof containers that have been surface decontaminated prior to removal from the containment zone, animal rooms, animal cubicles, or PM rooms, as described in SOPs for the safe and secure **movement** or **transportation** to a designated decontamination area or storage outside of the containment zone.

4.8.10 - **Decontamination technologies** and processes to be validated prior to initial use and when significant changes to the processes are implemented or new pathogens are introduced.

4.8.11 - Decontamination technologies and processes to be routinely verified, as described in SOPs. Frequency of **verification** to be determined by a **local risk assessment** (LRA).

4.8.13 - Contaminated bedding to be:

removed at a **ventilated cage changing station** or within a certified **biological safety cabinet** (BSC) prior to decontamination; or
decontaminated within containment cages.

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;
animal escape (if applicable);

failure of primary containment devices;

puff-back

30 days of the submission of an exposure notification report involving a human pathogen or toxin other than an SSBA.

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). Inventory to include pathogens, toxins, and other regulated infectious material stored outside of the **containment zone**.

5.1.1 - Performance and verification tests described in 5.1.2-5.1.7 to be conducted and documented at minimum annually, or more frequently as indicated by:

Table 2. CL2-SA FACILITY and OPERATIONAL PRACTICE REQUIREMENTS in addition to those for CL2

CL2-SA - rooms with small animals in primary containment caging e.g. Micro isolator lids, or HEPA filtered vented racks under negative pressure

CL2 Small Animal *in vivo* Requirements in addition to CL2 *in vitro* (CBS 2nd Edition 2015)
3.2.3 - Windows on the containment barrier to be non-opening and sealed.
3.2.4 - Window glazing material to provide the appropriate level of security as determined by a **biosecurity risk assessment**.

3.2.5 - Windows on the containment barrier to be positioned to prevent public viewing into **animal rooms**, **animal cubicles**, and **post mortem rooms** (PM rooms).

3.4.2 - Surfaces to be continuous with adjacent and overlapping materials.

3.4.4 -

a request of the Public Health Agency of Canada (PHAC) or the Canadian Food Inspection Agency (CFIA).

5.1.2 - Visual inspections of the **containment zone** to be conducted in order to identify faults and/or deterioration; when found, corrective actions to be taken.

5.1.3 - Visual inspections of small in-line filters to be conducted and filters to be replaced in accordance with maintenance schedules or as necessary to maintain function.

5.1.4 - **Decontamination technology** and processes to be validated through the use of **representative loads** in conjunction with application-specific biological indicators, chemical integrators, and/or parametric monitoring devices consistent with the technology/method.

5.1.5 - Class II **biological safety cabinets** (BSCs) to be certified in accordance with NSF/ANSI 49, where possible.

5.1.6 - Verification of the following manufacturer's specifications to be demonstrated, where the design of a BSC or custom ventilated enclosure does not permit certification in accordance with NSF/ANSI 49:

integrity of the **high efficiency particulate air (HEPA) filters** to be tested in accordance with the HEPA filter test method IEST-RP-CC034.3 or equivalent;

maintenance of a minimum average inflow velocity of 0.38 m/s (75 ft/min) through the front opening during normal operation to be verified;

airflow pattern inside the cabinet and at access opening to ensure no back streaming of air to be demonstrated;

alarms to be demonstrated to function as intended.

5.1.7 - Integrity of **primary containment devices** other than BSCs (e.g., **process equipment**, **closed systems**, **primary containment caging**) to be tested in accordance with testing procedures and acceptance criteria appropriate for the equipment and design.

5.2.1 - Performance and verification tests described in 5.2.3-5.2.11 to be conducted and documented at minimum every two years, or more frequently, where specified or as indicated by:

a change, repair, or modification to the **containment system**;
a condition of **licence**;
a condition of **animal pathogen import permit**; or
a request of the Public Health Agency of Canada (PHAC) or the Canadian Food Inspection Agency (CFIA).

[***Does not apply where SSBAs are present.***]

5.2.8 - **High efficiency particulate air (HEPA) filters** to be tested *in situ* by particle challenge testing using the scanning method in accordance with IEST-RP-CC034.3 or IEST-RP-CC006.3. When scan testing is not possible, probe testing is acceptable.

CL2 Ag *in vivo* for large animals in addition to CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
3.5.1 - IDA to be provided where: **pathogens** that are primarily infectious through inhalation are handled; or infectious aerosols or aerosolized toxins may be generated by procedures in use. [***Not required for SA zones.***]
3.5.3 - Monitoring device(s) that visually demonstrate IDA to be provided for the containment zone.
3.5.10 - Where IDA is provided, exhaust air to be: passed through a filter that prevents the **release** of **infectious material** or toxins; or 100% exhausted directly to the outdoors. [***Not required for SA zones.***]
3.6.1 - Exposed conduits, piping, and other services to be mounted to allow for **decontamination** of all surfaces.
3.6.5 - Sinks provided for handwashing to be equipped with "hands-

CL2 Ag *in vivo* for large animals in addition to CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
4.3.5 - Personnel working with animals to be trained in restraint and handling techniques.
4.4.3 - Personnel working in **animal rooms**, animal cubicles, or PM rooms to wear dedicated protective footwear and/or additional protective footwear, as determined by an LRA.
4.4.5 - Full body coverage dedicated protective clothing to be worn inside the **containment barrier** where human or **zoonotic pathogens** are handled.
4.4.9 - Respirators to be worn where there is a risk of exposure to infectious **aerosols** that can be transmitted through the inhalation route or to aerosolized toxins, as determined by an LRA.
4.5.9 - Personnel to verify correct reading of monitoring device(s) that visually demonstrate IDA, prior to entry into area where IDA is provided.
4.5.12 - Personal belongings not required for work to be left outside the containment zone or in change areas outside the containment barrier.
4.5.17 - Personnel to doff dedicated PPE (or additional layer of PPE, when worn) when exiting the containment barrier of: animal cubicles/PM rooms, except when exiting to the dirty corridor; ***or*** the containment zone. [***Not required for CL4 zones where positive-pressure suits are worn.***]
4.6.13 - **Verification** of **inward directional airflow** (IDA) to be performed routinely, as described in SOPs. [***Not required for CL2 SA zones.***]

CL2 Ag *in vivo* for large animals in addition to CL2 *in vitro*

CL2 Ag *in vivo* for large animals in addition to CL2 *in vitro*

Human pathogens and toxins: Laboratory Biosafety Guidelines, 3rd Edition, 2014 (PHAC)
Terrestrial animal pathogens: Containment Standards for Veterinary Facilities, 1st Edition , 1996 (CFIA)
Prions: Containment Standards for Laboratories, Animal Facilities and Post Mortem Rooms handling Prion Disease Agents, 1st Edition, 2005 (CFIA)

Canadian Food Inspection Agency (CFIA) Health of Animals Act

- The Act and its Regulations give the CFIA the legislative authority to control the use of pathogens which may cause disease in animals.
- The CFIA delegated control of indigenous animal pathogens in their pure form to PHAC and these are covered on Queen's PHAC Pathogen and Toxin Licence.
- CFIA has retained control of Foreign Animal Disease Pathogens (and parts thereof) and animal products or by-products at risk of containing foreign animal diseases.
- CL2 checklists, compliance letters and the issuance of import permits is how that this legislation is applied.

Canadian Environmental Protection Act

- Regulates activities that affect the environment. For example, air emissions, management of hazardous and non-hazardous solid wastes, use of ozone-depleting substadepleting substabs f1 0 0 1 108.14 305.69 (eig)13 TJETQq0.000011802 0 792 612 reW*

- Requires that employers provide safe working conditions, and that all employees be informed about potential hazards they may face on the job and how they can be minimized.
- The employee has the right to refuse unsafe work if faced with an unsafe condition.

Workplace Hazardous Materials Information System (WHMIS)

- Requires that all hazardous substances be labelled in a specified manner.
-