Queen's University Biosafety Manual 2021

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Biosafety Manual 2021

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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.

What is a biohazard?

A biohazard is any biological material that could be a health hazard to humans, animals, aquatic animal species, or plants

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

Work with low risk animal species (e.g. specific pathogen free rodents) and their tissues is classified as RG1 but is overseen by the University Animal Care Committee and does not require a Biohazard Permit.

Risk Group 2

Risk Group 2 *moderate individual risk and low community risk*.
A pathogen that:

- poses a moderate risk to the health of individuals and/or animals and a low risk to public health, livestock or poultry
- can cause serious disease in a human or animal but is unlikely to do so
- the risk of spread of diseases caused by these pathogens is low
- Effective treatment and preventative measures are available
- Examples include:
  - Bacteria such as *Salmonella typhi, Staphylococcus aureus, Bordatella pertussis*
  - Viruses such as *Herpes simplex virus, Adenovirus, Epstein-Barr virus*
  - Fungi such as *Aspergillus, Candida albicans*
  - Parasitic agents such as *Leishmania species, Giardia lamblia*
  - Prions

For Risk Group 2 organisms the primary exposure hazards are through the ingestion, inoculation and mucous membrane routes. Therefore, the risk mitigation measures in the lab are designed to interrupt these potential routes of infection.

Biological Toxins are classified as Risk Group 2 because they can be effectively handled in a containment level 2 laboratory.

Risk Group 2+

Risk Group 2+ This is the term commonly used to refer to microorganisms that require a level 2 physical containment facility with level 3 operational practices. These conditions are imposed for microorganisms:

- for which the hazard is higher than for Risk Group 2 organisms, but which usually are not transmitted by the airborne route or are lab-adapted so likely to have reduced virulence

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 u

virus has very low viability on environmental surfaces,

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 when force is applied to a liquid (e.g., pipetting, blending, sonicating, vortexing, centrifugation).

Fine aerosols are an inhalation hazard for agents that are infective via this route.

Local Risk Assessment

if PHAC has not assigned a risk group then see if another reliable source has done one (ATCC designation as BSL1 is not enough because their determination is for shipment)

- if you are uncertain of the risk group, or if you disagree with the ePATHogen database risk group determination then consult the Biosafety Officer (appeals to PHAC for a change can be made if there is evidence)
- If risk assessments of the organism are not available from a reputable source, then these must be performed by the researcher. In that case, contact the

medical surveillance (e.g. immunization, titre checks, first aid and

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 Occupational Health Services Walsh and Associates(4).pdf (queensu.ca).
- Charges will be billed to departments through the Department of Environmental Health and Safety and payment is the responsibility of the supervisor.

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 works with biohazards, one person not associated with biohazards research, preferably from outside the University and five Ex-Officio staff members.

A list of current Committee members and more detailed description of the composition and functions of the Biohazard Committee can be found in the Terms of Reference final version Dec 2006.pdf (queensu.ca) on the Department of Environmental Health and Safety website, under the Biosafety dropdown menu (Biohazard Safety | Office of Risk and Safety Services (queensu.ca)).

The University Biosafety Officer is one of the Ex-Officio staff members of the Biohazard Committee and is the Manager of Biohazard, Radiation and Chemical Safety in the Department of Environmental Health and Safety.

The Safety Technician (Biohazard and Chemical) is responsible for assisting with the day-to-day operations of the biohazard safety program and the chemical safety program in laboratories that also have biohazards. They perform biohazard laboratory inspections, maintain records related to the biosafety program, and assist with the development of SOPs.

Those wishing to use any biological material must contact the University Biosafety Officer in the Department of Environmental Health and Safety (ext. 77077) to determine whether they need to obtain a permit from the Queen's Biohazard Committee prior to importing or commencing work with the material. See Queen's Risk Group - Queens Biosafety SOP-05 Risk Group Definitions.pdf (queensu.ca) for a description of what biological material is regulated through the Biohazard Committee.

Approval from the Biohazard Committee is required before work begins and before grant funds will be released by the Office of Research Services.

There are two aspects to biohazard approval:

1. The first is approval of an application to the Biohazard Committee that includes a biohazard permit application form, a list of biohazardous materials (qualitative inventory), and a risk assessment and risk mitigation statement. Forms and submission is through the TRAQ/Romeo system on your Researcher Portal.
2. The second aspect of biohazard approval is an inspection, by two members of the Biohazard Committee, of the physical set up and operational practices in the

The Canadian

HPTA 18(6) - Licence holders are required to communicate all licence conditions to everyone conducting activities under that licence.

Notification must be provided to the PHAC without delay in the following circumstances:

- when a licence holder has reason to believe that a human pathogen or toxin has been released inadvertently from a facility.
- when a human pathogen or toxin that a person is not authorized to possess is inadvertently produced or otherwise comes into their possession.
- when an incident involving a human pathogen or toxin has caused, or may have caused, disease in an individual.
- when there is reason to believe that a human pathogen or toxin has been stolen or is

Biosafety Officer

At Queen's the Biosafety Officer is assisted in their role by the Safety Technician (Bio

- 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.

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- 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.
- I am familiar with the work described and the facilities and practices required for biohazard containment in this research.
- I approve of this work taking place in my department, using the precautions described.
- I will ensure that appropriate facilities are available for this work.
- If I become aware of a failure in the facility or a failure in the biohazard containment safety procedures or equipment, or a noncompliance safety issue associated with the work, I will ensure that they are reported to the Biosafety Office and assist in their correction.

Principal Investigators

Read and be familiar with the contents of this Biosafety Manual and ensure that it is followed in their laboratories.
Take the centralized training required of their personnel so that they know the material, and also so that by knowing what is covered in that training they can to better design the lab specific training.
Read and ensure compliance with any relevant Policies and Standard Operating Procedures released by the Department of Environmental Health and Safety.
Identify known and potential biological hazards within their laboratory.

- Obtain and maintain a valid Biohazard Permit for these hazards (annual renewals required, with full reapplication required every 4 years)
- maintain a list of the biohazardous materi35 O91.04 Tf1 owsqtri35Cabborators

Take Biosafety training, as described on the Environmental Health and Safety website and ensure that they:

- read the lab biohazard permit and associated documents (risk assessment, inventory , training statement, and any lab specific SOPs),
- read the Queen's Biosafety Manual and SOPs,
- Take quizzes as required (as described in lab training statement).
- Ensure that their training on Emergency Response Procedures is refreshed annually, and that they know where this document is posted in their laboratory.
- Comply with all University and Laboratory-specific biosafety SOPs and procedures.
- Take WHMIS training.
-

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 Procedures are:

- customized for the laboratory
- reviewed and updated annually (date and print a new version after review)
- posted in the laboratory and that all personnel know the location of this procedure
- training on these procedures is refreshed annually and documented

Report all exposure incidents or serious near misses involving biological or other hazards in writing to the Department of Environmental Health and Safety within 24 hours of the incident even if medical attention is not required.
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 when a human pathogen or toxin that your lab is not authorized to possess is inadvertently produced or otherwise comes into your possession.

All Other University Personnel and Students working as Biohazard Team Members

Laboratory workers must be trained about the hazards in the laboratory as described on the EHS website as biosafety training, understand and use the risk mitigation measures in place, and have their training documented and refreshed as required. Laboratory workers should be protected by appropriate immunization where possible, and antibody titres should be checked to determine whether there has been an adequate response to immunization. This requirement will be described in the biohazard permit. Ensure that if their health status changes they promptly review the implications for the hazards with which they work and have the risk mitigation measures re-evaluated through their supervisor, the BSO, and their personal physician or Queen's Occupational Health Care provider.

- This should not require you to reveal personal medical information to non-medical personnel.

Promptly inform their supervisor of any exposure to hazardous materials or other accidents or significant "near misses" in the laboratory and assist them in filling out forms to report these to the Department of Environmental Health and Safety.

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 their lab is not authorized to possess is inadvertently produced or otherwise comes into their possession.

TRAINING REQUIREMENTS

The inherent risks of working with hazardous agents can be reduced by:

knowledge of the hazardous agent and the procedure-associated hazards

Training on risk mitigation

good work habits and use of all the risk mitigation measures indicated for a particular activity

personal attributes of caution, attentiveness, and concern for the health of themselves and coworkers

CONTAINMENT AND ENGINEERING CONTROLS

It is an important basic tenant of safety that engineering controls (lab design and containment equipment), including proper laboratory ventilation, should be used first to limit exposure and only then supplemented with Personal Protective Equipment (PPE) and operational controls.

Lab Design

At Queen's University, laboratories must meet the design requirements of the Canadian Biosafety Standards (CBS), 3rd Edition, 2022; and, where applicable, other requirements that might be imposed by the Public Health Agency of Canada (PHAC), the Canadian Food Inspection Agency (CFIA) or other regulatory authority.

Unlike some legislation, the Human Pathogens and Toxins Act and Regulations do not permit "grandfathering" of the physical state of existing facilities. The CBS requirements are considered the minimum that must be met, and adopting the recommendations of the guidelines in the Canadian Biosafety Handbook (CBH) is encouraged where feasible. The

Lab specific training that is documented with a quiz is required for entry into Level 2+ labs.

- Lab personnel are responsible for all routine maintenance of a 2+ lab, including washing the floor.
- Because custodial assignments change frequently, it is usually not feasible for custodians to be trained to enter 2+ labs.

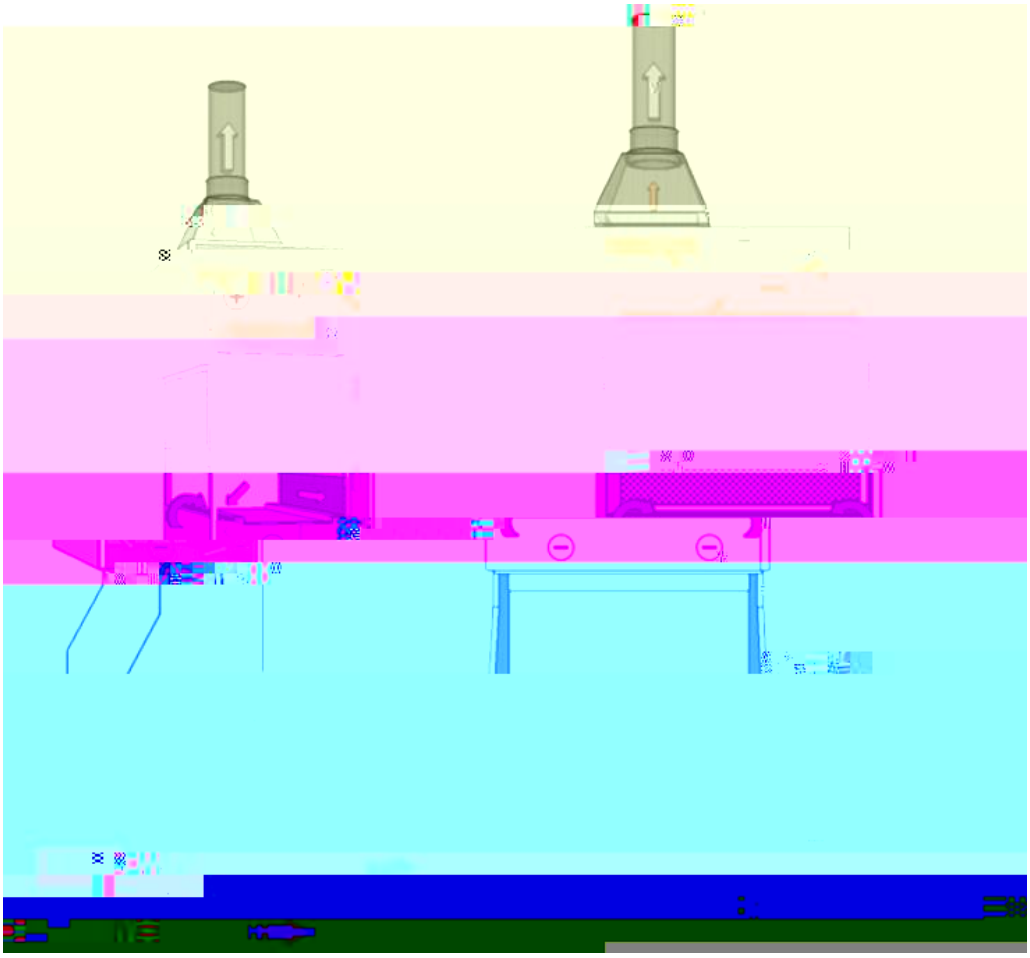


Illustration of a Class two Type A2 Biological Safety Cabinet (BSC)

Cabinet exhaust may be recirculated into the room and this is generally the case at Queen's. Exhaust may also be vented to the outside atmosphere through an air gap type (thimble) connection, as shown.

Diagram above from the CBH. In this figure, the BSC contains a thimble connection, and a positively-pressured contaminated plenum (between the blower and HEPA filters) surrounded by a negatively-pressured plenum. HEPA-filtered air from the top of the cabinet flows downwards towards the work surface. Above the work surface and halfway between the front and rear grilles, the HEPA-filtered downflow air splits in two. One half of the downflow air passes through the front grille while the other half passes through the rear grille. Room air is also drawn into the front grille. The room air and downflow air is drawn through the grilles and flows up the negatively-pressured plenum, through the blower, and into the positively-pressured plenum (between the blower and HEPA filters) at the top of the BSC. A portion of this air passes through the HEPA filter in the plenum before being recirculated towards the work area. The other portion passes through the HEPA filter located at the base of the thimble connection and is exhausted into the containment zone or directly to the outside atmosphere through the thimble connection.

Important tips for BSC use include:

Check the pressure (magnehelic) gauge every time you use the BSC so that you know that it is operating in the correct range.
Ensure the sash is at the correct height.
Work in the middle, and toward the back of the BSC, at or behind the air split if feasible so that contaminated air will be drawn away from you.

When using a fume hood be sure that:

The sash is at the correct height
you work well back in the fume hood
the exhaust is not blocked by extraneous material (do not store things in the fume hood)
surfaces are protected to permit easy clean-up of spills

Tubes

Tubes with proper closures are containment devices. For known infectio6(er) f3*nBT/F3 11.04 Tf1 0 0 1 333.77

purposes of verification. Chemical indicators and autoclave tape only indicate that the item has been exposed to steam, not that the steam, temperature and exposure time were enough to be effective at decontamination. Records must be maintained and kept for 5 years.

All autoclaves and autoclave users must comply with the following two Queen's University autoclave SOPs:

Autoclave Standard Operating Procedure, SOP-Lab-02.

SOP-Biosafety-09 describes the requirements for Autoclaves used for Biohazardous Waste Treatment.

Good Microbiological Practices for Biohazard Laboratories (Level 1 and 2)

In addition to physical containment, good laboratory practices are important for reducing the risk of laboratory acquired infections. In contrast, poor laboratory practices can significantly increase the risk of exposure to biohazards.

Good microbiological practices include the use of PPE, hand washing, disinfecting work areas, the use of procedures that minimize the creation of aerosols, and proper decontamination and disposal of materials. Of these, proper hand washing after removing gloves and before leaving the laboratory is considered the most important practice for preventing the spread of infectious agents.

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. Oral pipetting of any substance is prohibited in any laboratory.
5. Long hair is to be tied back, restrained, or covered, so that it cannot come into contact with hands, specimens, containers or equipment.
6. Access to laboratory and support areas is limited to authorized personnel.
7. Doors to laboratories must not be left open (this does not apply to an open area within a laboratory).
8. Open wounds, cuts, scratches and grazes should be covered with waterproof dressings. Ensure that first aid kits have waterproof dressings.
9. Laboratories are to be kept clean and tidy to avoid cross-contamination and to facilitate cleaning and disinfection. Storage of materials that are not pertinent to the work and cannot be easily decontaminated (e.g., journals, books, correspondence) should be

   materials work areas.
10. Protective laboratory clothing, properly fastened, must be worn by all personnel, including visitors, trainees and others entering or working in the laboratory; suitable footwear with closed toes and heels must be worn in all laboratory areas.
11. Where there is a known or potential risk of exposure to splashes or flying objects, whether during routine operations or under unusual circ(su)-1 0 0 1tnd r is ps (e.g.,)13()] TJd(e)12(i an)-

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. purses, bags) and street clothing (e.g., coats, boots) should be stored separately from PPE and from work stations where biological material is handled.

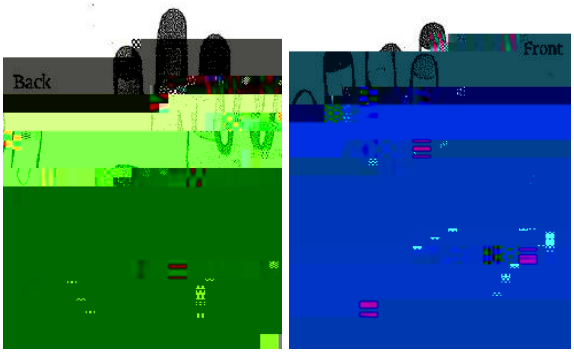
    a. PPE should be removed in a manner that minimizes the spread of contamination to the skin and hair.

14. Aseptic techniques should be used when manipulating open samples of RG1 biological material to provide basic containment and quality control. Pipet slowly without blowing bubbles to avoid generation of aerosols. Use closed, capped tubes for centrifugation.

15. If a known or suspected exposure occurs, contaminated clothing must be decontaminated before laundering (unless laundering facilities are within the containment laboratory/animal facility that has been proven to be effective in

blowing bubbles t317o45e

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).

- 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 equipment must be used; no work with open vessels containing infectious materials is conducted on the open bench.
2.

Microorganisms – Bacteria, Viruses, Fungi, Parasites

Microorganisms and the materials that contain them or may contain them are the focus of the Biosafety Program. Risk assessments of pure cultures of microorganisms may be quite straightforward because many have been characterized in detail. For some agents the information is readily available as PHAC Pathogen Safety Data Sheets. In addition, the PHAC/CFIA e-Pathogen database lists the risk group of 8000 infectious agents and notes if it is regulated by CFIA independently of PHAC. If a Risk Group is stated in this database then it must be followed. However,

existing host machinery and metabolic functions to replicate. Structurally, the simplest viruses consist of nucleic acid enclosed in a protein capsid (nucleocapsid). Enveloped viruses have a more complex structure in which the nucleocapsid is enclosed inside a lipid bilayer membrane. This membrane facilitates the virus's interaction with the host cells, and also increases susceptibility to decontamination.

Viruses are classified by their replication strategy and by the organization of their genome (i.e., double-

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
-

Those working with human blood and tissue must take the EHS training and quiz "Human Blood, Tissues and Bodily Fluids" (a.k.a. the "Blood Borne Pathogens Course"). They must read and follow SOP-Biosafety-08 Human Blood and Tissue .

Eukaryotic Cell Lines

Cell lines (or cell cultures) are commonly used in diagnostic, research, and industrial microbiology laboratories. Many cell lines do not inherently pose a risk to the individuals manipulating them in the laboratory; however, they have the potential to contain pathogenic organisms such as bacteria, fungi, mycoplasmas, viruses, prions, or recombinant virions. This can occur either naturally or through contamination by adventitious organisms, transformation or recombination.

Commercially available cultured cell lines are generally very well characterized and the presence of infectious contaminants is sometimes documented.

Freshly prepared cell lines from a primary culture may be at risk of contamination with infectious contaminants, especially if the cell line was obtained from a specimen known to be or suspected of being infected with a pathogen. There have been documented Laboratory Acquired Infections (LAIs) associated with the manipulation of primary cell cultures.

Cell lines that are known or potentially contaminated should be manipulated at the containment level appropriate for the contaminating organism of the highest risk.
Bacterial and fungal contamination in cell lines can be readily identified
Viruses are not as easily identified and can pose a sid12(n)-4(tami)11(n)-4(at)11(in)-6(g o)4(r)4(ga)13(n)-4

Animal Containment

Animal facilities are designed and operated in accordance with the the Canadian Council on Animal Care. Animal use in science is only permitted after a specific protocol has been approved by the University Animal Care Committee. If the animals might carry a zoonotic pathogen requiring Containment Level 2 or higher, or if biohazardous materials are administered to the animals, then approval by the Biohazards Committee is also required. A specific but brief SOP must be developed for each infectious agent to inform Animal Care Services personnel about the hazard and the procedures that they should use.

Small animals used for work with risk group 2 biohazardous material will be housed in containment cages. Containment facilities are designed and operated in compliance with the requirements of the CBS matrix for CL2-SA zones. Mice are housed in individually ventilated cages (IVC)under negative pressure to the room, with all exhaust and supply air HEPA filtered. Rats are housed either in cages with micro-isolator lids or, preferably, in IVCs under negative pressure to the room. Level 2 work with small animals will be performed in a biological safety cabinet in the housing room unless it is not feasible to do so.

Containment facilities for large animals (e.g. dogs, rabbits, sheep, Non-human primates) are unique, in part because of the large quantity of infectious microorganisms that may be present in the animal cubicle. Unlike a small animal housing room (CL2-

Individuals who are

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

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

Toxin	Trigger Quantity
Alpha toxin	5 mg

Biological Hazards –Sources of Information

Biosafety information regarding numerous biological hazards can be found in:

the PHAC Canadian Biosafety Handbook,
the PHAC Pathogen Safety Data Sheets (PSDS),
the PHAC e-Pathogen Risk Group Database of > 8000 microorganisms
and the American CDC and NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL; 6th

PERSONAL PROTECTIVE EQUIPMENT

It is important to select the appropriate personal protective equipment (PPE) for the specific work to be done. Engineering controls are more effective than PPE and so they must always be considered first, and their use implemented when feasible. Be aware that PPE can provide a false sense of security, pq0.>t53(ic)-4(u)larly ifat

Donning Order (Descending)	Single Gloves and Lab Coat	Double Gloves and Lab Coat	Doffing Order (Ascending)

Foot

Closed-toe and closed-heel shoes must be worn in all laboratories at all times.
Foot protection must be worn in compliance with Queen's University's Foot Protection Standard Operating Procedure, SOP-Safety-09.

Hand

Gloves of a suitable resistance material must be worn for any materials which are being handled.

Recommended glove practices:

Inspect gloves for cracks, tears and holes before wearing.
When donning gloves, ensure that they fit so that no skin will be exposed. Disposable gloves are available in different lengths. Do they fit over the cuffs of your lab coat?
Gloves should be changed when visibly contaminated and as soon as possible after handling infectious agents.

- Change gloves often if wearing for a long period of time.

Proper glove removal technique involves removing each glove without touching the outer contaminated surface.
Gloves can have pinholes or hands can become contaminated as gloves are being removed so hands and wrists should be washed thoroughly immediately after removing gloves.
Double gloving should be considered for some agents or procedures.
Reusing disposable gloves is not recommended.
Latex and vinyl gloves do not provide protection from sharps; nitrile has better abrasion, cut and punct F978>] TJETQ60 G[(W*nBis21 Tf1 0 0 1 108.02 656.5 Tm0 g0 G[(S)3(tan)-4(dar)6(d O)6(p)3

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

Disinfectant Type	Chemical Disinfectant	Natural Rubber Latex	Synthetic Rubber		Plastic Polymer
			Neoprene	Nitrile	Polyvinyl Chloride (PVC)
Oxidizing Agents	Sodium Hypochlorite (<15%)	G	G	F	VG

DECONTAMINATION AND WASTE DISPOSAL

Sterilization is a process that eliminates all living microorganisms, including bacterial spores. The probability of a microorganism surviving a sterilization process is less than one in one million (i.e., 10^{-6}), and is referred to as “sterility assurance”.

Disinfection is a less lethal process than sterilization that eliminates most forms of living microorganisms. The effectiveness of the disinfection process is affected by a number of factors, including the nature and quantity of microorganisms, the amount of organic matter present, the type and state of items being disinfected, water hardness, and the temperature.

Decontamination is the process by which materials and surfaces are rendered safe to handle and reasonably free of microorganisms or toxins. The primary objective of decontamination is to protect containment zone personnel and the community from exposure to pathogens that may cause disease. Depending on the situation, decontamination may require disinfection or sterilization.

Validation demonstrates that the equipment and method are effective at decontaminating, inactivating, or removing the specific pathogen(s) or toxin(s) in use. 14(ic)-5024 102.02 Tm/GS7 gs0 g/GS8 gs0 G

Decontamination processes and methods should be in accordance with applicable federal, provincial, territorial, and municipal regulations.

Decontamination procedures must be included in personnel training on the hazards and exposure/release mitigation strategies associated with the work being done (CBS R4.3.4). Training would include information on the products used, and the factors influencing their effectiveness.

Where possible, technologies that are routinely verified using biological indicators (e.g., autoclave) should be used instead of liquid chemical disinfectants.

Autoclaving

Infectious material and toxins, together with associated waste (e.g., petri dishes, pipettes, culture tubes, and glassware), can be effectively decontaminated in an autoclave. The effectiveness of decontamination by steam autoclaving is dependent on the temperature to which the material is subjected as well as the length of time it is exposed. Proper operation, loading, and monitoring of autoclaves are critical to ensure decontamination is achieved. Particular attention should be given to packaging, including the size of containers and their distribution in the autoclave. Items should be arranged in a manner that allows the free circulation and penetration of steam. Pre-vacuum autoclaves are preferred for decontamination because they resolve the air entrapment problems that prevent the penetration of steam and that are often encountered in gravity displacement autoclaves.

All personnel using autoclaves for decontamination or for sterilization must be trained on their use and the training must be documented. They must be in compliance with the general autoclave SOP-Lab-02. A location-specific operating procedure for each autoclave must be posted near the autoclave.

Any autoclave-based biohazardous waste treatment must also be in compliance with SOP-Biosafety-09 .

For autoclave-based decontamination of material:

1. Waste containers must be labelled. Plastic bags (unlabelled) to contain biohazard waste must be supported in a solid container. The container must be labelled with an orange biohazardous materials label. The purpose of the solid container is to hold the bag securely and to contain any leaks. Alternatively, if required by the host institution (e.g. in some areas associated with the hospital or clinics), a yellow container is use for biomedical waste.

2. Solid contaminated waste (excluding glass) should be placed in a clear or red/orange bag, inside a solid collecting container. Do not use bags with a biohazard label printed on them because personnel will then need to remember to deface the label (unless the label disappears when autoclaved).
3. When full, bags must be closed, and labelled with the name of contact person (the person disposing of the waste, and the supervisor) and room number. DO NOT OVERFILL BAGS (2/3 full only), and do not compress them, as this will inhibit steam penetration.
4. Double contain waste for removal from the lab (either double bag, or bag inside a closable solid container that can be autoclaved).
5. At the autoclave, bags for decontamination must be placed in the available trays and, immediately before autoclaving, opened to allow steam penetration.
6. Disinfected material that is no longer biohazardous must be placed in a regular garbage bag after ensuring that any biohazards warning labels are defaced.
7. The efficacy of the autoclave for decontamination of representative loads of biohazardous waste must be verified weekly using biological indicators (bacterial spores of Geobacillus stearothermophilus commercially available for this purpose) or chemical integrators (not just chemical indicators) as described in SOP-Biosafety-09 .
8. Annual validation of representative loads must be done using biological indicators.

Solid waste that would give off hazardous fumes in the autoclave and/or corrode the autoclave or damage the seals must NOT be autoclaved (e.g., hazardous chemicals, bleach, radioisotopes).

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)	

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

Animals

All animal carcasses must be placed in 6 mil dark plastic bags (body bags) and frozen prior to being sent for incineration. Bagged carcasses must be properly labelled and prepared for hazardous waste disposal.

Mixed Waste

For waste which is a mixture of chemical/radioactive and biohazardous waste, it might be possible to destroy the biohazard first by chemical means and then treat and/or dispose of the waste as appropriate for chemical or radioactive waste. However, when certain chemical hazards are present, disinfectant (eg. bleach) will create a worse chemical hazard. Therefore, it is important to know what you are doing. If you are not sure that this can be done safely and effectively, contact the Department of Environmental Health and Safety (ext. 32999) for assistance. Mixed waste should not be autoclaved and should not be sent for incineration without consultation with the Department of Environmental Health and Safety.

Disinfection Recommended Reference

For more specialized and detailed information on disinfection than that provided above, a book is recommended that is available at Queen's in the Bracken Library: Disinfection, Sterilization, and Preservation Editor: Seymour S. Block. Edition: 5th ed. Published: Philadelphia : Lippincott Williams & Wilkins, c2001. Location: Bracken Health Sciences Call Number: QV220 .D611 2001

E

national security?
Dual
Use

IMPORT, EXPORT, and MOVEMENT/TRANSPORT

An import permit will likely be required for material not covered by our PHAC

http://www.inspection.gc.ca/food/videos/airs-tutorial/eng/1528316420730/1528316421089

Export

Export of pathogens that fall under the Human Pathogens and Toxins Act is subject to the conditions of Queen's PHAC licence.

When exporting biological agents from Canada it is your responsibility to:

1. Inform the Queen's University Biosafety Officer
2. Ensure that you are in compliance with the import regulations of the country to which you are exporting.
3. Ensure that you are in compliance with international transportation regulations (see below).
4. Ensure that the person to whom you are shipping is aware of the hazards of the material.
5. Ensure that the person to whom you are shipping has the appropriate containment facilities to handle the material safely.

Transfer

For transfer within Canada, a transfer form is required to properly inform the BSO at the institution you will ship to (or receive material from). Contact the Queen's BSO.

When obtaining new biohazardous material on campus, the receiving lab's biohazard inventory and risk assessment must be updated by submitting an amendment to your biohazard permit through TRAQ.0 G[(bel)-2(ow)10())-3(.)] TJET>)ns.6ent m> BDC q0.00000912 0 612 792 reW*nBT/F1 11.04 Tf1 0

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-

BIOHAZARD ADMINISTRATION at QUEENS - LABORATORY PERMITS,

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.queensu.ca/risk/safety/biohazard
2. Inspection and approval of the laboratory or facility where the work will be conducted (before work begins).
3. The risk assessment will be edited with each significant amendment, so the risk assessment will be kept up to date.

The permit is valid for four years if renewed annually (and if amended as necessary). Four years after approval, a re-application must be submitted for review and approval by the Biohazard Committee.

The risk assessment will be edited with each significant amendment, so the risk assessment will be kept up to date. Nevertheless, re-application at the 4th renewal will still be required to update the information captured on the electronic biohazard form and to update the material with respect to any changes in biosafety practices or regulations.

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.

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

- 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

Flow cytometers or cell sorters in which the sample flows in a stream through air, rather than in tubing, present a risk to the operator of exposure to aerosols that may contain infectious microorganisms associated with the cells. They should be contained in a b/i6aaf[(tu)0 0 1 306.29 498.43 Tm0 g0

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 material from the tip of the pipette onto hard work surfaces; if this cannot be avoided then a disinfectant soaked paper should be placed on the working surface
The contents of the pipette or tip should be expelled gently down the sides of tubes or discharged slowly close to the surface of a liquid
Do not bubble air from a pipette to mix fluids
Avoid mixing by alternate suction and expulsion through a pipette, or work in a biosafety cabinet
For infectious level 2 material, submerge contaminated pipettes in disinfectant solution inside the BSC

- Submerge used non-disposable pipettes horizontally in disinfectant solution; dropping them in vertically may force out any liquid remaining in the pipette

Plastic vs. Glass

When feasible for work with infectious micro-organisms, plastic transfer pipettes, culture tubes, flasks, bottles, dilution tubes, etc. are preferable to glass, to reduce the risks of aerosol generation due to breakage and also to minimize the risk of cuts and accidental inoculation.

Pouring infectious material

Where feasible and depending on the risk of the material, avoid pouring off the supernatant fluid after centrifugation, cell washes, etc., even inside a biological safety cabinet, because this leads to contamination of the outside rim of the tube and to aerosol production (that will contaminate the surrounding area).

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.

Numbers in the left column are those used in the CBS for each item of the Standards.

Definitions of terms can be found in the CBS Glossary (Chapter 21).

Table 1 contains the requirements for CL2 laboratories. These are also required for CL2-SA and CL2-Ag facilities.

Table 2 contains additional requirements for CL2-SA facilities.

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 - Doors to the containment zone to be lockable.
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.
3.3.9 - Space to be provided for the storage of PPE in use.
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.
3.4.5 - Floors to be slip-resistant in accordance with function.

- 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 **iorkous material** that is not being corrected by that person after they have been made aware of it;

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

assiorksting with internal investigations**iorkidents**

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

4.1.4 - **Program iork** 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** (LRA) to be conducted to examine each task involving infectious material or toxins so that the **risks**

CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
4.1.12 - A medical surveillance program, based on an overarching risk assessment and LRAs, to be developed, implemented, and kept up to date.
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; animal work considerations; **decontamination** and **waste** management; the safe and secure movement and **transportation** of infectious material and toxins, and 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 or authority of any: **incident** that may have resulted in an exposure of an individual to a human pathogen or toxin in a facility; or disease that may have been caused by an exposure to a human pathogen or toxin in a facility.

CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
4.2.4 - Emergency medical contact card to be issued to containment zone personnel handling non-human primates or a pathogen identified by a **local risk assessment** (LRA).
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, including the signs and symptoms of **disease**(s) caused by the **infectious material** or toxins in use and the necessary precautions to prevent **exposure** to, or **release** of, pathogens or toxins.
4.3.3 - Personnel to be trained on the relevant physical design and operation of the **containment zone** and **containment systems**.
4.3.4 - Personnel to be trained on the correct use and operation of **laboratory** equipment, including **primary containment devices**.
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.
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.
4.4.1 - Appropriate dedicated PPE specific to each **containment zone**, to be donned in accordance with entry procedures and to be exclusively worn and stored in the containment zone.

CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
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.
4.5.5 - Access to supporting mechanical and electrical services for the containment zone to be limited.
4.5.8 - Current entry requirements to be posted at point(s) of entry to the containment zone, animal rooms, animal cubicles, and PM room.
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.
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 -

CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
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**.
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 - Sharps to be discarded in containers that are leakproof, puncture-resistant, and fitted with lids, or specially constructed for the disposal of sharps waste.
4.8.4 - **Primary containment devices** to be decontaminated prior to maintenance.
4.8.5 - All clothing and **personal protective equipment** (PPE) to be decontaminated when a known or suspected **exposure** has occurred.
4.8.7 - Contaminated liquids to be decontaminated prior to release to sanitary sewers.

4.8.8 - Contaminated equipment, materials, and waste to be:

decontaminated prief71nateg (en-US)r8.ie0u /MCID 972 201.6u 203.93 Tm0.32 rereW4.32 reW*nBT/F8 9.96 Tf1 0 0 1 93.744 173

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. Frequenc44 379s2.36 357.79 647

failure of primary containment devices;
puff-back from class II B2 BSCs, where present;
loss of containment;
emergency egress;
notification of key personnel and relevant federal regulatory agency (or agencies);
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.

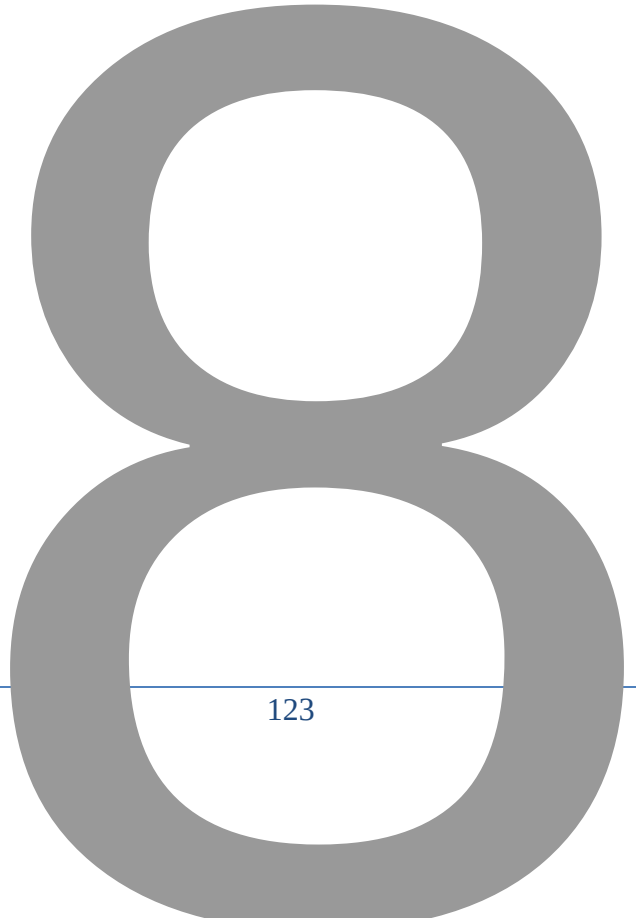
4.9.7 - Incidents involving pathogens, toxins, other regulated infectious material, infected an4(eraod)4(i)6(nf)4(ect)4(i)6(ous)15(m)-7(a

CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
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: a change, repair, or modification to the **containment system**; a condition of **licence**; or 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

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

3.4.4 - Backsplashes, when installed tight to the wall, to be sealed at the wall-bench junction and continuous with work surfaces.



CL2 Small Animal *in vivo* Requirements in addition to CL2 *in vitro* (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.
4.5.12 - Personal belongings not required for work to be left outside the containment zone or in change areas outside the containment barrier.
4.6.36 - Routine cleaning, as described in SOPs, to be carried out by containment zone personnel or other staff trained specifically for this task.
4.7.1 - Proper methods of restraint to be used to minimize scratches, bites, kicks, crushing injuries, and accidental self-inoculation. [***Not required for CL2 large scale production areas.***]
4.7.2 - **Primary containment caging** housing infected animals to be identified with labels. [***Not required for CL2 large scale production areas.***]
4.7.3 - Handling procedures to be employed to minimize the creation of **aerosols** and dissemination of dust from cages, refuse, and animals. [***Not required for CL2 large scale production areas.***]

CL2 Small Animal *in vivo* Requirements in addition to CL2 *in vitro* (CBS 2nd Edition 2015)
4.7.5 - Animals and carcasses to be securely moved into, out of, and within the containment zone. [***Not required for CL2 large scale production areas.***]
4.7.7 - Inoculation, surgical, and necropsy procedures to be designed and carried out to prevent injuries to personnel and minimize the creation of aerosols. [***Not required for CL2 large scale production areas.***]

4.7.8 - Inoculation, surgical, and necropsy procedures with animals in **small animal containment zones** (SA zones) to be carried out in a certified **biological safety cabinet** (BSC) or other nfp03340.512(t)3(82.36 379.2 g0.a)10(binet)] TŒT(proc)13(edur)12(es)4(w)6(i)-4(t)

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-

Table 3. CL2-Ag FACIL

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:

CL2 Ag *in vivo* for large animals in addition to CL2 *in vitro* Requirements (CBS 2nd Edition 2015)

4.6.36 - Routine cleaning, as described in SOPs, to be carried out by containment zone personnel or other staff trained specifically for this task.

4.7.1 - Proper methods of restraint to be used to minimize scratches, bites, kicks, crushing injuries, and accidental self-inoculation.

[***Not required for CL2***

CL2 Ag *in vivo* for large animals in addition to CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
4.7.9 - Animals to be disinfected and/or cleaned at site of injection or exposure following inoculation or aerosol challenge with **infectious material** or toxins. [***Not required for CL2 large scale production areas.***]
4.8.6 - PPE to be decontaminated prior to disposal or laundering unless laundering **facilities** are located within the containment zone and have been proven to be effective in decontamination.
4.8.14 - Animal cubicles, PM rooms, and dirty corridor, when present, to be decontaminated when grossly contaminated and at the end of an experiment.

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

5.2.4 - Emergency power and uninterruptible power supply (UPS) systems to be tested under representative electrical load conditions.

[***Not required in CL2 SA zones where Prions and SSBAs are not present.***]

5.2.5 - Operation of communication systems to be verified.

[***Not required in CL2 SA zones where Prions are not present.***]

Government Safe

It is important that people working with bi
what they may and may not do with those

The key document governing the use of bio
more detail in the BIOSAFETY OVERVIEW

The laws and regulations related to work w

Human Pathogens and Toxins Act (20

- Gives the Public Health Agency of Canada (PHAC) the authority to regulate the possession, use and transfer of human pathogens (risk group 2 and above), whether imported or domestic, and toxins (including genes, nucleic acids, soil, blood, or tissues until such time as it is determined that they do not contain blood)
- The HPTA was based on the rec

- 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.
- There must be a Material Safety Data Sheet (MSDS) available to accompany each hazardous substance used at the work site. MSDS for chemicals are available through Chemwatch.
- Also requires that all employees must receive training in WHMIS.
- MSDS sheets for some human pathogens are available through PHAC, called Pathogen Safety Data Sheets (PSDS).

Several provincial and municipal laws and regulations also affect the use or disposal of biohazards and associated material at the University.

Ontario Guideline C-4 defines biomedical waste and outlines its proper treatment and handling.

Memorandum of Understanding. Roles and Responsibilities in the Management of Federal Grants and Awards. An agreement between Queen's University and the Federal Granting Agencies

- Institution to monitor research and ensure that it adheres to the requirements of applicable laws and regulations
- Release funds to researchers only if the Institutional Biosafety Committee or Biosafety Officer has approved the project procedures and has provided a certificate to the laboratory
- Process to ensure Institutional Biosafety Committee or Biosafety Officer is notified promptly by the researchers if the research changes to involve the use of biohazards of a different level of risk
- Suspend funding due to a serious contravention of the Canadian Biosafety Standards; an applicable federal or provincial law; or any condition of approval imposed by the Institutional Biosafety Committee or Biosafety Officer
- Advise the Agencies in writing of any situation that results in a suspension of funds to a research project