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.

BIOSAFETY OVERVIEW and BASIC CONCEPTS

What is a biohazard?

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

The most obvious biohazardous materials are infectious or potentially infectious agents like bacteria, viruses, fungi, parasites and prions. However, tissues or cell lines that might contain such agents are also biohazards. Some toxins produced by microorganisms are also regulated as biohazards even though they may be more like chemical hazards. The Queen's University Biohazards Risk Group Definitions, SOP-Biosafety-05, defines the material that is regulated through the Queen's University Biohazards Committee.

Laboratory-Acquired Infections

There are several reasons why most people have worked with potentially infectious material for years without ever hearing of someone becoming infected. Although laboratory-acquired infections do occur, they often go unrecognized because of the generalized "flu-like symptoms" of many pathogens. Also, until recently there was no obligation to report laboratory-acquired infections so they usually were not known outside the lab or institution, unless they resulted in severe illness or death. It is now required in Canada to recognize when lab-acquired infections may have occurred, and to report them.

Case Studies

1. A researcher was using a strain of Salmonella as a host for DNA that coded for the production of an enzyme of interest for a pharmaceutical use. When asked if the strain of Salmonella was debilitated in some way, the researcher indignantly said, "Why of course it is." However, lab members reported that although they had been told it was debilitated, every new person in the lab had "a little diarrhea" when they first started working there, but got over it and didn't have any other problems. Fortunately, they never had any lab members who were immunocompromised or they might have had much more serious disease caused by the infection. Better lab practices were clearly needed. *(Anecdote provided by a Biosafety Officer at another University)*

2. On September 18, 2009, the Chicago Department of Public Health (CDPH) was notified by a local hospital of a suspected case of fatal laboratory-acquired infection with *Yersinia pestis*, the causative agent of plague. The patient, a researcher in a university laboratory, had been working with a pigmentation-negative (pgm-) attenuated *Y. pestis* strain. The

strain had not been known to have caused laboratory-acquired infections or human fatalities and was properly classified as Risk Group 2.
Although the route of transmission for the infection remains unclear, deficiencies in biosafety practices, including inconsistent use of gloves, could have resulted in inadvertent transdermal exposure.
A U.S. federal investigation determined that the cause of death likely was an unrecognized occupational exposure (route unknown) to the attenuated pgm- *Y. pestis*, leading to septic shock. The severe outcome experienced by the patient was unexpected, given that he had worked with an attenuated *Y. pestis* strain that 1) is widely used by laboratory researchers, 2) has not been associated with previous laboratory-acquired infections or fatalities, and 3) is excluded from select biologic agent requirements.
A postmortem diagnosis of hereditary hemochromatosis was made. One possible explanation for the unexpected fatal outcome in this patient is that hemochromatosis-induced iron overload might have provided the infecting *Y. pestis* strain, which is attenuated as a result of defects in its ability to acquire iron, with sufficient iron to overcome its iron-acquisition defects and become virulent. In addition, the patient had diabetes, a known risk factor for increased severity and complications from bacterial infections. *More details regarding this case are available in the CDC Morbidity and Mortality Weekly Report (MMWR) February 25, 2011.*

In both case studies above, the infections were caused by not using the appropriate CL2 containment practices for the organisms. Furthermore, the responses to infection were suboptimal.

- In the first case, lab personnel were unwise to accept becoming ill, even for a short period of time, as a necessary part of the introduction to the lab. The symptoms were an indication of improper technique and should have been reported so that improvements in facilities, equipment and/or operational practices could be made.
- Personnel should recognize that infections that cause mild disease in one individual can cause serious disease in others. Underlying medical conditions may not be recognized until someone becomes uncharacteristically ill, so **lab-acquired infections should not be tolerated and must be reported to their supervisor and the Biosafety Officer**.
- It is important for everyone to **remember the symptoms of the disease** that can be caused by the microorganisms with which they work, and to **inform their physicians** about it if they become ill. The fatal outcome from *Y. pestis* infection in the first case might have been averted had the researcher recognized the possibility that his symptoms were caused by a microorganism with which he worked and reported this to the physicians whom he consulted. **There are quite a few other examples in the literature of lab acquired infections where the outcome was worse because the patient didn't inform their physician about their work.**

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 hazard to health they pose, and how likely that hazard is to occur.

Risk Group 1 *low individual and community risk*
Risk Group 2 *moderate individual risk and low community risk*.
Risk Group 3 *high individual risk, low public health risk*
Risk Group 4 *high individual risk, high community risk*

A **microorganism/pathogen risk assessment** is a qualitative assessment based on science, policy, and expert judgment **to determine the risk group assignment** for an infectious agent, or for biological material that might contain that agent.

Factors considered in a pathogen risk assessment include:

- ***Pathogenicity/Virulence***: Is the pathogen able to infect and cause disease in humans or animals (i.e., pathogenicity)? What is the degree of disease severity in individuals (i.e., virulence)?
- ***Route of Infection:*** How does the pathogen enter the host (i.e., ingestion, inhalation, mucous membranes, subcutaneous, genitourinary)?
- ***Mode of Transmission*****:** How does the pathogen travel to the host (e.g. direct contact, indirect contact, casual contact, aerosolized droplet or airborne transmission, vectors, zoonosis, intermediate host)?
- ***Survival in the Environment*****:** How stable is the pathogen outside the host? Under what environmental conditions can it survive and for how long?
- ***Infectious Dose*****:** What amount of pathogen is required to cause an infection in the host (measured in number of organisms)?
- ***Availability of Effective Preventative and Therapeutic Treatments*****:** Are effective preventative measures available (e.g., vaccines)? Are effective treatments available (e.g., antibiotics, antivirals)?

- ***Host Range*:** What are the primary, intermediate, and dead-end hosts? Does the pathogen cause infection in a wide range of species, or is the host range more restricted?
- ***Natural Distribution*:** Is the pathogen present in Canada? Is it prevalent in a particular location, region, or human or animal population? Is the pathogen non-indigenous/exotic?
- ***Impact of Introduction and/or Release into the Environment or the Canadian Public*:** If the pathogen were introduced into the population or released into the environment (within Canada), what would be the economic, clinical, and biosecurity impact?

While most infectious material will clearly fall into one of the four risk groups outlined below, in some cases, the level of risk associated with the different risk factors can vary dramatically within a risk assessment. As a result, **certain risk factors may be considered more important when determining the final risk group.** For example, if a pathogen is unlikely to cause disease in humans or animals, it may be irrelevant that it can survive in the environment for a long period of time or that there is no available treatment.

Risk Group 1

Risk Group 1 *low individual and community risk*
A microorganism, nucleic acid, or protein that is either:

- not capable of causing human or animal disease; or
- capable of causing human or animal disease, but unlikely to do so.
    - Those capable of causing disease are considered pathogens that pose a low risk to the health of individuals and/or animals, and a low risk to public health, livestock or poultry.
    - **RG1 pathogens can be opportunistic and may pose a threat to immunocompromised individuals.**
    - RG1 agents are not regulated by the PHAC or the CFIA due to the low risk to public health, livestock or poultry.
- The **culture of RG1 microorganisms <u>is regulated</u> by the Queen's University Biohazards Committee** to ensure that due care is exercised and safe work practices (e.g., good microbiological practices) are followed when handling these materials.
- Examples include:
    - bacteria such as *Bacillus subtilis*, *Lactobacillus casei*, cloning strains of *E. coli (K12 strains)*
    - viruses such as *Baculovirus*
    - fungi such as *Schizosaccharomyces*

- 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
    - e.g. replication incompetent lentiviral vectors carrying a hazardous transgene
    - Lymphocytic choriomeningitis virus (lab adapted, non-neurotropic strains)

- for certain Risk Group 3 microorganisms (subject to PHAC approval). To work with RG3 microorganisms in a Containment Level 2/2+ lab, a RG3 licence from PHAC is required, even if they have published a decision that this level of containment is acceptable.

Although it is in common use, the 2+ designation is not recognized by Public Health Agency of Canada (PHAC) or the Canadian Food Inspection Agency (CFIA) so you will not see this short form used in official federal documents like the Canadian Biosafety Standards or the Canadian Biosafety Handbook, or on import permits.

- The level 3 operational practices required are only those that can be done in a level 2 facility.
- The personal protective equipment required depends on the organism and the work being done with it.
- According to the requirements issued by PHAC, **no one may enter a 2+ laboratory until they have been trained about the specific hazards and procedures involved and have demonstrated knowledge and understanding (i.e. by writing a lab-specific quiz).**
- The training requirement and other requirements might change in the future for some organisms, so be sure to read and comply with the stipulations on the Canadian import permit and the Queen's University Biohazard Permit for the organism you are using.
- **In brief, all work with 2+ organisms must:**
    - Be done in a biological safety cabinet
    - centrifugation must be done in closed tubes in sealed safety cups that are opened only in the biological safety cabinet
    - At Queen's, specific individualized operational procedures are written and approved by the Biohazard Committee for each 2+ laboratory as part of the biohazard permit application process.
- Everyone entering the 2+ lab must be trained on the hazards in the lab and procedures specific to the lab, and there must be written documentation of this training
        - this training requirement includes custodial personnel so in practice **custodians usually do not enter**.
    - **Any non-biohazard garbage must be surface decontaminated and placed outside the lab**
    - **Routine cleaning, including floors must be done by laboratory personnel.**
    - **A full surface decontamination including the floor must be done by lab personnel before annual sealing of the floor.**

Risk Group 3 and 4

Currently there are no level 3 or 4 containment facilities on Queen's University campuses so work requiring these containment levels cannot be done here. There are numerous level 3

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/or animals and a low risk to public health.
- likely to cause serious disease in a human or animal
- effective treatment and preventive measures are usually available and the risk of spread of disease caused by these pathogens is low for the public
- the risk of spread to livestock or poultry, however, can range from low to high depending on the pathogen.
- Examples include:
    - Bacteria such as *Bacillus anthracis*, *Coxiella burnetii, Yersinia pestis*
    - Viruses such as *Human Immunodeficiency Virus (HIV), Hepatitis B, Hepatitis C*
    - Fungi such as *Blastomyces dermatitidis*, *Histoplasma capsulatum*

Risk Group 4 *high individual risk, high community risk*
A pathogen that:

- poses a high risk to the health of individuals and/or animals and a high risk to public health.
- is likely to cause serious disease in a human or animal which can often lead to death.
- effective treatment and preventive measures are not usually available
- the risk of spread of disease caused by these pathogens is high for the public
- the risk of spread of disease to livestock or poultry, however, ranges from low to high depending on the pathogen.
- All Risk Group 4 agents are viruses, for example: *Ebola virus, Macacine alphaherpesvirus 1* (previously known as *Herpesvirus simiae*, *Cercopithecine herpes virus 1*, herpes B or monkey B virus)

Sources of Risk Group Information

The Public Health Agency of Canada (PHAC) has a database of approximately 8000 agents on which they have done risk assessments. Access to this database is freely available ePATHogen – Risk Group Database .

- **If an agent has been characterized as having a Risk Group of 2 or higher by PHAC then this risk group must be used when determining the containment facilities and practices for the work.**

- **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 <u>not</u> 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.
- If an agent has been classified as RG2 for humans and RG1 for animals and the researcher wishes to challenge the designation for humans, then they do not need to address questions on the form about animals.

The PHAC website has detailed and useful risk assessments of some microorganisms in the form of **Pathogen Safety Data Sheets** (PSDSs, an MSDS for pathogens) that are publicly available. The link to the PSDSs for infectious substances is also posted on the Biosafety dropdown menu at www.safety.queensu.ca/ under "Links, Sources, and Recommendations".

The American Type Culture Collection (ATCC) also assigns risk groups (that it calls "Biosafety Level") for the material that it supplies. **However, note that the ATCC sometimes assigns a Biosafety Level of 1 to material that would be considered Risk Group 2 by Canadian regulatory authorities,** or that would require containment level 2 facilities and practices at Queen's University. **Check with the Biosafety Officer to be sure.**

Risk Mitigation and Containment: How can biohazards be used safely?

Biohazards are contained so that they do not infect personnel inside the laboratory or escape into the environment outside the laboratory. The methods used for containment are more stringent for biological material in higher risk groups. Note that containment level is also called the biosafety level (BSL) and the BSL term is more commonly used in the United States.

The containment required depends in part on the risk group of the material and in part on what will be done with the material. The containment level required is often the same as the risk group, but sometimes the nature of the procedures or the quantity of the organism might increase or decrease the containment required. For example, HIV is a Risk Group 3 organism. However, because HIV cannot be spread directly by inhalation of aerosols and because this

virus has very low viability on environmental surfaces, work with HIV can be done under level 2+ containment conditions (with a PHAC RG3 Licence if it is cultured or concentrated from natural sources).

Local risk assessments must be performed by each Principal Investigator as part of the Biohazard Permit Application submission. The local risk assessment considers both the characteristics of the microorganisms (or biohazardous material/tissue) and the work that will be done with them **to determine the containment facilities and practices needed for the work.** Containment is intended to mitigate the risk, i.e. to reduce the probability that the hazard will have a negative effect on people or animals.

Biological containment. If possible, risk should first be mitigated by employing biological containment methods, which involve decreasing the potential consequences of exposure by using genetically modified or otherwise attenuated or lab adapted organisms that are less hazardous (e.g. using K12 derived strains of E. coli for molecular biology; using late generation replication-incompetent viral vectors; using lab adapted strains or attenuated strains of bacteria or viruses as models for more virulent strains).

Physical containment is provided by physical barriers that prevent or minimize the escape of biohazardous materials from the work area.

A containment laboratory must meet specific physical design requirements and provide equipment for containment as described in the Canadian Biosafety Standards(CBS), 3rd Edition 2022..

Before a laboratory is used for work with biohazardous materials it must be inspected by members of the University Biohazard Committee to ensure that the Standards are met.

Lab design requirements vary depending on the containment level and whether animals are intentionally infected.

Physical containment often requires labs with:

- surfaces that can be readily decontaminated
- sinks for hand washing
- eyewashes (and safety showers depending on lab use)
- lockable doors
- storage areas for personal protective equipment (PPE)
- specialized equipment like biological safety cabinets
- once-through ventilation so that air is not recirculated into offices
- labs having negative air pressure relative to the corridor

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.
- Aerosols carrying infectious agents can settle on bench tops and thereby become a hazard for ingestion or mucosal exposure through contamination of the hands.

There is general agreement that aerosols generated by procedures are the **probable sources of many laboratory acquired infections**, particularly in cases involving workers whose only known risk factor was that they worked with an agent or in an area where that work was done (i.e. there was no known exposure incident).

The effective **containment of any aerosols** that are produced due to the nature of the activity, and the minimization of aerosol generation where possible, are key considerations in determining the appropriate procedures and containment practices to employ.

If the production of significant Risk Group 2 aerosols is unavoidable, then aerosols must be contained by using equipment such as a biological safety cabinet (BSC).

Local Risk Assessment and Medical Surveillance

Risks of the Material and Procedures, and Risk Mitigation

Experience shows that established safe practices, equipment, and facility safeguards work. The Local biohazard risk assessment is a subjective process requiring consideration of the many characteristics of both the infectious agents (or biohazardous materials) and the procedures that will be used. Judgments are often based on incomplete information.

It is important to <u>think</u> about what you will be doing with biohazardous material and how you will reduce the probability that you or someone else might be exposed to and/or infected by the microorganisms you are culturing or the microorganisms that might be present in biological material:

- Some thinking will have been done for you and presented in the form of a written local risk assessment and risk mitigation strategy as part of a Queen's biohazard permit application.
- It is important that you **internalize this thought process and apply it to your day-to-day work** because:
    - Safe work practices protect you and your colleagues
    - unexpected situations may arise
    - you may need to make educated judgments about how to act
        - or decide whether it is necessary to **consult your supervisor or the Biosafety Officer**.

It is a requirement of the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) that each laboratory performs a detailed **local risk assessment** (LRA) to determine the biohazard containment level required for both facilities and operational practices for the biohazardous agents in use. Therefor the local risk assessment of all work with biohazardous material (Risk Group 1 and 2 and 2+), is to be documented as part of a Queen's University Biohazard Permit Application and all authorized personnel must read and follow it.

A local risk assessment will:

- identify the Risk Group of the microorganism (or tissue that might contain this microorganism)
    - review and use information from the PHAC PSDS if one is available
    - check the **PHAC** ePATHogen – Risk Group Database **of over 8000 agents to which they have assigned a risk group**
        - if a risk group has been assigned then use it and refer to the PHAC ePATHogen database

        - 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 Biosafety Officer for the PHAC Pathogen Risk Assessment Stakeholder Template.**

- describe the **potential hazard** associated with the microorganism, including symptoms of disease (which it is important for all lab members to know so that they will be aware of any potential lab acquired infection so that it can be diagnosed and treated appropriately)
- **what is being done with the material and where?**
    - consider the procedures' potential for generating aerosols that might spread infectious agents
    - indicate whether sharps will be used, and the precautions associated with them
    - *in vivo* use of infectious materials (*(i.e. introducing pathogens or material that might contain pathogens into animals)* increases the risk of exposure, so the facilities and operational practices for *in vivo* work must be described separately from that for *in vitro* work
- **describe the overall risk mitigation strategy and details of this strategy including:**
    - **physical containment** (i.e. lab design) can be indicated by stating which containment level 1 or 2 laboratories will be used for the different types of work, because the Biohazard Committee inspects all laboratories.
        - **If there is a change** in the containment level of the lab or if you have just moved into the lab then a **commissioning inspection** by the Biosafety Officer or the Safety Technician (Biohazard and Chemical) will be required.
    - **operational requirements**
        - containment equipment and supplies
            - equipment might include a Biological Safety Cabinet, centrifuge cups with aerosol resistant lids containing o-rings
            - supplies might include closed, screw-capped tubes
        - appropriate personal protective equipment (PPE)
            - describe what is to be worn and for which procedures and materials if there are different PPE requirements
        - decontamination and disposal methods

- medical surveillance (e.g. immunization, titre checks, first aid and medical response to accidental exposure)
- training needs

At Queen's the Principal Investigator's local risk assessment is documented and appended to the Biohazard Permit Application along with any applicable microorganism risk assessments from reputable sources (e.g. PHAC PSDS) and lab specific procedures/SOPs.

To assist laboratories when preparing a Biohazard Application, example/template local risk assessment documents are available in TRAQ/Romeo "Useful Links" and are attached to the Biohazard Permit application form in TRAQ. They can also be obtained from the Biosafety Officer.

- In general, more detail is required for material and activities that pose a greater risk.
- The risk assessment and associated documents are reviewed and approved by the Biohazard Committee.
- **After approval, these documents become an integral part of the training of lab personnel, so it is important that they are well written and thorough.**
- Following approval of a Biohazard Application or a Biohazard Amendment that changes the type or risk group of material used in the lab (reviewed by the Biohazard Committee), each member of the biohazard lab team is required to:
    - read the approved Biohazard application/amendment and associated documents
    - have any questions answered by their P.I. and/or the Biosafety Officer
    - submit the Biohazard Team Member Attestation form to the Biosafety Office, to indicate that they understand and will abide by the lab specific requirements for working safely with the biohazardous material.

If you are new to the lab, as part of your lab orientation you will be required to read the current approved biohazard permit application and all associated documents, and do the specified training. Once you have had your questions answered, you will be required to sign a Biohazard Team Member Attestation form that will be submitted to the Biosafety Office so that you can be authorized to work independently in the lab (at your supervisor's discretion).

Health and Medical Surveillance Program at Queen's

The health and medical surveillance program for a laboratory is intended to reduce the consequences of an exposure should it occur and to identify individuals who need to take special precautions. It needs to be appropriate and specific for the agents in use. As such it is reviewed by the Biohazard Committee as part of the Biohazard Permit Application process.

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 medical surveillance most commonly includes, as appropriate:

- Specific immunizations (e.g. Hepatitis B), and serum titre testing to confirm response to the immunization.
- A plan of what first aid and medical response is to occur in case of an incident involving exposure. The plan must be written, approved, and posted in the laboratory.
- Training to develop an awareness that changes in the health status of personnel can increase their personal risk from the biohazards in that laboratory.
    - If the organism being worked with has been attenuated or genetically altered to be less hazardous than wild-type, individuals should be aware of the mechanism of attenuation (if known) and any conditions that might make the attenuated organism more pathogenic for them.
- Changes in health status that might affect immune responsiveness (immune-compromised) should be reported.
    - For these individuals, **some risk group 1 microorganisms which do not normally cause disease can be pathogenic** and risk group 2 microorganisms can cause much **more severe disease than normal, or even death**.
    - Note that, without the need to reveal personal medical information, the occurrence of a change in an individual's health that might influence their susceptibility to infection should be reported to their supervisor so that, if necessary, appropriate adjustments in the operations or risk mitigation methods can be made in consultation with their personal physician and/or the Queen's University Occupational Health Services provider or other medical experts as necessary.
    - **Conditions of concern include:**
        - Pregnancy (pregnant women may need to take extra precautions or be reassigned to other duties early in their pregnancy because certain microorganisms can damage the fetus and because their own immune responsiveness may be altered)
        - Immune deficiency
        - Immune-suppressive drugs (e.g. with organ transplantation)
        - Anti-inflammatory medications
        - Cancer and treatment for cancer
        - Age (the elderly; also, very young children are more susceptible to infection, which is one of the reasons that they are not permitted in research laboratories)
        - Other conditions as determined by the person's physician

- **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

laboratory. The frequency of re-inspection depends on the containment level of the laboratory.

When **new biohazardous material** is introduced into the lab (e.g. a new cell line, new bacterial strain, new viral vector), or when there is a new procedure that might affect the risk of exposure (e.g. sonication, starting to use sharps, starting *in vivo* work), an **application to amend the existing biohazard permit must be made. The amendment application would include an amended risk assessment, inventory, and training documents as appropriate.**

Government Safety Regulations and Policies on Biohazardous Material

It is important that people working with biohazardous materials know about and understand the laws and the regulations that direct what they may and may not do with those materials and how they are to work with them safely. The Queen's Biohazard Manual and the associated Standard Operating Policies and Procedures (SOPs) summarize the information contained in these documents and the procedures that have been put in place to follow them at Queen's. However, the laws, regulations, and standards are the source documents and the standards to which you will be held.

The legislation, regulations and standards that relate to the use of biohazardous material is listed in Appendix III of this manual. **Consult Appendix III to ensure that you are aware of these documents.**

In Canada, all **facilities** that are **handling and storing** human **pathogens** or **toxins**, such as public health laboratories, teaching and research laboratories, diagnostic laboratories in hospitals, and vaccine production plants, are regulated by the Public Health Agency of Canada (PHAC) under the authority of the ***Human Pathogens and Toxins Act*** **(HPTA)** and the ***Human Pathogens and Toxins Regulations*** **(HPTR)**. Canadian facilities that import **animal pathogens**, infected animals, animal products or by-products (e.g., tissue, serum), or other substances that may carry an animal pathogen or toxin or parts thereof are regulated under the Canadian Food Inspection Agency (CFIA) ***Health of Animals*** **Act (HAA)** and ***Health of Animals Regulations*** **(HAR)**. PHAC and the Canadian Food Inspection Agency (CFIA) are jointly responsible for the importation and **transfer** of animal pathogens and toxins under the HAA and HAR.

Anyone working with human or terrestrial animal pathogens must have a licence from the Public Health Agency of Canada that permits the specific activities with that particular risk group of material. **Queen's University has a Pathogen and Toxin Licence that includes**:

- Risk Group 2 human pathogens and Toxin Licence under section 18 of the Human Pathogens and Toxins Act and
- Risk Group 2 Terrestraial Animal Pathogen Permit under section 160 of the Health of Animals Regulations

Queen's Pathogen and Toxin Licence was issued based on a description of our biosafety program. **If researchers have a permit from the Queen's Biohazard Committee, then they will be covered by the Queen's PHAC licence for the work with the type biohazards that it covers.**

Biohazardous material not permitted by the PHAC licence will require a separate permit from CFIA. Contact the Biosafety Officer for assistance with this if you work with:

- Foreign animal diseases (including parts of an agent e.g. SV40 large T antigen)
- Emerging animal diseases
- Aquatic animal pathogens
- Bee pathogens
- Any live animal, animal product and by-product infected by an animal pathogen

The key document governing the use of biohazardous materials that affect humans or terrestrial animals in Canada is the Canadian Biosafety Standard 3rd Edition, 2022 (CBS).

- The CBS sets out the physical containment, operational practice, and performance and verification testing requirements to ensure the safe handling and storing of human and terrestrial animal pathogens and toxins.
    - **The CBS describes the best practices for work with all human or terrestrial animal pathogens and, under Canadian law, must be followed for Risk Group 2 pathogens and above.**
    - The CBS updates and harmonizes three previous Canadian biosafety standards and guidelines for the design, construction and operation of facilities in which pathogens or toxins are handled or stored.
    - Replaces the following documents:
        - ***Canadian Biosafety Standards and Guidelines, 1st*** Edition, 2015 (PHAC)
        - ***Terrestrial animal pathogens:*** Containment Standards for Veterinary Facilities, 1st Edition, 1996 (CFIA)
        - ***Prions:*** Containment Standards for Laboratories, Animal Facilities and Postmortem Rooms handling Prion Disease Agents, 1st Edition, 2005 (CFIA)

- The Standards section may need interpretation. Note the explanatory notes appendix to the CBS, the recommendations below on how to use the CBS, and the additional information in the **Canadian Biosafety Handbook (CBH)**.
- Consult the University Biosafety Officer if you need assistance.

- **The Canadian Biosafety Standard (CBS) contains:**
  - **Matrices** that are the risk-based containment requirements for any laboratory using:
    - human pathogens (Risk Group 2 and above)
    - prions
    - terrestrial animal pathogens if they are imported into Canada
    - non-indigenous terrestrial animal pathogens
    - toxins from microorganisms listed in Schedule 1 of the HPTA (*these are also listed in Queen's Biohazard Permit application forms*)
    - Requirements for the importation, exportation, transfer between laboratories and institutions within Canada, and the transportation of the pathogens it regulates.
  - The CBS **appendix of explanatory notes** interprets each of the requirements in the matrices.

The Canadian Biosafety Handbook (CBH), 3rd Edition, 2022 is a national guidance document for the safe handling and storing of human and terrestrial animal pathogens and toxins.

- The *Canadian Biosafety Handbook* (CBH), 2nd Edition, 2016 is intended as a companion document to the *Canadian Biosafety Standard* (CBS), 2nd Edition, 2015. It is a guidance document for Canadian facilities where **human and terrestrial animal pathogens** are handled, that updates the guidelines originally published as Part II of the *Canadian Biosafety Standards and Guidelines* (CBSG), 1st Edition, 2013.
- The CBH includes concepts that are fundamental to the development and maintenance of a comprehensive, **risk**-based **biosafety** management program; however, it does not provide specific guidance or **standard operating procedures (SOPs)** for individual pathogens.
- Throughout the Queen's Biosafety Manual, you are directed to consult different sections of the CBH if you require more information on various topics.

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.

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 otherwise missing.
- when a licence holder decides to prohibit the holder of an HPTA Security Clearance from accessing a licensed facility, including the reasons for that decision.
- when the designated BSO has changed.
- where an SSBA is not received within 24 hours of the date and time when it was expected to be received; and where the holder of an HPTA Security Clearance is convicted of a criminal offence.

Biohazard Committee

- Advisory responsibility for the control of biohazardous material lies with the Biohazard Safety Committee and the University Biosafety Officer who is a member of this committee
- Meets on a regular basis and receives briefings on biohazard safety issues
- Reviews and approves requests to use biohazardous material on campus
- Responsible for reviewing and approving policy and procedures regulating the importing, handling and disposal of biohazardous materials
- Other responsibilities as detailed in the Terms of Reference final version Dec 2006.pdf (queensu.ca) for the Committee (available on the safety website)

Biosafety Officer

At Queen's the Biosafety Officer is assisted in their role by the Safety Technician (Biohazard and Chemical).

- Work with the Queen's University Biohazard Committee to translate the requirements of external bodies and legislation into specific policies and procedures for the University
- Promote a high standard of safe practice within University laboratories which handle biohazardous agents
- Provide information and advice on safe import, export, handling, decontamination and disposal of biohazardous agents
- Review applications for Biohazard Permits
- Monitor compliance by conducting site visits
- Act as the primary emergency response person for incidents involving biological materials

The roles and responsibilities of the Biosafety Officer are delineated in the Human Pathogens and Toxins Act and the Canadian Biosafety Standards.

CBS section 4.1.2:

A biosafety representative(s) (i.e., designated **biological safety officer** [BSO] in licensed **facilities**) with the knowledge appropriate for the **containment levels** and pathogens and toxins handled, to be designated for the oversight of biosafety and **biosecurity** practices including:

- verifying the accuracy and completeness of **licence** applications, **animal pathogen import permit** applications, and **transfer** applications for the **movement** of material imported under the ***Health of Animals Act*** (HAA) and ***Health of Animals Regulations*** (HAR), as applicable;
- communicating with the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) on behalf of the licence holder and animal pathogen import permit holder, as applicable;
- promoting and monitoring compliance with applicable legislation (including the ***Human Pathogens and Toxins Act*** [HPTA], ***Human Pathogens and Toxins Regulations*** [HPTR], HAA, and HAR), conditions of licence and applicable conditions of animal pathogen import permits; applicable biosafety and biosecurity standards, and the **Biosafety Manual** and **standard operating procedures** (SOPs), which includes, but is not limited to;
    - arranging and documenting appropriate biosafety and biosecurity training for personnel pertaining to human and **animal pathogens** and toxins, as applicable;
    - informing the PHAC of all occurrences of inadvertent possession of a human pathogen or toxin not already authorized by the licence;

    - 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 and revise Standard Operating Procedures as necessary
- Provide any necessary labeling, waste packaging and containers
- Facilitate the pick-up and disposal of hazardous waste on campus
- Provide Queen's WHMIS training and other training as required
- Ensure that biological safety cabinets are properly certified
- Provide support for the Biohazard Committee

Department Heads

- Read and be familiar with the contents of this Biosafety Manual and ensure that it is followed in their Department
- Read and ensure compliance with any relevant Standard Operating Policies and Procedures released by the Department of Environmental Health and Safety
- Make the Biohazards Safety Manual available to everyone in the Department, including support staff, summer students, co-op students, undergraduate honours students, contract workers, etc.
- Ensure that ALL faculty, students, technicians, and any others who may come into contact with biohazards within their Department are properly trained in the handling of biohazardous material
- Read and attest as follows for each approved Biohazard Permit in their Department:

- 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 materials in their laboratory:
        - to fulfill the inventory requirements of the CBS (R4.10.2)
        - to be provided to the Biohazards Committee as part of the Biohazard Permit process
        - to be updated when materials are added or destroyed
- Amend Biohazard Permit
    - When biohazardous materials or procedures change
        - Amendment event through TRAQ; attach revised risk assessment and inventory (use track changes to facilitate review)
    - When new grants are awarded
    - when new authorized personnel are to be added to the permit follow the current procedure in the Biosafety section of the EH&S website

- 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 other training is refreshed as required including:
        - training through Environmental Health and Safety
        - lab-specific training
- Ensure that all individuals under their supervision complete the biosafety training described on the Department of Environmental Health and Safety website under the Biosafety dropdown menu. This will include an online Biosafety Quiz and other training depending on the biohazards in the lab.
- Adequately train those who work in their lab in the lab-specific safe handling of biohazardous agents as described in the approved Biohazard Permit and associated documents
    - For Containment Level 2 laboratories, perform a training needs assessment at a minimum annually as required by the CBS. i.e. decide whether or not anything has changed in your program that requires an update of the training statement associated with your biohazard permit in the Romeo system. This review process will be performed and documented through the annual biohazard renewal application.
    - make these permits available to their lab personnel for their review and answer any questions that they may have about them
    - make available any amendments and their associated risk assessment summaries, and any laboratory-specific SOPs
    - Maintain documentation of training on the safe handling of biohazardous materials, chemicals, toxins etc., using the Student/Employee Orientation Checklist available in the Forms section of the Department of Environmental Health and Safety website .
        - You may add and delete items on this training checklist to make it appropriate for your laboratory by requesting a Word version.
- Ensure that all individuals working in their laboratory receive the appropriate immunizations, antibody titre checks, and any other medical surveillance that may be required.

- 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

Queen's Biosafety Training Program

The Queen's University Biohazard Committee requires that the Principal Investigator ensure that all personnel have both general and laboratory-specific training in the handling of biohazardous material.

An overview of the biosafety training program is provided below. Read the current detailed description of the requirements for biosafety training on the Training Requirements section of the Biosafety dropdown menu of the EH&S website.

The training program is designed:

1. So that training starts as soon as an individual joins a laboratory both for their personal safety and so that they can begin supervised work without waiting for a centralized course;
2. To provide centralized accreditation for general biosafety training through the Department of Environmental Health and Safety to ensure that certain information is provided and understood;
3. To promote safety and regulatory compliance (PHAC, CFIA, etc.);
4. To demonstrate an element of "due diligence" in the institution; and,
5. To assist the laboratory should there be an inspection by a regulatory agency.

T**ake one of the centralized biosafety quizzes** through the Department of Environmental Health and Safety to demonstrate their knowledge. The Biosafety quiz registration link is located at the bottom of the training section of the Environmental Health and Safety website, which will redirect you to login on onQ. Which course to take is specified in your biohazard permit training statement.

- **Those working in level 1 laboratories** are not required to know certain sections of this Biosafety Manual, so you are advised to consult the website.

- **Those working in level 2 laboratories** (even if working with Risk Group 1 material) are required to take the combined level 1 and 2 quiz.
- **Those working with human blood and tissue** must take the 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 .

Lab-specific hands-on training and reading including local biohazard risk assessments and SOPs are all important aspects of your biosafety training. This training must be documented using the New Employee/Student Safety Orientation Checklist.

- Note that the checklist should be modified by adding any specific training required for work in your laboratory (e.g. Cholera Toxin SOP, lentiviral vector SOP and quiz, hands on training on procedures associated with cell culture)

Emergency Procedures Training
A template document for Emergency Procedures is available on the Environmental Health and Safety Website.

- This document must be modified to be specific for your laboratory, **updated annually,** dated, and posted in the laboratory where everyone knows its location and its content.
- **Refresher training on these Emergency Procedures must be provided annually and documented**.

If you wish further information, videos and other biosafety training is available on the Public Health Agency of Canada (PHAC) website. These can be accessed at https://training-formation.phac-aspc.gc.ca/course/index.php?categoryid=2&lang=en Sign in or register and follow the instructions to access the videos.

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 Department of Environmental Health and Safety should be consulted early in the design process and before drawings are put out for tender.**

The descriptions below highlight some of the essential features of containment laboratories.

Level 1 Labs

Level 1 labs should:

- Be separated from public areas by a door which should be kept closed when biohazards are in use
- Have surfaces that can be readily cleaned and resistant to any disinfectants or other chemicals in use
- Have fly screens on any windows which can be opened
- Provide hooks for lab coats separate from personal clothing
- Have hand-washing stations, ideally near the exit
- Where indicated by the chemical hazards in the laboratory, must have Emergency eyewash facilities and emergency shower equipment in accordance with Queen's University's Emergency Eyewash Stations and Safety Showers Standard Operating Procedure SOP-LAB-03 .
- Have all appropriate door signage (e.g. biohazard sign, containment level, contact information, type of biohazardous material in use and any entry requirements such as PPE.

Level 2 Labs

A summary of the physical requirements for a level 2 laboratory is provided below. Laboratory personnel should check these systems and ensure that they are maintained. See Appendix II of this manual or the CBS Chapter 3 for more detail regarding the **CBS requirements for CL2 laboratory facilities**, including laboratory location and access; surface finishes and casework; heating, ventilation, and air conditioning; the containment perimeter and laboratory services (i.e. water, drains, gas, electricity, and safety equipment).

Level 2 labs must:

- Meet all the facility requirements described above for Level 1 laboratories
- **Doors must always be closed** with access limited to authorized personnel only, and doors must be **locked when the lab is not occupied**.
- Have non-absorptive work surfaces that are scratch, stain, chemical, moisture, and heat resistant.
- Where possible it is recommended that all labs have directional air flow into the lab (i.e. lab under negative pressure relative to the corridor). Inward directional air flow is required for some work with Risk Group 2 material, depending on a local risk assessment, and whether or not other primary containment devices are used. Considerations include whether the work will be *in vitro* or *in vivo* and whether small animal or large animal.
- An acceptable means of waste treatment or disposal must be provided.
- Biological safety cabinets are recommended and are often required, depending on the risk assessment.

Level 2+ Labs

Level 2+ is a commonly used term that is not officially recognized by the federal regulators, who prefer the term "level 2 facilities with level 3 operational practices". At Queen's we will continue to use the term "level 2+" with the understanding that it means a level 2 facility that meets or exceeds the minimum requirements stipulated in the CBS, and that enhanced operational practices are used as stipulated in the local risk assessment for the lab and its SOPs.

Level 2+ labs must (in addition to level 2 requirements):

- have directional air flow into the lab.
- all work with biohazardous materials done in a biological safety cabinet (BSC)
- centrifuge rotors must have aerosol-resistant lids that are opened only inside the BSC.

- 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.
        - Regular waste and glass waste should be surface decontaminated and put outside of door for custodians to pick up.
        - Prior to annual floor stripping and sealing, lab personnel must surface decontaminate all readily accessible surfaces in the lab including the floor.

Lab Design Changes

Any changes in lab design or location must be approved by the University Biological Safety Officer.

Containment Equipment and Supplies

Equipment and supplies to contain biological agents and thereby protect individuals and the environment from exposure include such items as biological safety cabinets, HEPA filtered ventilated animal caging, aerosol resistant covers for centrifuge rotors and sealed or screw capped tubes.

The use of key types of equipment commonly employed in the containment of biohazards is described below. The safe use of other equipment commonly used in conjunction with biohazardous material is described in Appendix I. **Read the sections in Appendix I for equipment used in your laboratory.**

Biological Safety Cabinets

Biological safety cabinets (Biosafety Cabinet, BSC) are the primary means of containment for working safely with biohazardous agents. BSCs are designed to reduce the risk of infection by isolating the activities in the BSC from the laboratory environment. BSCs are also frequently used to maintain an aseptic environment for cell culture.

- BSCs have laminar airflow directed through HEPA filters in a way that protects personnel from exposure to biohazardous material in the air in the cabinet when they are used correctly
    - A chemical fume hood is not a BSC.

- 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-03). **If you use a BSC you must read, understand, and follow that SOP. More information about BSCs can be found in Chapter 11 of the CBH.**

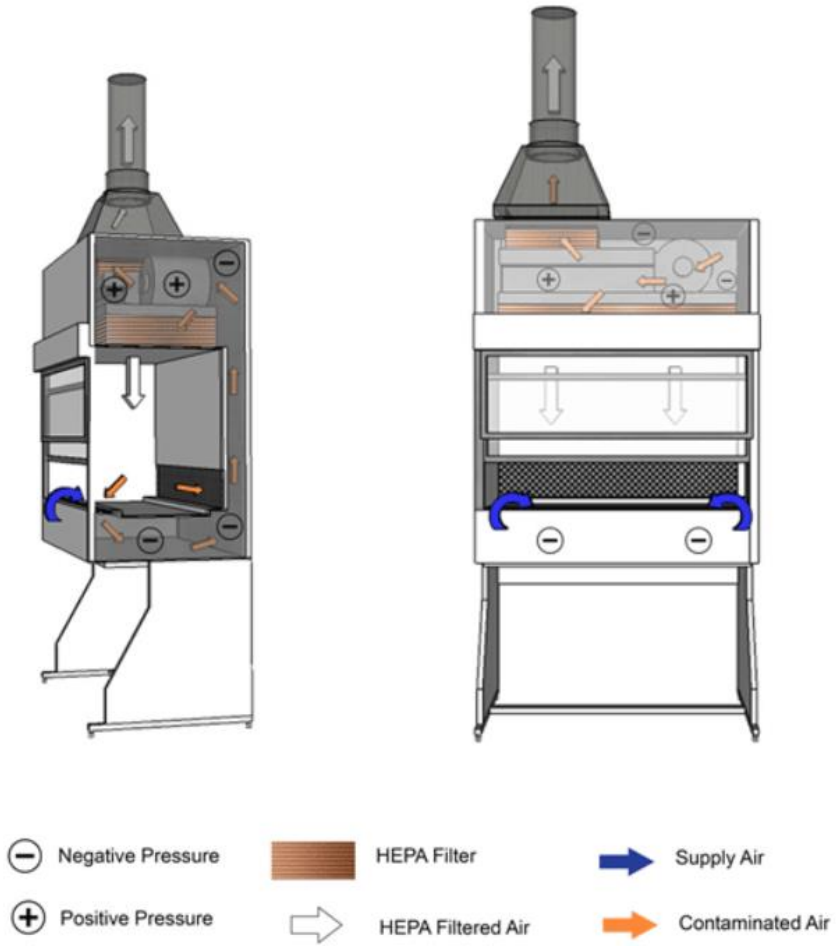


Illustration of a Class two Type A2 Biological Safety Cabinet (BSC)

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.
- If there are indications of cabinet malfunction, do not use your BSC, and call the Department of Environmental Health and Safety for assistance. Indications such as:
    - alarm sounds
    - no airflow
    - reduced pressure on magnehelic gauge (drop> 0.2)
    - unusual noises
- If the power fails or the BSC fails while you are using it, follow the Emergency Procedures posted in your lab.
- BSCs in CL2 laboratories must be inspected and certified:
    - Annually
    - Whenever they are moved (even a few feet within the lab)
- Clean the grille, catch basin and UV light periodically. If there is a spill in the catch basin, some BSC's will have a drain valve for easier cleaning. Please consult your BSC's manual for further detail.

- **BSCs are designed to have only one person working in them at a time**.
    - Long (six foot) cabinets are for experiments that need a large surface area, not for two people.
    - More than one person working in a BSC at one time can lead to disruption of the air curtain, potentially contaminating the cultures or personnel.
- **BSC must be properly located away from areas of high traffic, air vents and opening doors** because the air curtain is relatively weak and hazards can be sucked out of the BSC or contaminants from the room pushed in and contaminate your cultures.
    - Consult the Biosafety Officer before relocating a BSC, or if you think that the one you have might not be optimally located.

Risk Assessments and BSC use:

The use of a BSC to contain Risk Group 2 biohazardous aerosols is strongly recommended (and is usually required, depending on a risk assessment), rather than working on the open

bench and relying on good technique to reduce aerosol generation and personal protective equipment to prevent exposure.

- The decision about whether or not a BSC is required is based on the biohazardous material being used, the concentration and volume of pathogen in use, whether or not the procedures generate significant aerosols, and the qualifications and experience of personnel.

The type of Class II BSC to be used and whether or not it needs direct exhaust also needs to be part of your risk assessment.

Note that a BSC that exhausts directly into the room **does not provide protection against volatile chemicals or radioactive gases**. Depending on the type of BSC the hazard within the BSC may be increased (concentrated) due to recirculation.

If you would not work with a particular hazardous chemical on the open bench then do not work with it in a Class 2 Type A BSC either, **unless** the hazard is a particulate that you know would be trapped by the HEPA filter, **or unless** the BSC has been directly connected to the building exhaust, and even then the amount of hazardous material is limited to minute amounts (see the CBH and consult BSO).

HEPA filters are composed of a mat of randomly arranged fibres, in which particles become trapped. They can remove at least 99.97% of airborne particles 0.3 μm in diameter. Particles of this size are the most difficult to filter and are thus considered the most penetrating particle size (MPPS). Particles that are larger or smaller than 0.3 μm are filtered with even higher efficiency. However, HEPA filters do not prevent gases or radioactive gases from passing through.

Considerations for work *in vivo:*
For CL2 labs, small animal areas or animal cubicles CBS R4.6.24 states: A certified BSC to be used for procedures involving open vessels of infectious material or toxins that:

- may produce infectious **aerosols** or aerosolized toxins, when aerosol generation cannot be contained through other methods;
- involve **high concentrations** of infectious material or toxins; or
- involve **large volumes** of infectious material or toxins.
- Not required when collecting samples from or inoculating animals housed in an animal cubicle (i.e. for animals like a dog, sheep, or monkey that are too large to house in isolation caging / vented rack).

Fume Hoods

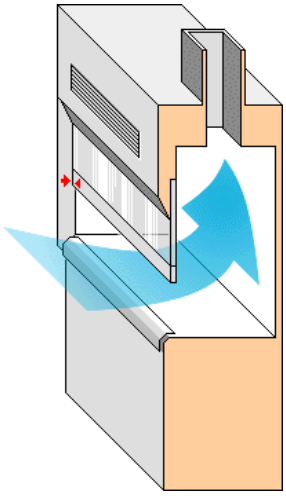


Image of fume hood depicting direction of airflow.

Fume hoods are for collecting potentially harmful chemical gases, vapours, mists, aerosols and particulates generated during the manipulation of **chemical substances**. These harmful substances are usually directly exhausted to the outside of the building where their dilution has been assessed as being sufficient protection by a Certificate of Approval from the Ministry of the Environment.

A biological safety cabinet, not a fume hood, must be used to contain biohazardous aerosols from infectious cultures because this traps potentially infectious microorganisms in a HEPA filter, and does not release them to the environment.

A fume hood may be used for fixing tissues that might contain biohazardous agents. Consult the Biological Safety Officer.

Some work may require a **biological safety cabinet with fume hood-like abilities** (for example, using biohazards with chemicals that produce toxic fumes or volatile compounds labelled with radioisotopes). There is currently only one such BSC on campus, a Class II/B2 cabinet in the Animal Care Facilities in Botterell Hall. There are now new types of BSC Class II/C1 that can switch modes from sending the exhaust out of the building to exhausting into the room. These have advantages over B2 in energy saving and exhaust system requirements.

All fume hoods must be in compliance with the Queen's University Fume Hood Standard Operating Procedure, SOP-LAB-01.

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 infectious material use tubes with outside screw-on closures.** Avoid using tubes with push-in and screw-in closures because when these tubes are opened, the film of liquid trapped between the tube and closure breaks and releases aerosols. Use a vortex mixer with partially filled tubes instead of inverting tubes; wait at least 30 seconds, (and preferably 5 minutes) after shaking a tube before opening the cap

- Open tubes of hazardous infectious material in a biological safety cabinet

Centrifuges

For **low speed centrifugation,** sealed centrifuge buckets with o-rings (safety caps) are recommended for level 2 material. Safety caps are strongly recommended for known infectious level 2 material (e.g., samples from infected patients, RG2 bacteria, viruses, and viral vectors). For level 2+ work, safety caps are **required** and centrifuge buckets must be opened only in a biological safety cabinet.

Microcentrifuges

Microcentrifuges should not be placed in the BSC for operation, because air convection during operation compromises the integrity of the containment provided by the BSC. Safety cups for microcentrifuges are now available. Tubes with o-rings should also be considered.

Autoclaves

Autoclaves used for the decontamination of biohazardous materials **must have their efficacy on a representative load validated annually using Biological Indicators and verified weekly** using either Biological Indicators or Chemical Integrators. Note that Chemical Integrators are not the same as Chemical Indicators or autoclave tape, neither of which are approved for the

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.**

The worker who is careful and proficient will minimize the generation of aerosols. A careless and hurried worker will substantially increase the aerosol hazard. For example, the hurried worker may operate a sonic homogenizer with maximum aeration whereas the careful worker will consistently operate the device to assure minimal aeration. Experiments show that the aerosol burden with maximal aeration is approximately 200 times greater than aerosol burden with minimal aeration. Similar results were shown for pipetting with bubbles and with minimal bubbles. **A hurried worker who moves quickly within or in front of a biological safety cabinet, will disrupt the air flow that is essential for containment.**

The following list of good microbiological practices outlines requirements for **all laboratories** handling biohazardous materials at Queen's University. Although the CBS and CBH are the current PHAC/CFIA documents, this list is also based on the previous Public Health Agency of Canada's Laboratory Biosafety Guidelines (3rd Edition, 2004), which more thoroughly described these practices. The reason for this change in the Federal documents is that the Public Health Agency of Canada does not have the authority to regulate Risk Group 1 microorganisms under the Human Pathogens and Toxins Act. Nevertheless, these practices remain the foundation of good work with infectious substances and their use is an important safety measure. Some of the guidelines listed below are covered in greater detail and/or clarified in other sections of this manual.

Good Microbiological Practices:

1. A documented procedural (safety) manual must be available for all staff, and its requirements followed; it must be reviewed and updated regularly.

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 minimized; paperwork and report writing should be kept separate from biohazardous 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 circumstances (e.g., accidents), eye and face protection must be used. Careful consideration should be given to the identification of procedures requiring eye and face protection, and selection should be appropriate to the hazard.
12. Gloves (e.g., latex, vinyl, co-polymer) must be worn for all procedures that might involve direct skin contact with biohazardous material or infected animals;
    a. gloves are to be removed when leaving the laboratory (or use a one glove technique if transporting hazardous material)
    b. disposable gloves should be discarded after use and never reused
    c. decontaminated with other laboratory wastes before disposal

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/zone and have been proven to be effective in decontamination).

16. The use of needles, syringes and other sharp objects should be strictly limited; use safety engineered sharps when feasible; needles and syringes should be used only for parenteral (through the skin) injection and aspiration of fluids from laboratory animals and diaphragm bottles; caution should be used when handling needles and syringes to avoid auto-inoculation and the generation of aerosols during use and disposal; where appropriate, procedures should be performed in a BSC; **needles should not be bent, sheared, recapped or removed from the syringe**; they should be promptly placed in a puncture-resistant sharps container (in accordance with Canadian Standards Association [CSA] standard Z316.6-95(R2000)) before disposal.

17. Hands should be washed with soap and water or otherwise disinfected (e.g., sanitized) after handling specimens that contain microorganisms (if gloves are not worn), after handling animals, immediately after removing gloves, and before leaving the work area. ***Note that even though gloves are worn hands still need to be washed.*** Hands must also be washed before leaving the laboratory and at any time after handling materials known or suspected to be contaminated.

18. Work surfaces must be cleaned and decontaminated with a suitable disinfectant at the end of the day and after any spill of potentially biohazardous material; work surfaces that have become permeable to biohazardous material (i.e., cracked, chipped, loose) must be replaced or repaired.

19. All items that come into contact with biological material, including liquid or solid wastes, should be decontaminated before disposal or reuse. Contaminated materials and equipment leaving the laboratory for servicing or disposal must be appropriately decontaminated and labelled or tagged-out as such.

20. Efficacy monitoring of autoclaves used for decontamination with biological indicators must be done regularly (i.e., consider weekly, depending on the frequency of use of the autoclave), and the records of these results and cycle logs (i.e., time, temperature and pressure) must also be kept on file for 5 years.

21. All contaminated materials, solid or liquid, must be decontaminated before disposal or reuse; the material must be contained in such a way as to prevent the release of the contaminated contents during removal (eg. double-bagged); centralized autoclaving facilities are to follow the applicable containment level 2 requirements.

22. Disinfectants effective against the agents in use must be available at all times within the areas where the biohazardous material is handled or stored. Bottles must have label with expiration date.

23. Leak-proof containers are to be used for the movement/transport of infectious materials within facilities (e.g., between laboratories in the same facility).

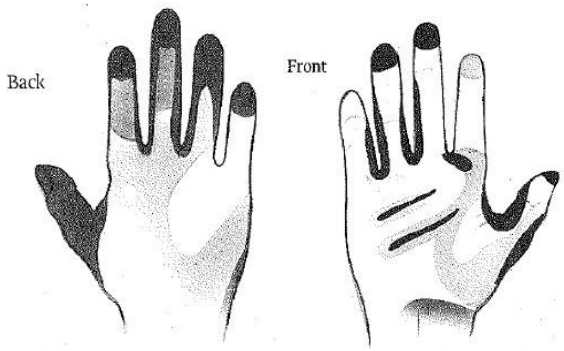
24. **Spills, accidents or exposures to infectious materials and losses of containment must be reported immediately to the laboratory supervisor and the University Biosafety Officer;** written records of such incidents must be maintained (in EH&S), and the results of incident investigations should be used for continuing education. **Such incidents involving level 2 material must be reported by the BSO to PHAC.**

25. An effective rodent and insect control program must be maintained. (*At Queen's if you see evidence of rodents or insects call the Department of Environmental Health and Safety who will notify the exterminator this is on contract with the university.)*

In addition, **consideration should be given to limiting the use of personal electronic devices in the laboratory**. These are a source of microbial contamination from outside the lab and may become contaminated by material used in the lab. If they must be used, **devices with touch-screens will work inside a plastic zip-lock bag** that can be disinfected before removing the device. **Ear buds should not be worn in the lab when working with level 2 material.**

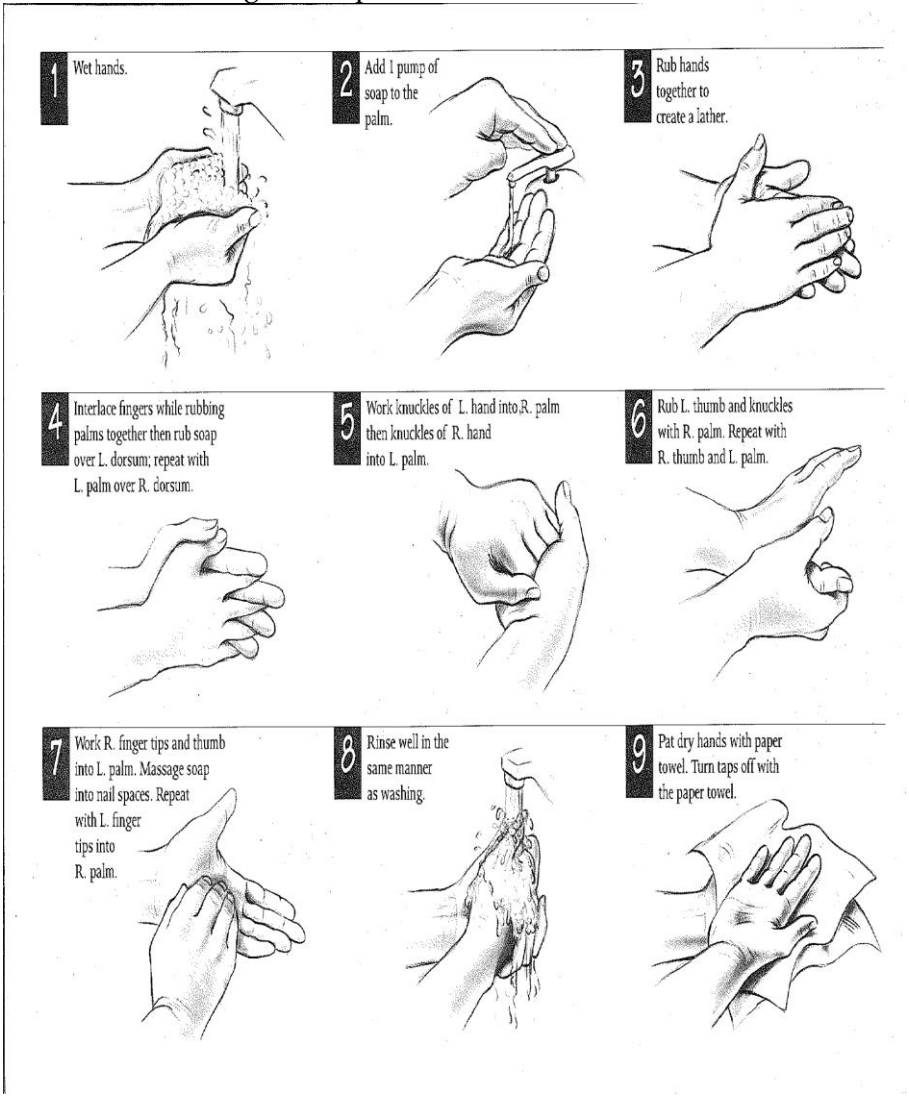
When an individual is at increased risk when working with the RG1 biological material (e.g. **immunocompromised individual** working with an opportunistic RG1 pathogen), consideration should be given to moving the work into a CL2 zone in a Biological Safety Cabinet. Without needing to reveal personal medical information, consult your supervisor, and the Biosafety Officer. Medical advisors will be consulted.

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. Laboratory supervisors, in consultation with the Biosafety Officer/Biosafety Committee, must perform a local risk assessment to determine which procedures and what concentrations and volumes necessitate the use of a BSC or other primary containment device.

When moving/transporting RG2 biohazardous material **outside of a containment zone** (e.g. moving out of containment to the autoclave, between floors in a building or between buildings), the material must be **labelled, double contained, and surface disinfected**.

Other level 2 requirements relate to:

- appropriate biohazard signage outside each laboratory,
- restriction of entry to those on official business,
- written emergency procedures,
- training and authorization (or direct supervision/accompaniment) of anyone working in the containment zone.

The specific operational requirements for work in CL2 laboratories and animal facilities have been extracted from the CBS Chapter 4 for your convenience (Appendix II). **If you work in a CL2 lab then read Appendix II.** If you do not understand any of the requirements, further explanation of each requirement is found in the CBH, or contact the Biosafety Officer.

Containment Level 2+ Operational Practices

In addition to the general and level 2 requirements for operational practice, containment level 2+ labs must:

- meet the operational requirements for level 3 labs that can be followed in a level 2 facility, as outlined in the matrices in the CBS Chapter 4 (2nd Edition, 2015)
- recognize that practices in level 2+ containment labs depend on the microorganisms in use
- specific procedures must be developed which ensure that the microorganisms are being handled safely

- 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. Centrifugation of infectious materials must be carried out in closed containers placed in sealed safety cups that are opened and unloaded in a BSC. Safety cups must be decontaminated before removing from the BSC.
3. The use of needles, syringes and other sharp objects is usually strictly limited because many of the pathogens requiring the additional CL3 operational procedures are transmitted through the percutaneous route of infection.

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, it is possible to appeal and have the RG designation changed if there is scientific evidence to support the appeal.

Note that judgment is required and a local risk assessment for the work is required because:

- the pathogenicity of different strains can vary markedly;
- within a Risk Group there is a range of pathogenicity;
- the work that will be done will affect the probability of exposure.

Bacteria are single-celled prokaryotic organisms lacking a nucleus and other membrane-enclosed organelles. Morphologically 0.5–5.0 μm in size, bacteria are spherical (cocci) or appear as rods (bacilli) that may be straight, curved, spiralled, or tightly coiled. Based on Gram staining and morphology, more than 4,000 bacterial species have been classified into one of the following three phenotypes: Gram-positive, Gram-negative or mycoplasma (bacteria lacking a cell wall). Bacteria vary in their requirements for oxygen, being described broadly as either aerobic, microaerophilic or anaerobic. Some bacteria can induce an extreme immune response (e.g., inflammation), secrete exotoxins, produce surface-associated endotoxins (i.e., lipopolysaccharides or lipooligosaccharides), or form spores that enhance survival and transmission outside the host for extended periods of time.

Bacteria that can **infect and cause disease** in humans and/or animals are referred to as **pathogenic bacteria.** Many pathogenic bacteria that colonize the body do not cause disease unless a disruption occurs in the host's immune system or natural barriers to infection, or the host is exposed to an excessively high dose of the pathogen, as may occur through activities conducted in a laboratory or an animal facility. Infections with certain pathogenic bacteria almost always result in illness.

Viruses are the smallest of replicating organisms. Their small size (20-300 nm) allows them to pass through filters that typically capture the smallest bacteria (e.g. 0.2 μm filters used to sterilize media). Viruses have no metabolism of their own and, once inside a host cell, they redirect

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-stranded DNA, single-stranded DNA, reverse transcribing, double-stranded RNA, negative-sense single-stranded RNA, positive-sense single-stranded RNA, and subviral agents). There are many families of viruses that are able to infect human and animal hosts. Some are species-specific while others infect a wide range of host species. Some viruses are able to produce a **persistent infection** (i.e., host cell remains alive and continues to produce virus over a long period of time) or a **latent infection** (i.e., there is a delay of months or years between viral infection of the host and the appearance of symptoms), or they may be **carcinogenic** (e.g., integration of an oncogene-carrying retrovirus into host genome).

Fungi are eukaryotic microorganisms that can be easily distinguished from bacteria and other prokaryotes by their greater size and the presence of organelles, including a nucleus, vacuoles and mitochondria. Of the 1.5 million estimated fungal species, approximately 300 are known to cause disease in human and/or animal hosts. Several species of yeast, which normally grow as single cells, and of moulds, which grows in branching chains, are known to be pathogenic to animals and humans. Differences in the virulence of these fungal species are used to categorize them into two main categories: frank fungal pathogens, which can cause disease in healthy hosts, and opportunistic fungal pathogens, which can cause disease in immunocompromised hosts.

The main risk associated with fungi is the exposure to spores that can be transmitted via the airborne route, inoculation, or casual contact, depending on the species. In addition, some fungal species may produce and disperse mycotoxins, which can be toxic. In general, human and animal tissue and blood samples are not considered a risk for the airborne dispersal of fungal spores.

Parasites include protozoa and helminths that live on or within a larger host organism at the host's expense. **Protozoa** are single-celled eukaryotic microorganisms that lack a cell wall and are generally motile; **helminths** are eukaryotic worms that may grow large enough to be visible to the naked eye. Parasites that live within the tissues or cells of their host are known as endoparasites and cause infections that are generally treatable. Some endoparasites can persist for many years in the human body, even following treatment, and will re-surface if the host becomes immunocompromised. Ectoparasites live on the external surface, or within the skin of their host, causing an infestation. The type and degree of injury inflicted on the host will vary based on the number of parasites present and can range from minor to severe.

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:

- the biology of the parent virus and associated risks of the viral vector
- the packaging system
- whether or not the vector system is supposed to be replication incompetent

- 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 syndrome, fatal familial insomnia, and kuru. There are no treatments and no vaccines available for these diseases.

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 present. However, even if the sample is from an apparently healthy individual, reasonable precautions should still be taken since unidentified pathogens that cause chronic or sub-clinical/asymptomatic infections could be present apparently healthy individuals (e.g. Hepatitis B, Hepatitis C, HIV, and SARS-CoV-2).

At Queen's University and many other Universities in Canada, work in research laboratories with tissues (including human blood) that might contain human pathogens requires a Biohazard Permit and is classified as Risk Group 2 material requiring Containment Level 2 facilities and practices. This designation is used because it is the terminology most familiar to personnel in an academic setting. In the health care setting, the term used for practices to prevent the infection of personnel with such material (or from patients themselves) is "Routine Practices". The principles and practices of Containment Level 2 match Routine Practices quite well, but the laws under which they are enforced differ.

Although classified as Risk Group 2, the actual risk (hazard times probability) and the operational practices and the response to an accident will vary depending on the following factors so these questions should be addressed in a risk assessment.

- What is the population that the samples are from and what are the associated risks? Is the population a generally healthy population?
- Is it screened for HIV, Hep B, Hep C, etc. and are samples from positive individuals excluded?
- Are the patients all known to be infected with a human pathogen (e.g. blood from an HIV positive population; vaginal swabs from patients with *Candida albicans* infection)?
- Although most human samples will be treated as risk group 2, it may be advisable to handle those known to contain certain pathogens under level 2+ containment (e.g. HIV positive).

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 significant hazard.
    - Some human cell lines have the potential to harbor a human bloodborne pathogen.
    - The handling of nude mice inoculated with a tumor cell line unknowingly infected with lymphocytic choriomeningitis virus resulted in multiple laboratory acquired infections.
- Growth conditions (e.g., pH, temperature, medium supplements) may cause altered expression of oncogenes, expression of latent viruses, interactions between recombinant genomic segments, or altered expression of cell surface proteins.
- Although mycoplasmas are commonly identified as sources of cell culture contamination, mycoplasma-contaminated cultures have never been reported as a source of a laboratory acquired infection. Nevertheless, the fact that a number of mycoplasmas are human pathogens renders them potential hazards in cell cultures.
    - Mycoplasmas can significantly alter the behaviour of cells, so **routine testing of cell lines for mycoplasma contamination is advisable from a scientific point of view**.

- **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.

- Are the cells fixed or lysed in a solution that would inactivate pathogens that might be present?
- Do the procedures create a risk of infectious aerosols?
    - If so then how are you going to contain these aerosols – in a BSC?
- Can the work be done safety on the open bench in a level 2 lab?
    - Is a barrier and/or eye and mucosal protection required?

Laboratory Animals

The care and use of animals at Queen's University is regulated by the University Animal Care Committee and the University Veterinarian. All work with biohazardous materials that involves animals or the use of animals that carry serious zoonotic pathogens **must also be approved by the Biohazards Committee** to ensure the protection of personnel.

Zoonoses

The term **"zoonoses"** describes diseases that are transmissible between living animals and humans (in either direction). Zoonoses are caused by zoonotic pathogens.

There have been several documented laboratory acquired infections (LAIs) involving zoonotic pathogens transmitted to humans by an infected animal.

The **risk of zoonoses is greater with activities involving first generation wild-caught animals** that may be infected with and carry a pathogen indigenous to the animal's natural environment. Due to the nature of these pathogens, additional precautions may need to be implemented whenever known or potentially infected animals are handled.

Documented zoonoses in humans have been caused by bacteria (e.g., *Salmonella* spp. can cause salmonellosis; *Yersinia pestis* can cause plague), viruses (e.g., rabies virus can cause rabies), parasites (e.g., *Toxoplasma gondii* can cause toxoplasmosis), and prions (e.g., BSE agent can cause vCJD).

In vivo work with Biohazardous Material

Work with biohazardous material in animals presents different hazards than work with the material *in vitro* for a number of reasons. There is a potential for the animal to:

- shed an infectious agent
- transfer infectious material into a worker by scratching or biting
- move while being injected, resulting in a needle-stick injury
- be infected with animal pathogens that are also pathogenic to humans (zoonotic pathogens)
- harbour retroviruses that could recombine with retroviral vectors.

Work with an animal that **involves risk group 2 pathogens** must be done in the level 2 containment rooms in the animal care facilities using protocols approved by the Biohazards Committee.

General protocols have been developed in the Animal Care facility for:

- the entry and exit of scientific staff, animal handlers, animals, biological samples, equipment, and food;
- and for the decontamination of equipment and wastes.

Each time a new agent is introduced *in vivo*, these protocols need to be reviewed as part of the risk assessment and modified if necessary to mitigate the hazard.

Animal Containment

Animal facilities are designed and operated in accordance with the ***Canadian Biosafety Standards (CBS) and*** the ***Policies and Guidelines of 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-SA), where the containment caging and the BSC provide primary containment, the large animal cubicle serves as both the primary and secondary barrier, so specific facility and operational containment requirements in the CBS matrix under the CL2-Ag column must be followed. CL2-Ag requires that an anteroom be provided at the point(s) of entry into/exit from the containment zone or from each animal cubicle and post-mortem room. Particular attention must be given to the use of protective clothing and equipment by staff entering an animal cubicle contaminated with large volumes of infected animal waste. The handler must have knowledge of the animal's general characteristics, such as mentality, instincts and physical attributes, and specific risk mitigation procedures need to be developed and followed.

Allergy

A high percentage of individuals who work with laboratory animals, particularly rodents, acquire a lab animal allergy. Such allergies can be serious, including the development of asthma, and may be career ending. Facilities and procedures should **minimize exposure to allergens**.

Individuals who are **already allergic to any animals should consider the routine use of a fit-tested respirator** (e.g. disposable N95), or at a minimum a procedure mask, to help prevent the development of allergies to laboratory animals or to reduce their exposure and symptoms if they are already allergic. Contact the Department of Environmental Health and Safety to be enrolled in the Respiratory Protection Program as described in SOP-Safety-05 on the Safety website.

Biological Toxins

Biological toxins are poisonous substances that are a natural product of the metabolic activities of certain microorganisms, plants, and animal species. Toxins can cause adverse health effects, severe incapacitation, or death in a human or animal, even when present at relatively low levels in host tissues. Some toxins can be artificially produced by chemical synthesis or by genetic engineering and rDNA technology. Toxins are classified according to the organism from which the toxin is derived (e.g., bacterial, fungal, plant, animal), although toxins are typically associated with bacterial disease.

Two types of bacterial toxins exist: exotoxins and endotoxins.

- **Exotoxins** are often heat-labile proteins and polypeptides that are produced and secreted or released by a variety of species, including both Gram-negative and Gram-positive bacteria.
- Bacterial **exotoxins** can be classified in five main groups based on their effect on the host, as follows:
    - damage to cell membranes
    - inhibition of protein synthesis
    - inhibition of release of neurotransmitters
    - activation of secondary messenger pathways
    - activation of host immune responses.
- **Examples of exotoxins** include tetanus toxin, produced by the Gram-positive bacterium *Clostridium tetani*, and cholera toxin, produced by the Gram-negative bacterium *Vibrio cholerae*.
- A family of heat-stable exotoxins exists, called **enterotoxins,** which exert their primary effects on the digestive tract. They include Staphylococcus Enterotoxin Type B produced by *Staphylococcus aureus*, heat-stable enterotoxins produced by enterotoxigenic *Escherichia coli* (ETEC), and cereulide produced by *Bacillus cereus*.
- **Endotoxins** are generally less toxic than exotoxins and are heat-stable. They are structural molecules (i.e., lipopolysaccharides or lipooligosaccharides) that 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

- 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 toxin**s" 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

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 edition, 2020)

Two recommended biosafety texts are available in the Queen's University Bracken Library:

Title: Biological Safety: Principles and Practices, Editors: Diane O. Fleming, Debra L. Hunt., Edition: 4th ed., Published: Washington, D.C. : ASM Press, c2006., **Location:** Bracken Health Sciences, Call Number: WA485 .B615 2006

Title: 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

PERSONAL PROTECTIVE EQUIPMENT

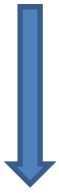
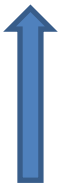
It is important to select the appropriate personal protective equipment (PPE) for the specific work to be done. Engineering controls are more effective than PPE and so they must always be considered first, and their use implemented when feasible. Be aware that PPE can provide a false sense of security, particularly if it is inappropriate or not maintained properly. Poorly chosen PPE may contribute to hazards by impairing performance e.g. stiff, bulky gloves reduce dexterity. The personal protective equipment to be used in a particular laboratory for work with biohazardous material must therefore be described in the Biohazard Permit application. The sections below describe considerations for different types of PPE.

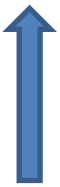
Dedicated PPE suitable for the containment zone and the work being done is donned (put on) upon entering the containment zone to protect personnel from contamination. These items are not worn or stored outside the containment zone, except following appropriate disposal or decontamination procedures, in order to reduce the risk of releasing potentially contaminated material from containment. The border of a containment zone in lower containment levels (i.e., CL1 and CL2) can include many areas that are connected by corridors, based on a local risk assessment (LRA). Users can specify in their procedures where certain PPE, such as lab coats, can and can't be worn in relation to their LRA.

PPE must be donned (put on) and doffed (removed) in an order that minimizes the risk of contamination of skin, hair, and personal clothing. The tables below provide examples for a standard CL2 lab, and for a situation where a number of types of PPE are worn (CL2-SA or CL2-Ag).

A written donning and doffing procedure for the PPE worn in your laboratory must be developed and posted:

- Figures with images may be used and are recommended. Such figures may be found on the internet but must accurately represent the PPE that your lab uses.
    - At a minimum use one of the tables below, amended for the PPE used in your laboratory.
    - If applicable, post the first table below near where you store PPE (which should be stored near entrance if possible).
    - The second table provides an example for a more complex situation.

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 • Lab coat (properly fastened) • Outer gloves (fitted over cuffs of lab coat)	

Donning Order (Descending)	Generic Example of the Donning Procedures when Multiple Layers of PPE are Worn	Doffing Order (Ascending)
	Inner gloves. Dedicated containment clothing, such as scrubs, dedicated footwear, shoe covers and when required, head covers. Back-closing gown or equivalent protective layer. Mask or respirator. Eye protection, including safety glasses, goggles, and face shield. Outer gloves, fitted over gown cuffs.	

Body

Lab coats should be worn in all labs, and may be worn in hallways of areas with restricted access if hazardous materials are being transported.

- Lab coats with knit cuffs are recommended so that a glove can be pulled up over the cuffs. Cuffs also reduce the hazard of catching a loose sleeve and causing a spill.
- Lab coats with snaps rather than buttons are recommended so that they can be removed quickly.
- There should be dedicated lab coats for level 2 laboratories that are left in that laboratory rather than being used in level 1 laboratories.
- Lab coats may not be worn in washrooms, lunchrooms, conference rooms, public elevators, or offices or other areas where food or beverages are consumed.

    - Lab coats may not be worn while eating, drinking, or chewing gum.
    - Lab coats are to be hung on hooks as close to the exit as feasible.
    - Lab coats may not be hung on top of another lab coat due to the risk of contaminating the inside of the coat.
- For highly infectious agents, surgical gowns with back closures and knitted cuffs offer superior protection. These should not be worn outside of the room in which they are used.
    - Disposable water resistant gowns are available in the stores in Botterell Hall.
- Plastic or rubber aprons or water resistant gowns are to be worn for activities that are likely to result in splashes of infectious agents.

Any lab coats which are known or suspected to be contaminated with pathogens must be successfully decontaminated, by autoclaving or soaking in bleach (or other suitable disinfectant), before laundering. Lab coats should not be taken home. Lab coats should be washed by an approved laundry service.

Eye

Goggles and a face shield should be worn as required to protect from possible splashes, aerosols, or other relevant hazards.

Face shields are considered **secondary protectors** and only provide adequate eye protection when worn with safety glasses or goggles.

- **Face shields should be worn when removing tubes from liquid nitrogen due to the risk of tubes exploding if liquid nitrogen has leaked into them.**

Safety glasses with side shields provide general eye protection but **safety goggles** offer superior eye protection from splashes.

It may be advisable in some cases to wear eye protection even when working at a biological safety cabinet to prevent individuals from touching their eyes with contaminated gloves.

The wearing of **contact lenses** does not provide adequate protection against biological, chemical, or particulate hazards. The wearing of contact lenses in the laboratory where chemical or biological hazards are used is permitted only when CSA approved protective eyewear is worn, and other forms of corrective eyewear are not suitable. Inserting or removing contact lenses is not permitted in any laboratory.

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 puncture resistance; fine metal mesh gloves are recommended where both dexterity and protection from sharps are needed (although they are not considered effective against needle sticks they can protect against cuts from blades and some animal bites).
- **Insulating gloves** or mittens should be worn when handling **high-temperature** materials (e.g., recently autoclaved materials) or **low-temperature materials** (e.g., metal boxes from a -80°C freezer or liquid nitrogen).

Some **toxic chemicals** will pass rapidly through some glove material. This has resulted in fatalities. Ensure that the glove material provides protection against any chemicals being used. A **general guide for choosing the appropriate glove type is provided in the table below** from the CBH (section 9.1). However, the quality of gloves varies so **for chemical hazards check the specifications including breakthrough time, permeation rate and degradation of physical properties for the specific chemical and gloves that you will be using from their manufacturer.**

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
	Iodine	G	G	G	G
	Hydrogen peroxide (30%)	VG	VG	VG	VG
	Sodium Hydroxide (50%)	VG	VG	VG	VG
	Ammonium hydroxide	G	G	G	VG
Phenolics	Hexachlorophene	G	G	G	--
Quaternary ammonium compounds	N, N Didecyl Dimethyl Ammonium Chloride	G	G	VG	--
Aldehydes	Glutaraldehyde (25%)1 Formaldehyde (37% in 1/3 methanol/water)	VG	VG	VG	VG
		F	G	VG	VG
Alcohols	Ethanol (92%) Isopropyl alcohol	F	VG	VG	G
		G	VG	VG	VG
Bisbiguanides	Chlorhexidine digluconate (4%)	F	F	VG	--

F Fair; these gloves show moderate degradation effects, a moderate permeation rate, and have breakthrough times less than 30 minutes.

G Good; these gloves show very little degradation upon exposure to the chemical, have breakthrough times of greater than 30 to 60 minutes, and slow permeation rates.

VG Very good; these gloves show very little degradation upon exposure to the chemical, have breakthrough times of greater than 60 minutes, and slow permeation rates OR were recommended as the preferred glove type by the tester.

-- No data available.

Respiratory

Respirator use must comply with SOP-Safety-05.

N95 or P100 respirators:

- are used for respiratory protection against infectious aerosols and micro-organisms that are infectious via the airborne route.
- are also effective in preventing exposure to lab animal allergens.
- may be disposable, ½ face reusable with filter cartridges, or full face, depending in part on the application, and on the time period for which they will be worn.
- Perform a seal check every time the respirator is donned.
- Never reuse disposable respirators or masks.
- Remove respiratory protection at the point at which a risk assessment deems it safe to do so upon exit from the containment zone.

Anyone requiring respiratory protection must be **properly fitted for a specific respirator model** and size through the Department of Environmental Health and Safety (ext. 32999). Mask fit should be re-checked at least every two years or if significant weight changes or other factors change the shape of the face. **Respirators are not effective if the individual is not clean-shaven.**

An alternative method of respiratory protection is a **Powered Air Purifying Respirator (PAPR)**. They are expensive but are comfortable to wear and can be worn by some people who cannot be fitted for a regular respirator.

Disposable **surgical masks** (procedure masks) are worn to:

- Protect the surgical subject from infection by the surgeon and/or to protect the surgeon from splash hazards such as spurting blood
- Protect the nose and mouth against a splash hazard from an animal, in which case they should be fluid resistant (rated for 160 mm hg)
- To discourage touching of the mouth and nose when working in a biological safety cabinet with infectious material that is infectious by the mucosal route

Disposable surgical masks may provide some protection to the wearer against droplet spread of infection, but **do not** provide significant protection from infection by the aerosol route and or against lab animal allergens.

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. Validation of all decontamination technologies and processes is required prior to initial use and whenever significant changes are implemented or new pathogens are introduced so that decontamination procedures and **standard operating procedures (SOPs)** can be established, amended, or updated as necessary

It is important that decontamination processes and procedures be **monitored (verified)** on a regular basis to confirm that established parameters have been met.

Facility personnel responsible for developing decontamination processes and methods should consider the following. Those working with materials regulated as level 2 pathogens under the scope of the CBS must do the following:

- Disinfectants effective against the infectious material used, and neutralizing chemicals effective against the toxins in use, must be available in the containment zone and used for contaminated or potentially contaminated material, including equipment, specimen/sample containers, surfaces, rooms and spills.
- Decontamination parameters (e.g., time, temperature, chemical concentration) consistent with the technology/method used must be validated to be effective against the infectious material and toxins of concern under the conditions present in that containment zone.
- Clear and strict procedures must be in place to support routine decontamination and routine verification.

- 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).

Chemical Disinfection

Chemical disinfectants are used for the decontamination of surfaces and equipment that cannot be autoclaved (or incinerated), specimen/sample containers to be removed from the containment zone, spills of infectious materials, and rooms and animal cubicles.

The use of disinfectants can impact worker safety directly (e.g., direct exposure to a hazardous chemical) or indirectly (e.g., exposure to viable pathogens when an inappropriate disinfectant is selected).

Containment zone personnel should learn about the products required for the disinfection of the infectious material and toxins with which they will be working, including the recommended directions for use:

- application method
- concentration

- contact time
- PPE
- first aid
- disposal

and chemical characteristics:

- toxicity
- chemical compatibility
- storage stability
- active ingredient
- concentration

The choice of a chemical disinfectant depends upon the resistance of the microorganisms concerned. To be effective, the disinfectant must be in contact with the biohazardous material for the **required contact time**.

The selection of an appropriate chemical disinfectant is dependent on a variety of factors, including the resistance of the infectious material or toxin, the application (e.g., liquid or gaseous), and the nature of the material to be disinfected (e.g., hard surface, porous materials). Consideration should also be given to organic load, concentration, contact time, temperature, relative humidity, pH and stability.

- Product effectiveness depends on the active ingredient(s) and the identity and concentration of other ingredients in the formulation. There are usually striking differences between the activities of disinfectants when used under actual laboratory conditions as opposed to the controlled, standardized testing methods.
- It is advisable for laboratories to **conduct in-use disinfectant efficacy testing** to evaluate a product's performance under their specific conditions of use. **See CBH 15.3 for testing methods.**
- If the microorganism survives, altering the contact time or concentration of the disinfectant, or both, may be required to achieve the desired level of disinfection. Factors that may affect the efficacy of the disinfectant are outlined in CBH Section 15.3.1.

Check the organism's Public Health Agency of Canada Pathogen Safety Data Sheets (PSDS, the MSDS for pathogens), if available, for its disinfectant susceptibility.

Common disinfectants which are often suitable include 10% bleach (freshly diluted, typically 30 minute contact time for liquid cultures and spills), 70% ethanol (10 minute contact time), glutaraldehyde, iodines, phenolics, and formaldehyde. Manufacturer's recommendations should be followed.

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 that alcohol is **not very effective against non-enveloped viruses or bacterial spores**, so it is important to think about what microorganisms are likely to be present in your samples and select a different disinfectant if appropriate.

Bleach

Although **bleach** (sodium hypochlorite, NaClO) is cheap and effective against many microorganisms, it is corrosive to stainless steel (such as in a biological safety cabinet) and corroded surfaces are difficult to decontaminate effectively.

- 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.

It can be effective in reducing airborne and surface contamination provided that:

- the lamps are properly cleaned,
- maintained and
- checked to ensure that the appropriate intensity is being emitted.

UV may be recommended in certain situations (e.g. bacteria in use are highly resistant to chemical disinfection or are spore forming); however, it is important to note that:

- the accumulation of dust, dirt, grease, or clumps of microorganisms reduce its germicidal effects.
- UV light is not effective against all organisms; and
- exposure to UV light is hazardous: it may result in severe eye damage and burns to the skin.
- UV light will degrade plastics

The routine use of UV lamps to decontaminate is not recommended. They should only be used secondary to chemical disinfection of surfaces. **UV lamps must be turned off whenever the laboratory is occupied, unless the BSC sash closes completely.**

Gamma irradiation and microwave irradiation can also be used for decontamination in some cases.

Incineration

Biohazardous waste that **must be disposed of by incineration includes** human or animal anatomical waste, material soaked with blood, biohazard sharps containers, and biohazardous waste that is contaminated with chemicals that would not be compatible with autoclave decontamination.

All hazardous waste, including biological waste to be incinerated, is picked up by an outside contractor directly from laboratories and shops at the request of Queen's employees. Waste needs to be labelled and tagged, and the appropriate forms must be filled out on the Department of Environmental Health and Safety website to request a pickup.

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, or autoclaved or incinerated as appropriate. (e.g.,

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.

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

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 Procedures is available on the Environmental Health and Safety Website.

This document must be modified to be specific for your laboratory. Remove sections that do not apply to your laboratory. It must be updated annually, dated, and posted in the laboratory where everyone knows its location and its content.

Refresher training of personnel on the Emergency Laboratory Procedures must be done annually in each laboratory and the training documented.

BIOSECURITY and DUAL-USE POTENTIAL

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 incidents. The plan includes physical security such as locked doors, and procedures to be followed by those working in and around biohazard research laboratories and by Queen's Security.

The biohazardous materials in use at Queen's are, for the most part, quite common in the Canadian environment and are not cultured in large quantities, so they are extremely unlikely to represent a significant community-wide biosecurity risk.

The largest risk is that of individual illness due to an accidental laboratory acquired infection from certain risk group 2 materials, and this risk is mitigated by the facilities and procedures described in this manual.

It would be possible for someone to take certain risk group 2 materials and intentionally (or accidentally) make someone ill, either inside or outside the containment zone. Such infections would be treatable and highly unlikely to produce fatalities. Nevertheless, unauthorized access to such materials should be prevented.

Biosecurity practices for laboratory personnel:

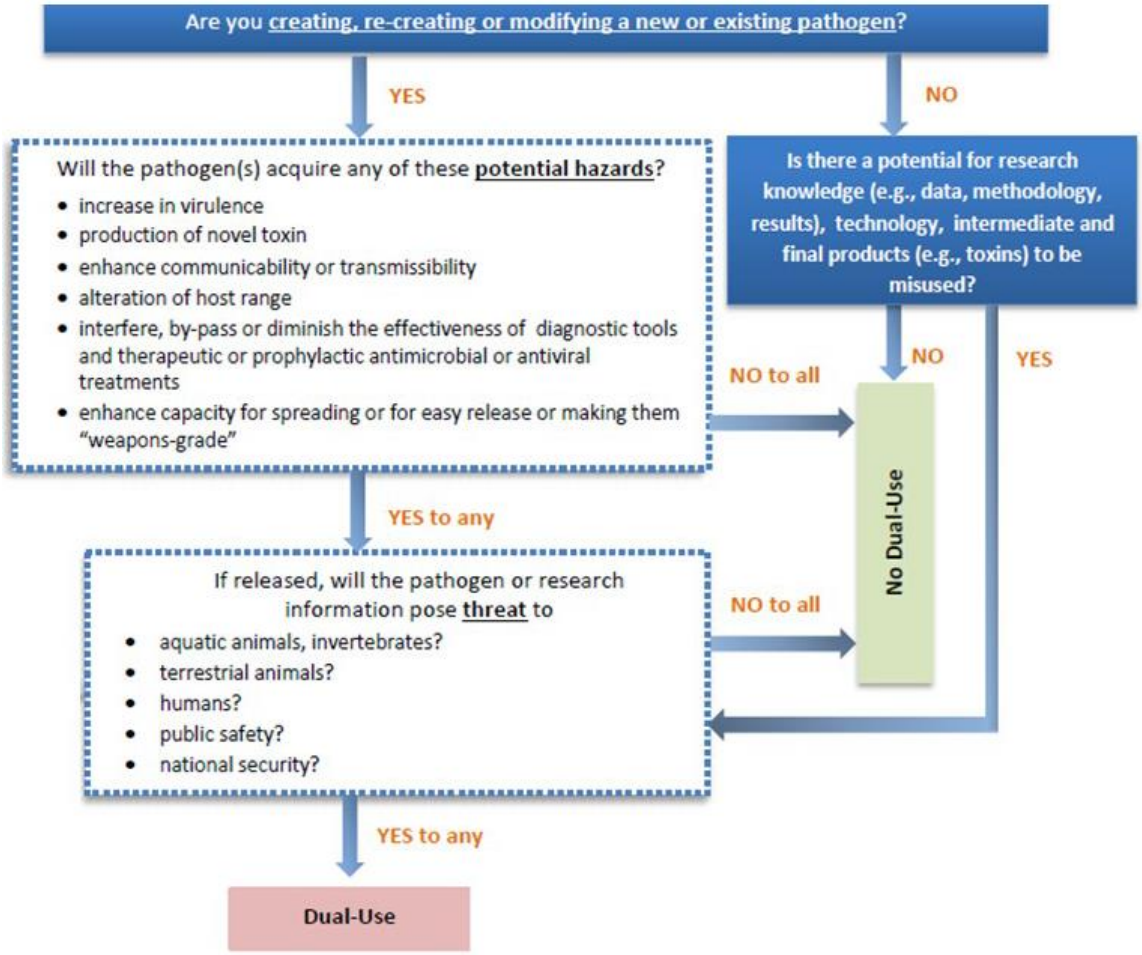
- Lock laboratory doors when the lab is not occupied.
- Lock freezers or other devices in which biohazardous material is stored outside of areas with restricted access.
- Maintain the usefulness of locks by not copying keys, not giving keys, fobs, or other electronic security access devices to unauthorized personnel, and by returning these when you no longer work at the University.
- Challenge anyone unfamiliar to you who is unaccompanied in areas that do not have public access; if not comfortable challenging them then report to your supervisor or Campus Security.
- Do not give biohazardous material to anyone who is not an authorized user on your biohazard permit without permission of your Principal Investigator.
    - Transfer of biohazardous material from one Principal Investigator's lab to another at Queen's requires an amendment to the recipient's biohazard permit.

- 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.


Are you creating, re-creating or modifying a new or existing pathogen?
YES
NO
Will the pathogen(s) acquire any of these potential hazards?
increase in virulence
production of novel toxin
enhance communicability or transmissibility
alteration of host range
interfere, by-pass or diminish the effectiveness of diagnostic tools and therapeutic or prophylactic antimicrobial or antiviral treatments
enhance capacity for spreading or for easy release or making them "weapons-grade"
Is there a potential for research knowledge (e.g., data, methodology, results), technology, intermediate and final products (e.g., toxins) to be misused?
NO
YES
NO to all
No Dual-Use
YES to any
If released, will the pathogen or research information pose threat to
aquatic animals, invertebrates?
terrestrial animals?
humans?
public safety?
national security?
NO to all
YES to any
Dual-Use

IMPORT, EXPORT, and MOVEMENT/TRANSPORT OF PATHOGENS OR MICROORGANISMS

The following is an outline of requirements. For further assistance contact the Biosafety Officer.

Importation or Purchase from Canadian Suppliers of Imported Material

If the biohazardous material you would like to order is not specifically listed on your approved biohazard inventory in the TRAQ/Romeo System, submit an amendment event form through TRAQ with an updated biohazard inventory and risk group document and an updated risk assessment.

- You may only order after the amendment is approved.
- If there is an immediate need to order the material, email the Biosafety Officer in the Department of Environmental Health & Safety for assistance.

The Biosafety Officer will guide you through the importation process, but an overview is provided below to give you an idea of timelines.

The use and importation of human and zoonotic pathogens and most non-zoonotic terrestrial animal pathogens is regulated by the Public Health Agency of Canada (PHAC) in collaboration with the Canadian Food Inspection Agency (CFIA). These pathogens can be imported using the Queen's PHAC licence which is readily available (obtained by contacting the Biosafety Officer).

- The use of biohazardous materials must be approved and listed on the license issued to the University by the Public Health Agency of Canada (PHAC).

- **The University's current PHAC RG2 Licence does not include**:
    - **prions or Security Sensitive Biological Toxins above the trigger quantity.**
        - Contact the Biosafety Officer as soon as you anticipate a need to work with this material because the required preparations will take months.
    - **Risk Group 3 or 4 agents.**
        - A few risk group 3 agents can be used in containment level 2 facilities with additional precautions as indicated in written statements (Directives) from PHAC.
            - However, work with these agents still requires a PHAC RG3 Licence to possess the agent.
            - Contact the Biosafety Officer months in advance of when you wish to begin work with such agents.
        - The university currently does not have the facilities required to work with microorganisms that require Containment Level 3 or 4 facilities.

- **An import permit will likely be required for material not covered by our PHAC HPTA RG2 licence, increasing the work involved and the lead-time required.**
- The Canadian Food Inspection Agency (CFIA) regulates the use and importation of **exotic non-zoonotic terrestrial animal pathogens, aquatic animal pathogens, and plant pests**. Also, some **animal blood, serum, animal products or by-products require a CFIA import permit because they might contain pathogens exotic to Canada**.
- The country of origin will be a factor in determining if an import permit is required for animal material. Consultation with CFIA will be required and the Biosafety Officer can assist. Before an import permit can be requested, a My CFIA account must be set up online with the assistance of the Safety Technician (Biohazard and Chemical).
- **CFIA processing time for CL2 compliance letters, import permits and courtesy letters is up to 10 business days.** Therefore, contact the Biosafety Officer well ahead of when you want to import material regulated by CFIA.
- If you do not already have a CFIA CL2 compliance letter, filling out the required checklist usually takes about 1 ½ hours with the assistance of the Biosafety Officer and/or the Safety Technician (Biohazard and Chemical).
- The import permit will stipulate the containment requirements.
- A copy of the CFIA facility approval and the import permit must be sent to the Biosafety Officer for the Principal Investigator's Biohazard Permit file.

Some Canadian Suppliers have import permits for biohazardous materials requiring CL2. E.g. Cedarlane supplies ATCC cells. **They require proof of the appropriate federal licences or permits before they will ship this material.**

It is important to keep in mind that the Biosafety Officer must be consulted during the import or export process of pathogens and microorganisms.

AIRS (Automated Import Reference System)
The Automated Import Reference System (AIRS) helps you find the import requirements for Canadian Food Inspection Agency (CFIA) regulated goods

AIRS

http://www.inspection.gc.ca/food/imports/airs/eng/1300127512994/1326599324773

Tutorial

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.

Movement and Transportation

When moving/transporting RG2 biohazardous material **outside of a containment zone** (e.g. moving out of containment to the autoclave, between floors in a building or between buildings), the material must be **labelled, double contained, and surface disinfected**.

When transporting biohazardous material **off the Queen's campus**, or packaging for transport, you must comply with Transportation of Dangerous Goods Regulations.

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.

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 SOP-Biosafety-05 Queen's University Biohazard Risk Group Definitions .

Permits will be issued to a Principal Investigator for all the work with biohazardous materials under their supervision, even if it occurs in a shared facility (e.g. shared cell culture rooms will be listed on your biohazard permit; work in an animal facility).

- The permit is for the specific biohazardous materials, the procedures used with them, and the facility.
- New materials, procedures, projects, and award titles are to be added to the permit by submitting an amendment event application (through TRAQ).

Biohazard permitting at Queen's is administered through the TRAQ/Romeo system. Initial training in the technical aspects of the use of the electronic biosafety permitting system can be provided in person (or virtually) upon request and is provided by the TRAQ/Romeo system team in collaboration with the Biosafety Officer. Training manuals and videos are also available on the TRAQ website.

Technical questions about the system should be addressed to the TRAQ help desk.

Contact the Biosafety Officer if you have questions about biosafety matters, the review process, or if you would like assistance with your local risk assessment.

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.

- Biohazard Permit electronic forms and their use **is described in detail in the Biosafety section of the EH&S website**. These forms are also available on your Queen's TRAQ Researcher Portal http://www.queensu.ca/traq/signon.html

Personnel who work in the lab unaccompanied (i.e. without direct supervision; given keys to the lab) must be added to a list of authorized lab team members associated with your Biohazard Permit record in the Department of Environmental Health and Safety (outside of the TRAQ system).

- The process for adding or removing authorized biohazard lab team members is described on the EH&S website.
    - Do not add undergraduate project students who must always be directly supervised by an authorized lab member and who should not be given keys to the lab.
    - Nevertheless, undergraduate students must be trained about the hazards.

A**ll 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 of inspection is determined by the Biohazard Committee and relates to the level of risk.

Equipment Decommissioning

Prior to shipping out for service, or relocating to another laboratory, any equipment that has been used with biohazardous material must be thoroughly decontaminated and labelled as decontaminated before being removed from the containment zone. Consult the Biosafety Officer if you have any questions.

Prior to disposal, any research equipment or furniture that may have been in contact with or may contain biohazardous or other hazardous substances must be decommissioned by the Department of Environmental Health & Safety. Queen's University must ensure that all hazards are removed to prevent any spread of contaminants into the environment and to comply with existing regulations. There is no cost to individual departments, and a few simple procedures, outlined here, must be followed: https://www.queensu.ca/risk/safety/chemical.

Laboratory Decommissioning

Any laboratory that is undergoing **significant renovations** may need to be decommissioned first. Contact the Department of Environmental Health and Safety regarding any renovations to your laboratory.

Any Principal Investigator **closing a laboratory**, leaving the university, or transferring to another location within the University must be in compliance with the Queen's University Standard Operating Procedure for Laboratory Decommissioning, SOP-LAB-04.

A Laboratory Procedures Decommissioning Checklist, to be filled out and signed by officials from the Department of Environmental Health and Safety, the Principal Investigator, and the Department Head to document that the lab has be properly cleared of all hazardous material.

Failure to follow the required procedure may result in significant financial charges if members of the Department of Environmental Health and Safety are required to decontaminate the lab and package and remove hazardous material.

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.

To avoid contaminating your centrifuge:

- Check glass and plastic centrifuge tubes for stress lines, hairline cracks and chipped rims before use. Use unbreakable tubes whenever possible.
- Avoid filling tubes to the rim.
- Use caps or stoppers on centrifuge tubes. Do not use lightweight materials such as aluminum foil as caps.
- To reduce aerosol generation upon opening, use screw-capped tubes and bottles rather than plugs or snap caps when feasible.
- **Use sealed centrifuge buckets (safety cups) or rotors:**
    - **Load and unload in a biological safety cabinet.**
    - **Decontaminate the outside of the cups or buckets before and after centrifugation.**
    - **Inspect o-rings regularly and replace if cracked or dry.**
- Ensure that the centrifuge is properly balanced.
- Do not open the lid during or immediately after operation, interfere with the interlock safety device or attempt to stop a spinning rotor by hand or with an object.
- Clean spills promptly.

Cryostat

Frozen sections of unfixed human tissue or animal tissue infected with an infectious agent pose a biohazard exposure risk. Freezing tissue often does not inactivate infectious agents. Freezing propellants under pressure should not be used for frozen sections as they may cause spattering of droplets of infectious material. Gloves and a lab coat or gown should be worn during preparation of frozen sections. Depending on the infectious agent, consider whether a mask and eye protection needs to be worn. When working with biohazardous material in a cryostat, the following is recommended:

- Consider the contents of the cryostat to be contaminated and decontaminate it frequently with a disinfectant suitable for the agent(s) in use.
- Consider trimmings and sections of tissue that accumulate in the cryostat to be potentially infectious and remove them during decontamination.
- Decontaminate the cryostat with a tuberculocidal & virucidal disinfectant regularly and immediately after cutting tissue known to contain bloodborne pathogens, M. tuberculosis or other infectious agents.
- Handle microtome knives with extreme care. Stainless steel mesh gloves should be worn when changing knife blades.
- Consider solutions for staining potentially infected frozen sections to be contaminated.

Cryogenic Liquids

The storage, handling and dispensing of cryogenic liquids (e.g. liquid nitrogen, helium and argon) can lead to serious workplace injuries. Hazards related to these activities include oxygen deficiency, contact with extremely cold materials, oxygen condensation, or pressure build-up. Suitable engineering and operational controls must be applied wherever cryogenics are used.

This manual only describes the hazards and safety considerations for the manipulation of vials of pathogens or cells stored in liquid nitrogen. Consult the EHS SOP-CHEM-09 on cryogenic liquids for more information about the hazards of cryogens and risk mitigation.

If liquid nitrogen enters a vial during storage, then upon warming it can explode. This has caused eye and hand injuries. Always wear protective gloves, goggles and a full face-shield when removing vials from liquid nitrogen, until they have been safely opened in a biological safety cabinet.

- Check the recommendations from the manufacturer of the cryovials that you use.
    - Some cryovial manufacturers recommend using internally threaded cryovials for storage in the vapour phase of liquid nitrogen only, but many labs store vials in the liquid phase.
    - If storage in the liquid phase is required, consider how to reduce the risk.
        - Nunc sells tubing to seal vials that must be stored in the liquid phase.
        - It has been reported in a web-based article on the University College London site that vials with "male" caps that fit inside the vial, have a relatively thick thread and a sealing O-ring are less likely to explode than vials with a "female" cap. However, preventing the contamination of the material in the vial when inserting and removing a "male" cap requires care.
        - If vials have been stored in the liquid phase, then manufacturers recommend moving the vials to the vapour phase for 24 hours to allow any liquid nitrogen inside the vial to evaporate before removing the vial from the tank to open.
        - A more practical approach might be to slightly loosen the cap immediately upon removal from liquid nitrogen (if appropriate for the material involved – consider how pathogenic it is) and/or to immediately place the vial in a closed, shatter-proof container in case of explosion.

If the cryovials leak then viruses, bacteria and cells can escape, contaminating the liquid nitrogen and potentially contaminating other vials in the tank.

- 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 biosafety cabinet.

The risk associated with cell sorters and flow cytometers (depending on their design) can be attributed to both the nature of the sample (i.e., the presence and nature of the infectious material or toxins contained within the sample) and to the equipment itself (e.g., use of droplet-based cell sorting, which uses jet-in-air technology, and has the potential to produce a large amount of aerosolized droplets). Droplet-based cell sorting involves the injection of a liquid stream carrying the cells through a narrow nozzle vibrating at a high frequency. High-speed cell sorters with jet-in-air technology use even higher pressures and nozzle vibration frequencies, and consequently produce a larger amount of aerosolized material.

- **If you need to conduct flow cytometry or cell sorting with unfixed samples, the activity must be listed on your Biohazard Permit.**
- **Unless the biohazardous material has already been assessed for the cytometry/sorter facility/service you will use, you must contact the University Biosafety Officer for assistance with a local risk assessment**

Freeze-Driers (Lyophilizers)

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

- Load samples in a biological safety cabinet.
- Check glass vacuum containers for nicks and scratches.
- Use only glassware that was designed for high vacuum use.
- Use a disinfectant-containing trap for the vacuum pump exhaust (and/or a HEPA filter).
- After completion of the run, decontaminate all accessible surfaces.

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 labelled with radioisotopes). Contact the Biological Safety Officer if this is the case.
    - Depending on the quantity of hazardous chemical involved a Type II A2 BSC with a thimble connection to the building exhaust system might be suitable, or a Type II B2 BSC with full fume hood capabilities might be required.

- Note that in some instances hazardous chemicals may be added to biohazardous materials in a fume hood (e.g. Trisol), but only after a risk assessment is approved by the Biosafety Officer/Biohazard Committee.

Microbiological transfer loops

- Avoid flaming a loop in an open flame – use a loop microincinerator or pre-sterilized plastic loops
- If flaming is necessary, to eliminate the spattering and aerosolization associated with flaming of loops, char the material before fully inserting the loop into the flame: i.e., before flaming, hold the loop close to (but not into) the flame.
- Do not use a flame in a biological safety cabinet (flames can disrupt the protective air curtain and damage the HEPA filter)
- Streak plates where the surface of the medium is smooth (i.e. avoid bubbles)

Microscopes

- Microscope eyepieces may provide a potential route of transmission of both bacterial and viral infections. Large outbreaks of conjunctivitis have been attributed to the sharing of microscopes among employees.
- Disinfect the eyepieces, knobs, stage, and any other contaminated parts. Select a disinfectant that will be non-toxic, effective on the pathogens in use and not harm the microscope. Consult the manufacturer.

- 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 break.

Needles and Syringes

Hypodermic needles and syringes present hazards of spill, autoinoculation and aerosol generation, and should be used only when absolutely necessary, such as for parenteral injection or withdrawal of body fluids.

Safer alternatives to sharp needles:

- When withdrawing liquids from septum-capped or diaphragm bottles, consider using an opener made especially for this type of bottle; this allows for use of a pipette rather than a syringe/needle assembly.
- Use cannulas or blunt-end needles for introduction or removal of fluids through small apertures in equipment.

When working with syringes and needles, the following precautions are recommended:

- Perform all operations with infectious material in a biological safety cabinet (except when not feasible as with large animals).
- Fill syringes carefully; avoid frothing or introduction of air bubbles.
- Shield needles with disinfectant-soaked cotton wad when withdrawing from stoppers.
- Use luer-lock needles and syringes or units in which needles are integral to syringes. If possible, use one of the newer "safe" alternatives to needles and syringes, such as those with needles that automatically retract when the plunger is fully depressed.
- Do not bend, shear, or recap needles.
    - If any of the above activities are required, a lab-specific procedure for doing so that minimizes the risk of needle-stick must be documented and approved
- Place used needles and syringes in puncture-resistant sharps containers and surface decontaminate before disposal through Environmental Health and Safety.

Pipettes and Mechanical Pipetting Aids

Improper handling of pipettes has led to laboratory acquired infections. These are avoidable by using a mechanical pipetting aid (never pipette by mouth) and by using proper pipetting procedures to avoid the generation of hazardous aerosols.

- A pipetting device used with biohazardous material should be autoclavable and be provided with aerosol protection (filter) to reduce the possibility of contaminating the pipetting aid
- Check the quality of seal formed with pipettes to be used; liquid should not leak from the pipette tip
- Plug the top end of pipettes with cotton or use aerosol resistant disposable pipettes

- 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).

- the use of pipettes to transfer fluids is preferable
- Pouring may be necessary, particularly if large volumes are involved:
    - disinfectant soaked absorbents can be used to wipe the rims of tubes
      infected material can be poured through a funnel, the end of which is below the surface of disinfectant in the discard container (the top of the funnel should be slightly larger than the discard container so it rests securely and disinfectant should be poured through the funnel after use)

Vacuum and Aspirator Equipment

Those using Vacuum and Aspirator Equipment must comply with the Queen's University Standard Operating Procedure, SOP-Biosafety-01

- Note the requirement for a HEPA filter in the line leading into the vacuum line: cartridge-type in-line filters provide an effective barrier to escape of aerosols into vacuum systems, and are commercially available for this purpose (discard used filters as biomedical waste).

Water baths and bead alternative to water

Water baths kept at 37°C are an ideal environment for microbial growth. To prevent contamination, especially the growth of disinfectant-resistant biofilms, clean regularly.

- Use only distilled water.
- Do not use abrasives because they will cause small scratches where microbes can grow and be difficult to decontaminate.
- Consider adding a disinfectant, such as a quaternary ammonium compound or phenolic detergent, to the water.
- Or raise the temperature to 90°C or higher for 30 minutes once a week for decontamination purposes.
- Do not use sodium azide to prevent growth of microorganisms in water baths (sodium azide forms explosive compounds with some metals).

Metal beads used as alternative to water in heating baths should be cleaned and decontaminated periodically.

APPENDIX II

CBS Requirements for Containment Level 2 *in vitro* and *in vivo*

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 3** contains additional requirements for CL2-Ag.

These tables **do not present the operational requirements that are exclusively for prion work** (for those look for "P" in the CBS matrices).

Table 1. CL2 *in vitro* 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.
3.6.4 - Sinks to be provided and located to facilitate handwashing upon exit from the containment zone. [***Not required for CL4 zones where positive-pressure suits are worn.***]

CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
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 elimination of puff-back cannot be achieved, the risk associated with puff-back to be mitigated through physical and operational means.
3.7.4 - Process equipment, **closed systems**, and other primary containment devices to be designed to prevent the **release** of infectious material or toxins.
3.7.6 - BSCs, where present, to be located as far as possible from high traffic areas, doors, openable windows, and air supply/exhaust diffusers.
3.7.11 - **Decontamination technologies** for the **decontamination** of materials to be provided within the **containment zone**, or **standard operating procedures** (SOPs) to be in place to safely and securely move or transport waste out of the containment zone to a designated decontamination area.
3.7.14 - Decontamination technologies to be provided with monitoring and recording devices that capture operational parameters.
3.7.15 - An autoclave, where present, to be capable of operating at the appropriate temperature for decontamination, as determined by **validation**.
3.7.17 - Vacuum systems to be equipped with a mechanism that prevents internal **contamination**.

3.7.18 - Two-way communication system(s) to be provided inside the containment barrier that allows communication between inside the containment barrier to outside the containment zone, in accordance with function.

4.1.1 - A biosafety program to be in place for the oversight of safety and containment practices.

4.1.2 - A biosafety representative(s) (i.e., designated **biological safety officer** [BSO] in licensed **facilities**) with the knowledge appropriate for the **containment levels** and pathogens and toxins handled, to be designated for the oversight of biosafety and **biosecurity** practices including:

- verifying the accuracy and completeness of **licence** applications, **animal pathogen import permit** applications, and **transfer** applications for the **movement** of material imported under the ***Health of Animals Act*** (HAA) and ***Health of Animals Regulations*** (HAR), as applicable;
- communicating with the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) on behalf of the licence holder and animal pathogen import permit holder, as applicable;
- promoting and monitoring compliance with applicable legislation (including the ***Human Pathogens and Toxins Act*** [HPTA], ***Human Pathogens and Toxins Regulations*** [HPTR], HAA, and HAR), conditions of licence and applicable conditions of animal pathogen import permits; applicable biosafety and biosecurity standards, and the **Biosafety Manual** and **standard operating procedures** (SOPs), which includes, but is not limited to;
    - arranging and documenting appropriate biosafety and biosecurity training for personnel pertaining to human and **animal pathogens** and toxins, as applicable;
    - informing the PHAC of all occurrences of inadvertent possession of a human pathogen or toxin not already authorized by the licence;
    - 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**.

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** (LRA) to be conducted to examine each task involving infectious material or toxins so that the **risks** are identified and safe work practices developed and documented.

4.1.9 - A **training needs assessment** to be conducted.

4.1.10 - A Biosafety Manual to be developed, implemented, kept up to date, made available to personnel inside and outside of **containment zone**, and to contain institutional biosafety policies, programs, and plans, based on an overarching risk assessment and LRAs. The Biosafety Manual to include:

- the program intent;

- 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
    - SOPs for safe work practices specific to the containment zone.

4.1.11 - A biosecurity plan, based on a biosecurity risk assessment, to be developed, implemented, evaluated and improved as necessary, and kept up to date. The biosecurity plan to include mitigation strategies for the risks associated with:

- physical security;
- personnel suitability and reliability;
- accountability for pathogens, toxins, and other regulated infectious material;
- **inventory**;
- incident and emergency response; and
- information management.

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.

CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
4.6.6 - Open wounds, cuts, scratches, and grazes to be covered with waterproof dressings.
4.6.7 - Traffic flow patterns from areas of lower **contamination** (i.e., clean) to areas of higher contamination (i.e., dirty) to be established and followed, as determined by a **local risk assessment** (LRA).
4.6.8 - Dedicated paper/computer work areas to be utilized for paperwork and report writing.
4.6.9 - Use of needles, syringes, and other sharp objects to be strictly limited and avoided when suitable alternatives are available.
4.6.10 - Bending, shearing, re-capping, or removing needles from syringes to be avoided, and, when necessary, performed only as specified in SOPs.
4.6.11 - Work surfaces to be cleaned and decontaminated with a disinfectant effective against the pathogen(s) in use, or a neutralizing chemical effective against the toxin(s) in use, at a frequency to minimize the potential of exposure to infectious material or toxins.
4.6.14 - Verification of the integrity of **primary containment devices** to be performed routinely, as described in SOPs.
4.6.15 - BSCs, when present, to be certified upon initial installation, annually, and after any repairs, modification, or relocation.
4.6.18 - Good microbiological laboratory practices to be employed.
4.6.19 - Samples of pathogens, toxins, or other regulated infectious material to be opened only in containment zones that meet the **containment level** requirements to which that infectious material or toxin has been assigned.
4.6.20 - Containers of pathogens, toxins, or other regulated infectious material stored outside the containment zone to be labelled, leak-proof, impact resistant, and kept either in locked storage equipment **or** within an area with **limited access**.
4.6.24 - A certified BSC to be used for procedures involving open vessels of infectious material or toxins that:

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

[***Not required when collecting samples from or inoculating animals housed in an*** *animal cubicle***.**]

4.6.26 - Procedures to be followed to prevent the inadvertent spread of contamination from items removed from the BSC after handling infectious material or toxins.

4.6.27 - Personnel to wash hands after completing tasks that involve the handling of infectious material or toxins and before undertaking other tasks in the containment zone.

4.6.28 - Centrifugation of infectious material where inhalation is the primary route of infection to be carried out in sealed safety cups (or rotors) that are unloaded in a BSC.

4.6.30 - Use of on-demand open flames in a BSC to be strictly limited and avoided when suitable alternatives are available; sustained open flames to be prohibited in a BSC.

4.6.31 - Procedures, as determined by an LRA, to be in place to prevent a leak, drop, spill, or similar event during the **movement** of infectious material or toxins within the containment zone or between containment zones within a building.

4.6.33 - Collecting samples, adding materials, or transferring culture fluids from one closed system to another to be performed in a manner that prevents the release of aerosols or the contamination of exposed surfaces.

4.6.34 - Experimentally infecting cells or other specimens derived from the person conducting the experiment to be prohibited.

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 and labelled as decontaminated prior to cleaning, disposal, or removal from the containment zone or prior to removal from the **animal rooms**, **animal cubicles**, or **post mortem rooms** (PM rooms), as described in SOPs; or

- 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** 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 animals, or involving failure of **containment systems** or control systems to be reported immediately to the appropriate internal authority.

4.9.8 - Incident investigation to be conducted and documented for any incident involving pathogens, toxins, other regulated infectious material, infected animals, or failure of containment systems or control systems, in order to determine the root cause(s).

4.9.9 - The Public Health Agency of Canada (PHAC) to be informed without delay via the submission of an **exposure notification report** following:

- an exposure to a human pathogen or toxin; or
- recognition of a **disease** that has or may have been caused by an exposure to a human pathogen or toxin.

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

- 15 days of the submission of an exposure notification report involving a **security sensitive biological agent** (SSBA); or

CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
• 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**.
4.10.5 - Records of regular inspections of the containment zone and corrective actions to be kept on file.
4.10.6 - Records of building and equipment maintenance, repair, inspection, testing, or certification, including performance verification and testing records, in accordance with containment zone function, to be kept on file.
4.10.7 - Equipment used for performance verification and testing of containment systems and essential biosafety equipment to have a valid calibration certificate at the time of testing; calibration certificates to be kept on file.
4.10.9 - Records of **validation** and routine verification of **decontamination technologies** and processes to be kept on file.
4.10.10 - Records and documentation pertaining to: • **licence** activities involving human pathogens and toxins to be kept on file for a minimum of 5 years; and • **animal pathogen import permit** requirements for **animal pathogens**, toxins, and other regulated infectious material to be kept on file for a minimum of 2 years following the date of disposal, complete **transfer**, or inactivation of the imported material.
4.10.11 - Records of **incidents** involving pathogens, toxins, other regulated infectious material, infected animals, or losses of containment to be kept on file for a minimum of 10 years.

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 - **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;
- integrity of BSCs designed with positive-pressure plenums to be demonstrated by determining that exterior surfaces of all plenums, welds, gaskets, and plenum penetrations or seals, are free of leaks (to be performed during initial installation, if any panels are removed, or if the cabinet is relocated); and
- 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.

Table 2. CL2-SA FACILITY and OPERATIONAL PRACTICE REQUIREMENTS in addition to those for CL2 *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 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). [***Not required for CL2 large scale production areas.***]
3.3.3 - Where unique hazards exist, project-specific signage to be posted at the **animal room**, **animal cubicle**, and **post mortem room** (PM room) point(s) of entry.
3.3.5 - Restricted access into the containment zone to be provided through a controlled access system.
3.3.7 - Non-reproducible keys to be used when key-locks are used as the controlled access system.
3.3.10 - Dedicated change area to be provided at personnel entry to the containment zone to allow for separation of personal clothing from dedicated containment zone clothing (i.e., **"clean" change area** separated from **"dirty" change area**).
3.3.11 - Anteroom(s) to be provided at the point(s) of entry into/exit from the containment zone.

CL2 Small Animal *in vivo* Requirements in addition to CL2 *in vitro* (CBS 2nd Edition 2015)
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.
3.4.6 - Floors in **animal rooms**, **animal cubicles**, **post mortem rooms** (PM rooms), and corridors to withstand loading, in accordance with function. [***Not required for CL2 large scale production areas.***]
3.4.7 - Continuity of seal to be maintained between the floor and wall.
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-free" capability. [***Not required for CL4 zones where positive-pressure suits are worn.***]
3.6.18 - Services and equipment critical to maintaining containment and **biosecurity** to be supported by emergency power.
3.7.8 - **Primary containment caging** to be provided to house infected animals. [***Not required for large scale production areas.***]
3.7.10 - Animal cages and **animal cubicles** to be designed to prevent animal escape. [***Not required for CL2 large scale production areas.***]

CL2 Small Animal *in vivo* Requirements in addition to CL2 *in vitro* (CBS 2nd Edition 2015)
4.3.5 - 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 appropriate **primary containment device**. [***Not required for CL2 large scale production areas.***]
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.***]
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 - **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;
- integrity of BSCs designed with positive-pressure plenums to be demonstrated by determining that exterior surfaces of all plenums, welds, gaskets, and plenum penetrations or seals, are free of leaks (to be performed during initial installation, if any panels are removed, or if the cabinet is relocated); and

CL2 Small Animal *in vivo* Requirements in addition to CL2 *in vitro* (CBS 2nd Edition 2015)
• 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.

Table 3. CL2-Ag FACILITY and OPERATIONAL PRACTICE REQUIREMENTS in addition to those for CL2 *in vitro*

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

CL2 Ag *in vivo* for large animals in addition to CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
3.1.4 - **Laboratory work areas** to be located outside of animal cubicles.
3.1.5 - Cold storage area or equipment to be provided in, or adjacent to, the PM room.
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). [***Not required for CL2 large scale production areas.***]
3.3.3 - Where unique hazards exist, project-specific signage to be posted at the **animal room**, **animal cubicle**, and **post mortem room** (PM room) point(s) of entry.
3.3.5 - Restricted access into the containment zone to be provided through a controlled access system.
3.3.7 - Non-reproducible keys to be used when key-locks are used as the controlled access system.
3.3.10 - Dedicated change area to be provided at personnel entry to the containment zone to allow for separation of personal clothing from dedicated containment zone clothing (i.e., **"clean" change area** separated from **"dirty" change area**).
3.3.12 - Anteroom(s) to be provided at the point(s) of entry into/exit from:

CL2 Ag *in vivo* for large animals in addition to CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
• the containment zone; ***or*** • each animal cubicle and PM room.
3.3.17 - Anteroom **critical door(s)** to be provided with a physical or operational mechanism that prevents the simultaneous opening with: • the door leading into the anteroom from outside of the containment zone; and • the door(s) leading from the anteroom into the **laboratory work area**/animal room/animal cubicle/PM room.
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.
3.4.6 - Floors in **animal rooms**, **animal cubicles**, **post mortem rooms** (PM rooms), and corridors to withstand loading, in accordance with function. [***Not required for CL2 large scale production areas.***]
3.4.7 - Continuity of seal to be maintained between the floor and wall.
3.4.8 - Continuity of seal to be maintained between the wall and ceiling.
3.4.9 - Interior surface materials to restrict penetration of gases and liquids used for decontamination and/or **laboratory** purposes.
3.4.10 - Protruding obstructions to be minimized and appropriately shielded in animal cubicles and corridors.

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-free" capability. [***Not required for CL4 zones where positive-pressure suits are worn.***]
3.6.18 - Services and equipment critical to maintaining containment and **biosecurity** to be supported by emergency power.
3.7.10 - Animal cages and **animal cubicles** to be designed to prevent animal escape. [***Not required for CL2 large scale production areas.***]

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* 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 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.***]
4.7.4 - Entry/exit procedures to be employed to minimize the release of aerosolized or **airborne pathogens** from **animal cubicles** and **post mortem rooms** (PM rooms).
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.***]
4.7.6 - Animal carcasses to be removed from cubicles/PM rooms via the dirty corridor, or if removed via the clean corridor, to be divided into smaller portions (as necessary) and placed into labelled, leakproof, and impact resistant transport containers.
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.***]

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 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.***]
5.2.3 - Operation of **controlled access systems** and security systems to be verified to be functioning as intended. [***Not required in CL2 SA zones where Prions and SSBAs are not present.***]

CL2 Ag *in vivo* for large animals in addition to CL2 *in vitro* Requirements (CBS 2nd Edition 2015)
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.***]
5.2.7 - Testing to be performed at all **critical doors** on the containment barrier, where **inward directional airflow** (IDA) is provided, to verify, using a smoke pencil or other visual aid that does not influence the direction of airflow, that IDA is maintained in accordance with **facility** design. [***Not required in CL2 SA zones.***]
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.
5.2.9 - Operation of mechanical or electronic door **interlocks** and associated manual overrides to be verified.
5.2.12 - Integrity of the seals of containment barrier penetrations, **animal cubicle** penetrations, and **post mortem room** (PM room) penetrations to be tested with a smoke pencil or other aid that does not influence the direction of airflow.

Government Safety Regulations and Policies on Biohazardous Material

It is important that people working with biohazardous materials know about and understand the laws and the regulations that direct what they may and may not do with those materials and how they are to work with them safely.

The key document governing the use of biohazardous materials in Canada is the Canadian Biosafety Standards, so it is described in more detail in the BIOSAFETY OVERVIEW section of this manual.

The laws and regulations related to work with biohazardous material in Ontario, Canada are listed below:

- **Human Pathogens and Toxins Act (2009), (HPTA) and the Human Pathogens and Toxins Regulations, (HPTR)**
  - Gives the **Public Health Agency of Canada (PHAC)** the authority to control the use of all human pathogens (**Risk Group 2 and above**), whether imported or not, **except those in their natural environment** (i.e. it does not regulate pathogens in soil, blood, or tissues until such time as those pathogens are isolated from the natural environment e.g. cultured from blood)
  - The HPTA was based on the requirements of the Laboratory Biosafety Guidelines (2004) and the subsequent Canadian Biosafety Standards and Guidelines 1st Edition (2013).
  - The HPTA and the Human Pathogens and Toxins Regulations under that HPTA came fully into force in December 2015 with the release of the Canadian Biosafety Standards 2nd Edition (2015).
  - The HPTA contains a list of regulated toxins (Schedule 1) and a list of material that no one in Canada may possess (Schedule 5; at this time only variola virus i.e. smallpox virus is prohibited)

- **Canadian Biosafety Standards, 3rd Edition, 2022** forms the basis of regulations under the Human Pathogens and Toxins Act.
  - **The CBS describes the best practices for work with all human or terrestrial animal pathogens and, under Canadian law, must be followed for Risk Group 2 pathogens and above.**
  - Updates and harmonizes three previous Canadian biosafety standards and guidelines for the design, construction and operation of facilities in which pathogens or toxins are handled or stored:

- *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 substances, sewer disposal of substances and management of spills.

- **Transportation of Dangerous Goods legislation**
  - Transportation of Dangerous Goods Act, 1985
  - Transportation of Dangerous Goods Act, and regulations R.R.O. 1992
  - ICAO Technical Instructions for the Safe Transport of Dangerous Goods by Air.
  - IATA Dangerous Goods Regulations, International Air Transport Association, 1999
  - CNSC Transport Packaging of Radioactive Materials Regulation 2000
  - IAEA Regulations for the Safe Transport of Radioactive Material TS-R-1 1996
  - Ontario General Waste Management Regulation, Reg. 347 and 558/00

- **Occupational Health and Safety Act of Ontario**

- 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