

Queen's University Biosafety Manual

2013

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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 animals. The goal is to prevent laboratory acquired infections (LAIs) in laboratory workers and to protect those outside the laboratory from the deleterious 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 such material, as set out in the [Canadian Biosafety Standards and Guidelines \(CBSG\)](#) and other **regulations and guidelines**. Some of the text in this manual is copied directly from the CBSG and presented here for your convenience. Nevertheless, it is recommended that review the CBSG to become familiar with it as a resource document after you read the Queen's Biosafety Manual. In particular, read the Table of Contents at the beginning of the CBSG so that you have an overview of the valuable information that it contains.

Ensure that there are links to both the CBSG and the Queen's Biosafety Manual, in a Safety folder on the Desktop of your lab computers. If you do this, and make all lab members aware of it, you are not required to print these documents.

There is legislation in Canada regarding **Aquatic Animal Pathogens and Plant Pests**, however this material is beyond the scope of this Biosafety Manual. For further information on such material, contact the Biosafety Officer.

The term "**Biological material**" refers to:

- microorganisms, proteins, and nucleic acids, along with other biological matter that may contain microorganisms, proteins, and nucleic acids, whether or not they are infectious or toxic.
- Pathogens are a specific group of microorganisms, proteins, or nucleic acids that are capable of causing disease
- the overarching term "microorganism" also includes microbes that do not normally cause disease (i.e., Risk Group 1 (RG1) microorganisms).
- "Infectious material" is used throughout the Canadian Biosafety Standards and Guidelines to refer to the pathogenic subset of biological material, including the pathogens themselves and any biological matter that may contain them (e.g., blood, tissue).

The degree of hazard associated with a particular biological material is assessed qualitatively by:

- how likely it is to cause disease in people,
- how serious that disease is likely to be,
- and whether or not effective treatment is available.
- The designation, called a Risk Group (RG), indicates how hazardous the material is and the measures that are required when working with it to mitigate the hazard. Risk Groups are described in the first section of this manual.

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 biohazard applications dealing with microorganisms that infect humans or both humans and animals (zoonotic pathogens). 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 necessary 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 particular equipment and procedures that they will employ.

- **When first starting work**, check the sign(s) on the door to your lab(s) to determine the containment level(s).
- **Note that the training you require is determined by the containment level of the lab in which you will be working**, not by the material with which you will be working. So if you are working in a level 2 lab with only level 1 material, you still need to do the level 2 training.
- See the section of this manual on training for more information.
- **Be aware of the biohazard door signs in other labs that you may need to enter**, and ensure that you follow any required entry procedures or precautions while in the lab.

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 similar to 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.

Risk Groups - How hazardous is it?

Laboratory-acquired infections can happen in laboratory personnel handling human pathogens, zoonotic agents (animal pathogens that are also pathogenic to humans), cell cultures, and animal and diagnostic specimens. The outcome of accidental infections ranges from complete recovery, to permanent disability, to death depending on the organism, the individual and the medical treatment 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. A **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 the might contain that agent. The risk assessments also consider both the characteristics of the microorganisms 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.

Factors considered in a risk assessment that is performed to determine the risk group 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 gain entry into 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?
- **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.**
 - Neither of the RG1 subsets is regulated by the PHAC or the CFIA due to the low risk to public health, livestock or poultry.
- The culture of RG1 microorganisms and work with research animal tissues that are likely to contain large quantities of RG1 bacteria (gut tissue), are regulated 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. Work with other tissues from research animals and with the animals themselves is overseen by the University Animal Care Committee.
- Examples include:
 - bacteria such as *Bacillus subtilis*, *Lactobacillus casei*, cloning strains of *E. coli* (*K12 strains*)
 - viruses such as *Baculovirus*
 - fungi such as *Schizosaccharomyces*

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
- is able to 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*, *Bordetella 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*

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 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, nonneurotropic strains)
- for Risk Group 3 microorganisms that are used in low concentration and not cultured.
 - e.g. HIV positive blood may be used at containment level 2+, but HIV may not be cultured except in a level 3 containment facility.

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 and Guidelines, 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 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

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*, Herpesvirus simiae (herpes B or monkey B virus)

Sources of Risk Group Information

Sources of Risk Group Information

The Public Health Agency of Canada (PHAC) website has detailed and useful risk assessments of some microorganisms in the form of Pathogen Safety Data Sheets (PSDSs, an MSDS for pathogens) (<http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php>). 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 (PHAC) or that would require containment level 2 facilities and practices at Queen’s University.** So check with the Biosafety Officer to be sure.

If a commonly accepted risk group has not been assigned then when applying for a biohazard permit, the investigator must assign a risk group and justify the assignment to the Queen’s University Biohazard Committee.

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 most 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. All culturing of HIV must occur in a level 3 containment facility. However, work with HIV infected blood is considered less hazardous and can be done under level 2+ containment conditions.

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. Lab design requirements vary depending on the containment level and whether or not 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

A containment laboratory must meet specific physical design requirements and provide equipment for containment as described in the [Canadian Biosafety Standards and Guidelines \(CBSG\)](#), 2013, Chapter 3 of the Standards section.

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.

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 and Guidelines \(CBSG\)](#), 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).

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.

- Fine aerosols are an inhalation hazard for agents that are infective via this route.
- Aerosols carrying infectious agents can settle on bench tops and become an ingestion or mucosal exposure hazard through contamination of the hands.

There is general agreement that aerosol generation by procedures is the probable source 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). **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).**

Health and Medical Surveillance

Laboratory-Acquired Infection Case Studies

1. A researcher was using a strain of Salmonella as a host for DNA coding 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. (*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 an attenuated pigmentation-negative (pgm-) attenuated *Y. pestis* strain. The strain had not been known to have caused laboratory-acquired infections or human fatalities and would be 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 *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 of the case studies above, the infections were caused by not using the appropriate CL2 containment practices for the organisms, and 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.

Medical Surveillance Program at Queen's

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

The risks to lab personnel should be reviewed in order that they each gain an understanding of the biological hazards as they relate to personal immune system susceptibility and medical conditions. Appropriate risk mitigation methods must then be employed. The program may also include but is not limited to the following: 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, rabies), 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 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 your 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
 - 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 your 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 <http://www.safety.queensu.ca/walsh/> .
 - Charges will be billed to departments through the Department of Environmental Health and Safety and payment is the responsibility of the supervisor.

Local Risk Assessment – the material and what is being done with it

Biological risk assessment is the basis for the safeguards developed by the federal agencies of many countries and by the microbiological and biomedical community to protect the health of laboratory workers and the public from the risks associated with the use of hazardous biological agents in laboratories. Experience shows that established safe practices, equipment, and facility safeguards work. Biological risk assessment is a subjective process requiring consideration of the many characteristics of agents and procedures, and judgments are often based on incomplete information.

It is important to think 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.

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.

A local risk assessment will:

- identify the Risk Group of the microorganism (or tissue that might contain this microorganism)
- 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)
- indicate whether the material will be used only *in vitro*, or also *in vivo*
 - what is being done with the material and where; consider the procedures’ potential for generating aerosols that might contain and spread infectious agents
 - indicate whether or not sharps will be used and the precautions associated with them
 - *in vivo* use of infectious materials 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 and engineering controls (i.e. lab design) This can be indicated simply 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.
 - operational requirements
 - containment equipment and supplies
 - equipment might include e.g. Biological Safety Cabinet, centrifuge cups with aerosol resistant lids containing o-rings
 - supplies might include e.g. 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. Example local risk assessment documents are available in TRAQ/Romeo “Useful Links” or from the Biosafety Officer, to assist laboratories when preparing a Biohazard Application.

- 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.
- 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 that are posted on the TRAQ/Romeo site
 - have any questions that they might have answered by their P.I. and/or the Biosafety Officer
 - submit the Biohazard Team Member Attestation form through the TRAQ/Romeo system, to indicate that they understand and will abide by the 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 register so that you have access to read the approved biohazard permit application and all associated documents on the TRAQ/Romeo site, have your questions answered, and sign the Biohazard Team Member Attestation form so that you can be authorized to work in the lab.

The information required in the local risk assessment is described in a template document available in “Useful Links” through the TRAQ/Romeo system. If you have questions after reading this document, or if you would like a pre-review of your risk assessment before formal submission to the Biohazard Committee, contact the University Biosafety Officer (ext. 77077).

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 regulations from the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) and other applicable legislation, listed below.

The University Biohazard Committee 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 [Biohazard Committee Terms of Reference](#) on the Department of Environmental Health and Safety website, under the Biosafety dropdown menu (www.safety.queensu.ca).

The **University Biosafety Officer**, one of the Ex-Officio staff members of the Biohazard Committee, is a member in the Department of Environmental Health and Safety.

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. **Approval from the Biohazard Committee is required 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, and a risk assessment and risk mitigation statement. This process has been paper based and is transitioning to electronic submission through the TRAQ/Romeo system in late 2013.
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.**

Any PHAC- or CFIA-imposed containment requirements will be monitored by the Queen's Biohazard Committee and will require a Queen's Biohazard Permit Application and laboratory inspection.

See Queen's [SOP-Biosafety-05](#) for a more detailed description of what biological material is regulated through the Biohazard Committee.

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.

The key document governing the use of biohazardous materials in Canada is the Canadian Biosafety Standards and Guidelines, so it is also described here.

- It is a 343 page document that contains useful information.
- The Standards section may need interpretation, so note recommendations below on how to use the CBSG and consult the University Biosafety Officer if you need assistance.
- Throughout the Queen's Biosafety Manual you are directed to consult different sections of the CBSG if you require more information on various topics.

- [Canadian Biosafety Standards and Guidelines \(CBSG\), 1st Edition, 2013](#) will form the basis of regulations under the Human Pathogens and Toxins Act.
 - **The CBSG 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.
 - Replaces the following documents:
 - ***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)

The CBSG contains:

- **The Standards (matrices in Part I of the CBSG)** that are the risk-based containment requirements (physical and operational) 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
 - Requirements for the importation, exportation, transfer between laboratories and institutions within Canada, and the transportation of the pathogens it regulates.
- **A Transition Index**, (in the middle of the CBSG), that provides information on:
 - why a requirement from the Standards is needed
 - supplementary information
 - where to find further guidance on the subject in the Guidelines.
 - The Transition Index does not include additional requirements but rather provides information and recommendations only.
 - **It is strongly recommended that when reading an item in the Standards, you always also read the Transition Index for that item for clarification.**
- **The Guidelines (Part II of the CBSG)** that provide overall guidance on how to achieve the biosafety requirements outlined in Part I (the Standards). The guidelines describe concepts and information that are fundamental to the development and maintenance of a comprehensive, risk-based biosafety management program.

RESPONSIBILITIES

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](#) for the Committee (available on the safety website)

Biosafety Officer

- 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

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

Principal Investigators

- Read and be familiar with the contents of this Biosafety Manual and ensure that it is followed in their laboratories.

- 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)
 - 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 with their associated risk assessment summaries, and any laboratory-specific SOPs
 - maintain a list of the biohazardous materials in their laboratory:
 - to fulfill the inventory requirements of the CBSG (R4.1.12)
 - to be provided to the Biohazards Committee as part of the Biohazard Permit process through the Romeo system
 - to be updated when materials are added or destroyed
- 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 CBSG. 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.
 - Maintain documentation of training on the safe handling of biohazardous materials, chemicals, toxins etc., using the checklist [[PDF](#)][[Word](#)] available on the Department of Environmental Health and Safety website (<http://www.safety.queensu.ca/>).
 - Add and delete items on this training checklist to make it appropriate for your laboratory.
- 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.
- 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 biohazards attend [WHMIS training](#) offered by the Department of Environmental Health and Safety. Information regarding the timing of these training sessions can be obtained on the Department of Environmental Health and Safety website (<http://www.safety.queensu.ca/>) under Training
- 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 stolen or missing Risk Group 2 material to Department of Environmental Health and Safety within 24 hours of the incident.

All Other University Personnel and Students working as Biohazard Team Members

- Take [Biosafety training](#), as described on the Environmental Health and Safety website and ensure that they:
 - read the lab biohazard permit and associated documents (on the TRAQ/Romeo system),
 - read the Queen’s Biosafety Manual and SOPs,
 - take quizzes as required.
- 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
- Laboratory workers should be protected by appropriate immunization where possible, and antibody titres should be checked to determine whether or not there has been an adequate response to immunization.
- 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.

TRAINING REQUIREMENTS

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

- training
- knowledge of the hazardous agent and the procedure-associated hazards
- 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, as a condition of obtaining a biohazard permit, that the Principal Investigator ensure that all personnel have both general and laboratory-specific training in the handling of biohazardous material.

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.

Lab-specific hands-on training and reading including local 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)

A detailed description of the requirements for biosafety training is to be read from the [Training Requirements](#) section of the Biosafety dropdown menu.

All personnel working in biohazard laboratories **must read this Biosafety Manual and take one of the centralized biosafety quizzes** through the Department of Environmental Health and Safety to demonstrate their knowledge. [Biosafety quizzes](#) are located in the [training section of the Environmental Health and Safety website](#) .

- **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.

Emergency Procedures

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 on the Biohazard Permit Renewal form.

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 <http://lab-bio.pensivo.com/index.php?fuseaction=public.home&id=1>. Click on “Not registered?” and follow the instructions to access the videos. Once signed in, click “Tools and Resources” at the top right corner of the page and select “Videos”. If you cannot see the Control Functions for the video player, make sure that your browser is at 100% zoom or higher.

CONTAINMENT AND ENGINEERING CONTROLS

It is a 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 and Guidelines \(CBSG\), 1st Edition, 2013](#)**; 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.

When new facilities are constructed or extensively renovated, the CBSG Standards must be met, and adopting the recommendations of the Guidelines 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 so that personnel will know what facility systems they should ensure are maintained. More detail regarding the **CBSG 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), are in the [CBSG Standards Chapter 3](#).

Level 2 labs must:

- Meet all the facility requirements described above for Level 1 laboratories
- Doors must be closed at all times 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 meet the recommendation for directional air flow into the lab (i.e. lab under negative pressure relative to the corridor). This may be required depending on the nature of the work (*in vitro* vs. *in vivo* small animal vs. large animal) and a local risk assessment.
- 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+ labs have the same physical requirements as level 2 labs and also should have directional air flow into the lab. The lab must have a biological safety cabinet and centrifuge rotors must have aerosol-resistant lids.

Lab Design Changes

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

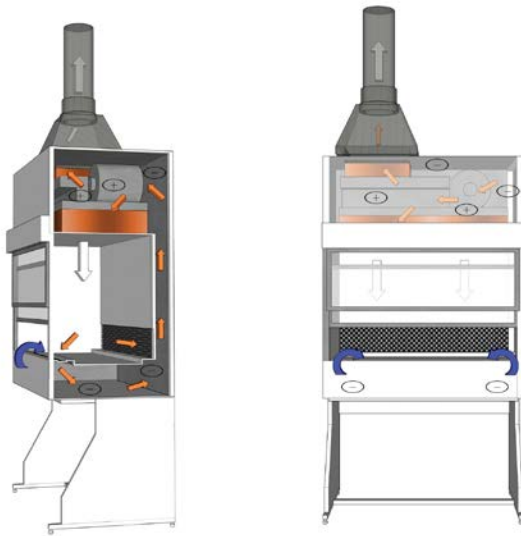
Containment Equipment and Supplies

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 (BSCs) 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.

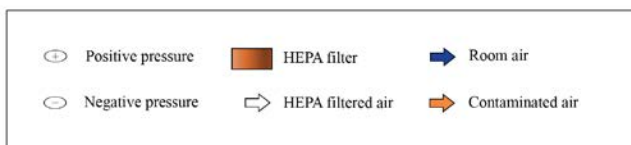


Class II Type A1 (with a negatively pressured plenum)/Type A2 BSC.

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 from the CBSG provided courtesy of Smith Carter Architects and Engineers Incorporated .



- 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.
 - 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.
- 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)
 - 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 CBSG.
- **Important tips for BSC use include:**
 - Make sure that you check the magnehelic guage every time you use the BSC so that you know that it is operating in the correct range.
 - Do not use your BSC, and call the Department of Environmental Health and Safety for assistance if there are indications of cabinet malfunction 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
 - **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.

In general, the use of a BSC to contain Risk Group 2 biohazardous aerosols is 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.

When working with biohazard risk group 2 materials, an important consideration in the risk assessment is whether or not a BSC is required for the work. The decision is based on the actual

material being used, the concentration and volume of pathogen in use, and whether or not the procedures generate significant aerosols (see CBSG R4.6.23).

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. It will be determined based on the use of hazardous volatile chemicals or radioisotopes.

Fume Hoods

Fume hoods are NOT to be used for level 2 biohazard containment when aerosols will be generated.

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 since this traps potentially infectious microorganisms in a HEPA filter.

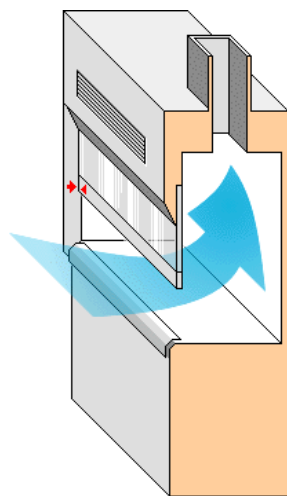
A fume hood may sometimes be used for fixing tissues that might contain biohazardous agents. Consult the Biological Safety Officer.

Some work may require a **biological safety cabinet with fume hood abilities** (for example, using biohazards with chemicals that produce toxic fumes or volatile compounds labeled with radioisotopes).

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, 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 tubes with outside screw-on closures.

- Use a vortex mixer instead of inverting tubes; wait at least 30 seconds after shaking a tube before opening the cap
- Open tubes of hazardous infectious material in a biological safety cabinet only

Centrifuges

For **low speed centrifugation**, as is commonly used in cell/tissue culture, sealed centrifuge buckets with o-rings (safety caps) are recommended for level 2 material and for all cell lines. Safety caps are strongly recommended for known infectious level 2 material (e.g., viruses, viral vectors, and bacteria). 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 caps for microcentrifuges are now available.

Autoclaves

Autoclaves used for the decontamination of biohazardous materials must have their efficacy on a representative load verified using Biological Indicators weekly and records must be maintained.

Queen's University [SOP-Biosafety-09 describes the requirements for Autoclaves used for Biohazardous Waste Treatment](#) and labs must be in compliance.

All autoclaves and autoclave users must be in compliance with the Queen's University [Autoclave Standard Operating Procedure, SOP-Lab-02](#) .

OPERATIONAL PRACTICES

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

Both physical containment and good laboratory practices are important for reducing the risk of laboratory acquired infections. Note that laboratory technique can significantly alter 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 general practices outlines requirements for **all laboratories** handling infectious substances (**both level 1 and 2**) at Queen's University. Although the CBSG is now in force, this list is based on the previous Public Health Agency of Canada's Laboratory Biosafety Guidelines (3rd Edition, 2004), which more thoroughly **described good microbiological practices** than does the CBSG. 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 or restrained 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.
9. Laboratories are to be kept clean and tidy. 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; gloves are to be removed when leaving the laboratory and decontaminated with other laboratory wastes before disposal; 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.
14. 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).
15. The use of needles, syringes and other sharp objects should be strictly limited; 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.
16. Hands must be washed after gloves have been removed, before leaving the laboratory and at any time after handling materials known or suspected to be contaminated.
17. 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 (i.e., cracked, chipped, loose) to biohazardous material must be replaced or repaired.
18. Contaminated materials and equipment leaving the laboratory for servicing or disposal must be appropriately decontaminated and labeled or tagged-out as such.
19. 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.
20. 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; centralized autoclaving facilities are to follow the applicable containment level 2 requirements.

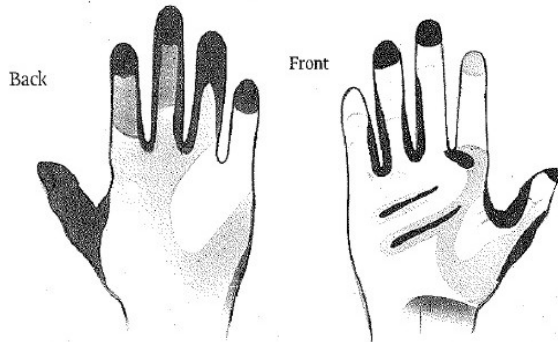
21. Disinfectants effective against the agents in use must be available at all times within the areas where the biohazardous material is handled or stored.
22. Leak-proof containers are to be used for the movement/transport of infectious materials within facilities (e.g., between laboratories in the same facility).
23. Spills, accidents or exposures to infectious materials and losses of containment must be reported immediately to the laboratory supervisor; written records of such incidents must be maintained, and the results of incident investigations should be used for continuing education.
24. 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 on contract.*)

In addition, consideration should be given to limiting the use of personal electronic devices in the laboratory.

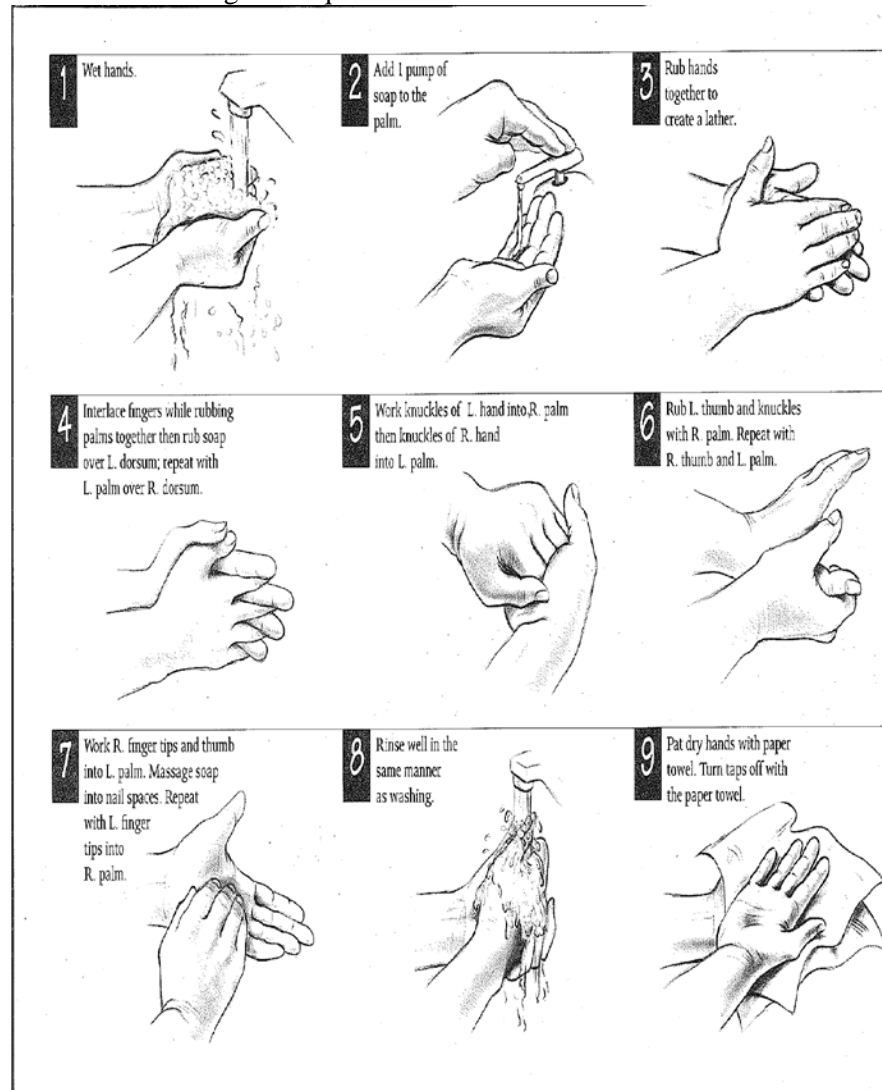
Where there is an increased risk when working with the RG1 biological material (e.g. immunocompromised individual working with an opportunistic RG1 pathogen), consideration should be given to moving the work into a CL2 zone in a Biological Safety Cabinet. Without needing to reveal personal medical information,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

Hand washing

In the diagram below, 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.



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 CBSG Standards Chapter 4.

At level 2, the major addition to the good microbiological practices described above, is that BSCs must be used for procedures that may produce 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 **labeled, 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 or 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 CBSG Standards Chapter 4 for your convenience (Appendix II). If you work in a CL2 lab then read Appendix II. Further explanation of each requirement is found in the [CBSG Transition Index](#) (the middle section of the CBSG between the Standards and the Guidelines).

Containment for Human Blood, Tissues and Bodily Fluids

At Queen's University and many other Universities in Canada, work in research laboratories with human tissues, blood and fluids that might contain human pathogens is classified as requiring Containment Level 2. This designation is used because it is the terminology most familiar to research laboratory personnel.

In medical facilities, the term used for practices to prevent the infection of personnel with such material (or from patients themselves) is "[Routine Precautions](#)" (also known as "Standard Precautions"), which are a combination of the historical "Universal Precautions" and "Body Isolation".

The principals and practices of Containment Level 2 and Universal Precautions are essentially the same where feasible, but the laws under which they are enforced differ. The PHAC Human Pathogens and Toxins Act (HPTA) does not regulate the use of pathogens in their natural environment, so it only applies when a pathogen is cultured from human blood or tissue.

However the need to use appropriate containment practices for material that potentially contains human pathogens (or is known to contain such pathogens), is enforced through the Occupational Health and Safety Act of Ontario and is regulated through the Queen's Biohazard Committee.

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 CBSG Standards Chapter 4 (1st Edition, 2013)
- 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
 - procedures must be approved by the Biohazard Committee
 - 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 unloaded in a 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.

Operational Requirements for Specific Materials

Microorganisms – Bacteria, Viruses, Fungi, Parasites

Microorganisms and the materials that contain 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](#). Nevertheless judgment is required because the pathogenicity of different strains can vary markedly; because within a Risk Group there is a range of pathogenicity; and because 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 also 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. 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 pathogens, which can cause disease in healthy hosts, and opportunistic 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.

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?
- requirements imposed by an import permit issued by PHAC or CFIA must be followed

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.

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.

If working with neurological material that might contain prions, consult the CBSG and the Biosafety Officer about any additional mitigation measures that will be required and include these in the local risk assessment associated with your Biohazard Permit.

Human Blood, Tissues and Bodily Fluids

Human blood, tissues, and bodily fluids can contain blood borne pathogens that are a risk for infection by the mucosal or parenteral route. Certain tissues and bodily fluids may contain other pathogens that colonize these tissues. 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 could be present (e.g. Hepatitis B, Hepatitis C).

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 level of care in containment 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 positive for HIV or positive for some other human pathogen?
- 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 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**, even if they have been classified as Risk Group 1 because they have not been shown to contain a pathogen. It is **recommended to use containment level 2 facilities and work practices when working with all cell lines**. This is usually relatively easy to do since continuous cell culture is done under sterile 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:

- 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 safely 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)
- harbor 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 for work with small and large animals are designed and operated in accordance with the Canadian Biosafety Standards and Guidelines (CBSG) and the Guide to the Care and Use of Experimental Animals, published by the Canadian Council on Animal Care, and other CCAC guidelines and policies.

Small animals will be contained in cages with micro-isolator lids or, preferably, in vented racks under negative pressure with HEPA filters. Level 2 work with small animals will be done in a biological safety cabinet in the housing room unless it is not feasible to do so and then only after a specific protocol has been approved by the University Animal Care Committee and the Biohazards Committee.

Containment facilities for **large animals** (e.g. dogs, rabbits, sheep) are unique, in part because of the large quantity of infectious microorganisms that may be present in the animal cubicle. Unlike a laboratory room, where the BSC and containment caging provide primary containment, the large animal cubicle serves as both the primary and secondary barrier, so **specific facility and operational containment requirements in the CBSG Standards matrix under the CL2-Ag column must be followed**. 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.

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** to prevent the development of allergies to a laboratory animal 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**, that 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:

Biological toxins produced by microorganisms are the only type of toxins regulated under the HPTA. They are listed on Schedule 1 of the HPTA and must be listed on a Biohazard Permit for local oversight. The Biosafety Officer will register their use with the Public Health Agency of Canada.

- Check whether any of the toxins you are using are listed in HPTA Schedule 1 (see [Queen's Toxin Registration form](#) under the Biosafety dropdown menu, or in "Useful Links" in TRAQ/Romeo).
 - If so then you must have a Biohazard Permit application for their use.
 - 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 the Biosafety Officer will assist you with if so requested.
- To purchase Schedule 1 toxins, your lab will need a Containment Level 2 Compliance Letter from PHAC. The Biosafety Officer will assist you with the application.
- Factors to consider when doing a risk assessment for a biological toxin are described in the CBSG section 4.2.1.1.
 - An SOP for the handling of the toxin will be required as part of a Biohazard Permit application or amendment.
 - An SOP might already have been written by another investigator, so contact the Biosafety Officer for assistance.
- Decontamination/inactivation of toxins by thermal or chemical means is described in the CBSG section 16.9
- Further information, including details about the inactivation of specific biological toxins, can also be found in Appendix I of the U.S. Centers for Disease Control and NIH document, [Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, 2007 \(BMBL\)](#)

Other Biological Hazards – Sources of Information

Biosafety information regarding numerous biological hazards can be found in the [CBSG](#), the [PHAC PSDS](#), the [BMBL](#)

A recommended text is 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

PERSONAL PROTECTIVE EQUIPMENT

The selection of appropriate personal protective equipment (PPE) for the specific work is important. 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.

Personnel don (put on) dedicated PPE suitable for the containment zone and the work being done, upon entering the containment zone to protect them 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 determination of 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. 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 particular PPE worn in your laboratory must be developed and posted:

- If applicable, post the first table below near where you store PPE.
- If more complicated than the first table below, a written donning and doffing procedure for the particular PPE worn in your laboratory must be developed and posted outside the lab as a reminder.
 - See the second table as an example for a more complex situation
 - Figures with images may be used

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)	

In the table below the numbers in brackets refer to the Regulatory requirement number in the Operational Standards section of the CBSG. This table is from the CBSG.

Generic Example of the Donning Procedures when Multiple Layers of PPE are Worn		
Donning Order (Descending)	<p>Remove any personal accessories, such as jewellery and identification cards (R4.5.8, R4.6.4)</p> <p>Remove personal clothing (R4.5.9)</p> <p>Dedicated containment clothing, such as scrubs, dedicated footwear, shoe covers and when required, head covers (R4.4.1, R4.4.3)</p> <p>Inner gloves (R4.4.4)</p> <p>Protective back-closing gown or equivalent layer, when required (R4.4.5)</p> <p>Mask or respirator, when required (R4.4.8)</p> <p>Eye protection, including safety glasses, goggles, or face shield, when required (R4.4.2)</p> <p>Outer gloves, fitted over gown cuffs, when required (R4.4.6).</p>	Doffing Order (Ascending)
	<p>Don personal clothing and any personal accessories which were removed prior to entering containment zone</p> <p>Dedicated containment clothing, such as scrubs, dedicated footwear, shoe covers and when required, head covers (R4.5.12)</p> <p>Inner gloves</p> <p>Protective back-closing gown or equivalent layer, when required (R4.5.13)</p> <p>Mask or respirator, when required</p> <p>Eye protection, including safety glasses, goggles, or face shield, when required</p> <p>Outer gloves when worn</p>	

Body

Lab coats should be worn in all general labs, and may be worn in hallways if hazardous materials are being transported.

- Lab coats with knit cuffs are recommended so that a glove can be pulled up over the cuffs.
- Lab coats with snaps rather than buttons are recommended so that they can be removed quickly.
- For highly infectious agents, surgical gowns with back closures and knitted cuffs offer superior protection.
- Plastic or rubber aprons are to be worn for activities that are likely to result in splashes of infectious agents.

- 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, or offices or other areas where food or beverages are consumed.
 - Lab coats may not be worn while eating, drinking, or chewing gum.

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 for any reason. Lab coats should be washed by an approved laundry service.

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 as appropriate for any materials which are being handled. In general, vinyl, latex or nitrile gloves are suitable protection against infectious agents, but remember to check compatibility with any chemical hazards that you are also using.

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. 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 so hands should be washed thoroughly immediately after removing gloves.
- Double gloving should be considered for some agents or procedures.
- Reusing gloves is generally not recommended.
- Latex and vinyl gloves do not provide protection from sharps and needles; nitrile has better abrasion, cut and puncture resistance; fine metal mesh gloves are recommended where both dexterity and protection from sharps are needed.

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 guide for choosing the appropriate glove type can be found through [North Scientific](#).

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).

Eye

Goggles and/or 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 other forms of corrective eyewear are not suitable and CSA approved protective eyewear is worn. Inserting or removing contact lenses is not permitted in any laboratory.

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 also 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.

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.

Anyone requiring such 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 rechecked 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.**

Disposable **surgical 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 **do not** provide significant protection from infection by the aerosol route or against lab animal allergens.

DECONTAMINATION AND WASTE DISPOSAL

Sterilization is a process that completely eliminates all living microorganisms, including bacterial spores. The probability of a microorganism surviving a sterilization process is considered to be 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, 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.

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 CBSG must do the following according to the requirement of the Standard cited in brackets (R##):

- 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 (R4.8.2) 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 (R4.8.10) 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 (R4.1.8, R4.1.9) and routine verification (R4.8.11).
- 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 (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 either a gravity displacement autoclave or a pre-vacuum 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 resolve the air entrapment problems that prevent the penetration of steam and 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 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. Solid contaminated waste (excluding glass), should be placed in a clear bag, inside a solid collecting container which must be labeled with an orange biohazardous materials label.
2. When full, bags must be closed, and labeled with the name of contact person (the person disposing of the waste, not the supervisor) and room number. **DO NOT OVERFILL BAGS** (2/3 full only), and do not compress them, as this will inhibit steam penetration.
3. Double bag for removal from the lab.
4. At the autoclave, bags for decontamination must be placed in the available trays and, immediately before autoclaving, **opened to allow steam penetration**.
5. Disinfected material that is no longer biohazardous must be placed in a regular garbage bag after ensuring that any biohazards warning labels are defaced.
6. The efficacy of the autoclave for decontamination of representative loads of biohazardous waste must be monitored weekly using **biological indicators (bacterial spores of *Geobacillus stearothermophilus*)** commercially available for this purpose) as described in [SOP-Biosafety-09](#) (CBSG R4.8.10, R4.8.11).

Solid waste that would give off hazardous fumes in the autoclave 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**.

There are usually striking differences between the activities of disinfectants when used under actual laboratory conditions as opposed to the controlled, standardized testing methods used to generate efficacy data for product registration. It is therefore difficult to make generalizations about contact times and concentrations needed to kill specific pathogens. When working with microorganisms where an assay is possible, it is advisable for laboratories to conduct in-use disinfectant efficacy testing to evaluate a product's performance under specific conditions of use.

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.

Susceptibility	Microorganism	Disinfectants reported to be effective
Extremely resistant	Prions	Unusually resistant to chemical disinfectants. High concentrations of sodium hypochlorite (NaOCl) or heated strong solutions of sodium hydroxide (NaOH) (see Section 16.10).
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.

Susceptibility	Microorganism	Disinfectants reported to be effective
	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.

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.

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.

Although **bleach** is cheap and effective against many microorganisms, it is corrosive to stainless steel (such as in a biological safety cabinet) and 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., tissue, blood, bedding, feces) protects microorganisms and toxins from contact with disinfectants and can neutralize many germicides (e.g., NaOCl). **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 16 of the CBSG](#). That chapter 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 2 in the [BMBL 5th Edition](#).

Appendix I of the [BMBL](#), tables 1 and 2, have information about the physical and chemical inactivation of some **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, 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.

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 labeled 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.

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

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.

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 be in compliance 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.

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,
 - b. labeled 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 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 labeled and prepared for hazardous waste disposal.

Mixed Waste

For waste which is a mixture of chemical/radioactive and biohazardous waste, it is often 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. If this is not possible, or 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 incinerated 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.

A [template word document for Emergency Procedures](#) is available on the Environmental Health and Safety Website <http://www.safety.queensu.ca/emergency/>.

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 of personnel on the Emergency Procedures must be done annually.

BIOSECURITY

Biosecurity breaches, 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 also 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 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 of the 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 a CL2 lab.
- 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 who is walking unaccompanied in the 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 within 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 to Principal Investigator and the Department of Environmental Health and Safety.

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

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

Detailed information about the Import and Export of Biological Material is available in [SOP-Biosafety-02](#).

Importation or Purchase from Canadian Suppliers of Imported Material

The Biosafety Officer will guide you through the importation process. Filling out the required forms will take 2 or 3 hours if you do not already have a CL2 compliance letter. **Federal agency processing time for CL2 compliance letters, import permits and courtesy letters is up to 20 business days.** So contact the Biosafety Officer well ahead of when you want to import the material.

The Public Health Agency of Canada (PHAC) in collaboration with the Canadian Food Inspection Agency (CFIA), regulates use and importation of human and zoonotic pathogens and most non-zoonotic terrestrial animal pathogens.

The Canadian Food Inspection Agency (CFIA) regulates use and importation of exotic non-zoonotic terrestrial animal pathogens, aquatic animal pathogens, and plant pests.

Investigators wishing to import human or animal pathogens requiring level 2 or 2+ containment must have a valid Queen's Biohazard Permit for the specific organism or material, and a **valid PHAC and/or CFIA permit before importation.**

Some Canadian Suppliers have import permits for biohazardous materials requiring CL2. E.g. Cedarlane supplies ATCC cells. **They require proof that the laboratory has a current Federal CL2 Compliance letter before they will ship this material.**

The completed lab checklist to obtain a Federal CL2 Compliance letter and/or Import Permit application must be signed by the Biosafety Officer and sent to the appropriate agency for approval.

The import permit will stipulate the containment requirements. A copy of the facility approval and the import permit must be sent to the Biosafety Officer for the Principal Investigator's Biohazard Permit file.

Some animal blood, serum, products or by-products also require an import permit because they might contain pathogens exotic to Canada. The country of origin will be a factor in determining whether or not an import permit is required for animal material. Consultation with CFIA will be required and the Biosafety Officer can assist.

If importing non-pathogenic biological material, both CFIA and PHAC will issue a “**courtesy letter/non-path letter**” to indicate that material is non-pathogenic. It is advisable to request such a letter using the import permit application form if you are importing from a collaborator rather than a commercial supplier, to ensure that material will not be delayed (and perhaps destroyed) when entering Canada.

Export

Export of pathogens falls under the Human Pathogens and Toxins Act and will be controlled by regulations under this act when they are written.

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

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

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 **labeled, 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 campus), 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 alter the classification of an infectious substance from Risk Groups 1-4 to 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 or not your sample qualifies. Information can be found in the [Biohazard Module of the TDG training slides](#).

BIOHAZARD ADMINISTRATION at QUEENS - LABORATORY PERMITS, COMMISSIONING and DECOMMISSIONING

Biohazard permitting at Queen’s transitioned from a paper-based application to an electronic system during the fall of 2013. Previously approved paper applications have been scanned and uploaded so that they can be viewed through the system. Renewals, amendments, re-applications and new biohazard applications must now be submitted through that system.

Training in the technical aspects of the use of the electronic biosafety permitting system is being provided by the TRAQ/Romeo system development team. 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.

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.

The types of material requiring a Biohazard permit are described in [SOP-Biosafety-05 Queen’s University Biohazard Risk Group Definitions](#) .

The content of the biohazard permit application is the responsibility of the Principal Investigator and must be submitted under their signature. However they may delegate the work of preparing the form and risk assessment(s) to a member or members of their team. Part of the role of the Biosafety Officer is to assist in the preparation of these applications when requested.

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.

Under the old paper-based system, the re-application was designed to ensure that the risk assessment and associated documents still fully considered the biohazard risks in your laboratory, to consolidate any amendments into one risk assessment document and to update the material with respect to any changes in biosafety practices or regulations.

After we transition to the new electronic system, 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.

Under the electronic system, **all laboratory personnel will be required to read the approved biohazard permit documents and submit an electronic 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.

Biohazard Amendment

Changes to an approved biohazard permit may be made with a biohazard amendment event form. 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 approve 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. 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 labeled 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 in order 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: <http://www.safety.queensu.ca/decom/decomrequest.htm>.

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 Principle 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 been 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 re-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 and avoid vigorous shaking and blowing bubbles with your pipette when resuspending packed cells and/or work in a biological safety cabinet to contain aerosols.

For **low speed centrifugation** as is commonly used in tissue culture, 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+ work 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 convection 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 [Emergency Response Procedures](#) posted in your lab, in the section on Equipment Associated Emergencies.

To avoid contaminating your centrifuge:

- Check glass and plastic centrifuge tubes for stresslines, 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. Avoid using 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 which can be loaded and unloaded 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 risk because accidents can occur and aerosols may be generated. 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 type disinfectant regularly and immediately after tissue known to contain bloodborne pathogens, *M. tuberculosis* or other infectious agents is cut.
- 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.

Cryogenics and Liquid Nitrogen

- If liquid nitrogen enters a vial during storage, then upon warming it can explode. This has caused eye and hand injuries. Always wear protective goggles or 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 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 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. Cell sorters are used to physically separate a defined subpopulation of cells from a larger, heterogeneous population.

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, 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 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 collecting 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 (for example, using biohazards with chemicals that produce toxic fumes or volatile compounds labeled with radioisotopes). Contact the Biological Safety Officer if this is the case.

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
- 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 non-corrosive to the microscope.
- 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 condition of gaskets, caps and bottles before using.
- Open tubes in a biological safety cabinet, or if that is not possible then allow aerosols to settle for at 10 minutes after use before opening containers
- 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
 - May cover 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.
- **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. 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.
- Fill syringes carefully; avoid frothing or introduction of air bubbles.
- Shield needles with disinfectant-soaked cotton pledgets 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 while minimizing the risk of needle-stick must be documented
- Place used needles and syringes in puncture-resistant containers and decontaminate before disposal.

Pipettes and Mechanical Pipetting Aids

Improper handling of pipettes has led to a number of 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
- Submerge used non-disposable pipettes horizontally in disinfectant solution; dropping them in vertically may force out any liquid remaining in the pipette

- For infectious level 2 material, submerge contaminated pipettes in disinfectant solution inside the bsc

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](#)
- In particular, 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

- To prevent contamination, clean regularly
- 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).

APPENDIX II

CBSG Operational Practice Requirements for Containment Level 2 *in vitro* and *in vivo*

The tables below present the containment level 2 operational practice requirements as stated in Chapter 4 of Part I, the Standards, in the Canadian Biosafety Standards and Guidelines (CBSG) 1st Edition, 2013.

For all PHAC (HPTA) or CFIA designated:

- **CL2 laboratories**
- **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)**

- Note that this table is provided here in isolation from the CBSG for convenience. However, it is **strongly recommended** that when first reading an item in the Standards, or if you have any doubt about its intention, you **also read the Transition Index for that item for clarification**. If still in doubt, consult the University Biosafety Officer.
- Numbers in the left column are those used in the CBSG for each item of the Standards.
- Definitions of terms can be found in the CBSG 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 requirements for CL2-Ag. Note that these requirements are in addition to those in the first two tables.
- These tables **do not present the operational requirements that are exclusively for prion work** (for those look for “P” in the CBSG matrices).

Table 1. CL2 OPERATIONAL PRACTICE REQUIREMENTS

4.1	Biosafety Program Management
4.1.1	A biosafety program management system to be in place to oversee safety and containment practices.
4.1.2	Contact information to be provided to the relevant federal regulatory agency (or agencies), and kept up to date.
4.1.3	Program intent to be submitted to the relevant federal regulatory agency (or agencies) in accordance with importation and/or certification/recertification requirements.
4.1.4	An overarching risk assessment to be conducted and documented to identify the hazards and appropriate mitigation management

	strategies for the proposed activities involving infectious materials or toxin.
4.1.5	A Local Risk Assessment (LRA) to be conducted and documented to examine each activity, identify risks, and develop safe work practices.
4.1.6	A respiratory protection program to be in place, when respirators are in use.
4.1.7	A biosafety representative(s), commonly a BSO, to be designated for the oversight of biosafety and biosecurity practices including: <ul style="list-style-type: none"> • monitoring of biological material that enters, is held within, or leaves the containment zone; • facilitating compliance with all relevant federal regulatory requirements; • provision or coordination of employee training; • development and maintenance of the Biosafety Manual and SOPs; • facilitating compliance with the Biosafety Manual and SOPs; and • determining personnel authorized to work in the containment zone.
4.1.8	A Biosafety Manual to be developed, implemented, kept up to date, made available to personnel inside and outside of containment zone, and contain institutional biosafety policies, programs, and plans, based on a documented overarching risk assessment and/or LRAs; the Biosafety Manual to include: <ul style="list-style-type: none"> • program intent; • biosafety program; • brief description of the physical operation and design of the containment zone and systems; • SOPs for safe work practices for handling infectious material, toxins, and/or infected animals, including: <ul style="list-style-type: none"> ○ PPE requirements; ○ Entry/exit procedures for personnel, animals and materials; ○ use of primary containment devices; ○ animal work considerations; ○ decontamination and waste management; ○ movement and transportation of biological material procedures; • medical surveillance program, where applicable; • biosecurity plan; • training program; • emergency response plan (ERP) and incident reporting procedures;

	<ul style="list-style-type: none"> housekeeping program; and facility and equipment maintenance program for components of the containment zone.
4.1.9	The Biosafety Manual to be supplemented and updated with SOPs specific to the nature of the work being conducted in the containment zone and to each project or activity, as applicable.
4.1.10	A biosecurity risk assessment to be conducted.
4.1.11	A Biosecurity Plan, based on a biosecurity risk assessment, to be implemented, evaluated and improved as necessary, and kept up to date.
4.1.12	Inventory of infectious material and toxins handled or stored in the containment zone to be maintained, and kept up to date. Infectious material or toxins stored outside the CL2 zones to be included in the inventory.
4.1.13	Records pertaining to importation requirements to be kept for 2 years following the date of disposal, complete transfer or inactivation of the imported infectious material or toxin, and made available upon request.
4.2	Medical Surveillance Program
4.2.1	A medical surveillance program, based on an overarching risk assessment and LRAs, to be developed, implemented, and kept up to date.
4.2.3	Containment zone personnel to immediately notify their supervisor of any illness caused by, or that may have been caused by, the infectious material or toxin(s) being handled or stored.
4.3	Training and Retraining Program
4.3.1	A training needs assessment to be conducted.
4.3.2	A training program, based on a training needs assessment, to be implemented, evaluated and improved as necessary, and kept up to date.
4.3.3	Personnel to be trained on the relevant components of the Biosafety Manual/SOPs, as determined by the training needs assessment.
4.3.4	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 toxin(s) in use and the necessary precautions to prevent exposure to, or release of, infectious material or toxins.
4.3.5	Personnel to be trained on the relevant physical operation and design of the containment zone and systems.
4.3.6	Personnel to be trained on the correct use and operation of laboratory equipment, including primary containment devices.
4.3.7	Personnel working with animals to be trained in restraint and handling techniques.
4.3.8	Visitors, maintenance/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.
4.3.9	Personnel to demonstrate knowledge of and proficiency in the SOPs on which they were trained.
4.3.10	Trainees to be supervised by authorized personnel when engaging in activities with infectious material and toxins until they have fulfilled the training requirements.

4.3.11	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.
4.3.13	Training and refresher training to be documented; records to be kept on file.
4.4	Personal Protective Equipment
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.
4.4.2	Face protection to be worn where there is a risk of exposure to splashes or flying objects.
4.4.3	Personnel working in animal rooms, cubicles, or PM rooms to wear dedicated protective footwear and/or additional protective footwear.
4.4.4	Gloves to be worn when handling infectious material, toxins or infected animals.
4.5	Entry and Exit of Personnel, Animals and Materials
4.5.1	Containment zone doors and animal room/cubicle doors to be kept closed.
4.5.2	Access to containment zone to be limited to authorized personnel and authorized visitors.
4.5.4	Current entry requirements to be posted at entry to containment zone, animal rooms/cubicles, and PM room point(s) of entry.
4.5.6	Personal clothing to be stored separately from dedicated PPE.
4.5.7	Personal belongings to be kept separate from areas where infectious material or toxins are handled or stored.
4.5.10	Personnel to doff dedicated PPE (in accordance with SOPs) in a manner that minimizes contamination of the skin and hair when exiting the containment zone.
4.5.11	Personnel to wash hands after handling infectious materials or toxins, and when exiting the containment zone, animal room/cubicle, or PM room.
4.6	Work Practices
General	
4.6.1	Contact of the face or mucous membranes with items contaminated or potentially contaminated with infectious material or toxins to be prohibited.
4.6.2	Hair that may become contaminated with working in the containment zone to be restrained or covered.
4.6.3	Type of footwear worn to be selected to prevent injuries and incidents, in accordance with containment zone function.
4.6.5	Oral pipetting of any substance to be prohibited.
4.6.6	Open wounds, cuts, scratches, and grazes to be covered with waterproof dressings.
4.6.7	Traffic flow patterns from clean to dirty areas to be established and followed, as determined by an 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.
4.6.10	Bending, shearing, re-capping, or removing needles from syringes to be avoided, and, when necessary, performed in accordance with

	SOPs.
4.6.11	Work surfaces to be cleaned and decontaminated with a disinfectant effective against the infectious material in use, or a neutralizing chemical effective against the toxins 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 in accordance with SOPs.
4.6.15	BSCs, where present, to be certified upon initial installation, annually, and after any repairs or relocation. Certification to include verification of correct operation by <i>in situ</i> testing in accordance with NSF International (NSF)/ANSI 49, or, where not applicable, with manufacturer specifications.
Handling Infectious Material and Toxins	
4.6.18	Good microbiological laboratory practices to be employed.
4.6.19	Samples of infectious material or toxins 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 infectious material or toxins stored outside the containment zone to be labelled, leakproof, impact resistant, and kept either in locked storage equipment or within an area with limited access .
4.6.23	A certified BSC to be used for procedures that: <ul style="list-style-type: none"> • 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. <p><i>[Not required when working with large-sized animals.]</i></p>
4.6.25	Gloves to be removed before exiting the BSC.
4.6.26	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.
4.6.28	Sustained open flames to be prohibited in a BSC; on-demand open flames to be avoided.
4.6.29	Procedures, based on an LRA and in accordance with SOPs, 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.
4.6.30	Large scale cultures of infectious material or toxins to be contained within a closed system or other primary containment device.
4.6.31	Sample collection, addition of materials, or transfer of 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.
4.6.32	Experimentally infecting cells or other specimens derived from the person conducting the experiment to be prohibited.
Housekeeping and General Maintenance	
4.6.33	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.
4.6.35	An effective rodent and insect control program to be maintained.

4.6.38	Personnel to conduct periodic visual inspections of the containment zone to identify faults and/or deterioration; when found, corrective actions to be taken.
4.6.39	Records of regular inspections of the containment zone and corrective actions to be kept on file.
4.6.40	Records of building and equipment maintenance, repair, inspection, testing or certification, in accordance with containment zone function, to be kept on file.
4.8	Decontamination and Waste Management
4.8.1	Gross contamination to be removed prior to decontamination of surfaces and equipment, and disposed of in accordance with SOPs.
4.8.2	Disinfectants effective against the infectious material in use and neutralizing chemicals effective against the toxins 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, fitted with lids, and specifically designed for sharps waste.
4.8.4	Primary containment devices to be decontaminated prior to maintenance.
4.8.5	All clothing and PPE to be decontaminated when a known or suspected exposure has occurred.
4.8.7	Contaminated liquids to be decontaminated prior to release into the sanitary sewer.
4.8.8	Contaminated materials and equipment to be decontaminated and, in accordance with SOPs, labelled prior to cleaning, disposal, or removal from the containment zone, animal rooms/cubicles, or PM rooms.
4.8.10	Decontamination equipment and processes to be validated (in accordance with SOPs) using representative loads, and routinely verified using application-specific biological indicators, chemical integrators, and/or parametric monitoring devices (e.g., temperature, pressure, concentration) consistent with the technology/method used.
4.8.11	Efficacy monitoring of decontamination equipment and processes to be performed routinely, based on an LRA, and records of these actions to be kept on file.
4.8.13	Contaminated bedding to be removed at a ventilated cage changing station or within a certified BSC prior to decontamination, or to be decontaminated within containment cages.
4.8.14	Animal cubicles, PM rooms, and the dirty corridor, when present, to be decontaminated when grossly contaminated and at the end of an experiment.
4.9	Emergency Response Planning
4.9.1	An ERP, based on an overarching risk assessment and LRAs, to be developed, implemented, and kept up to date. The ERP is to describe emergency procedures applicable to the containment zone for: <ul style="list-style-type: none"> • Accidents/incidents; • medical emergencies; • fires • chemical/biological spills (small and large; inside/outside BSC and centrifuge); • power failure; • animal escape (if applicable); • failure of primary containment devices; • loss of containment;

	<ul style="list-style-type: none"> • 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.
4.9.5	Incidents involving infectious material, toxins, or infected animals, or involving failure of containment systems to be reported immediately to appropriate personnel.
4.9.6	Incident investigation to be conducted and documented for any incident involving infectious material, toxins, infected animals, or failure of containment systems, in order to determine root cause(s).
4.9.7	Records of incidents involving infectious materials, toxins, infected animals, or losses of containment to be kept on file.

Table 2. Additional Procedures required for CL2-SA beyond CL2

4.5	Entry and Exit of Personnel, Animals and Materials
4.5.8	Personal belongings not required for work to be left outside the containment zone or in change areas outside the containment barrier.
4.6	Work Practices
General	
4.6.13	Verification of inward directional airflow to be performed routinely and in accordance with SOPs.
Housekeeping and General Maintenance	
4.6.34	Routine cleaning, in accordance with SOPs, to be carried out by containment zone personnel or other staff trained specifically for this task.
4.7	
Animal Work Considerations	
4.7.1	Proper methods of restraint to be used to minimize scratches, bites, kicks, crushing injuries, and accidental self-inoculation.
4.7.2	Primary containment caging housing infected animals to be labelled.
4.7.3	Handling procedures to be employed to minimize the creation of aerosols and dissemination of dust from cages, refuse and animals.
4.7.4	Animals and carcasses to be securely transported into, out of, and within the containment zone.
4.7.6	Inoculation, surgical, and necropsy procedures to be designed and carried out to prevent injuries to personnel and minimize the creation of aerosols.
4.7.7	Inoculation, surgical, and necropsy procedures with animals in SA zones to be carried out in a certified BSC or other appropriate containment devices. <i>(does not apply to CL2-Ag)</i>
4.7.8	Animals to be disinfected and/or cleaned at site of injection or exposure following inoculation or aerosol challenge with infectious material or toxins, where possible based on work.
4.8	Decontamination and Waste Management
4.8.13	Contaminated bedding to be removed at a ventilated cage changing station or within a certified BSC prior to decontamination, or to be decontaminated within containment cages.

Table 3. Additional Procedures required for CL2-Ag (beyond CL2-SA)

4.4	Personal Protective Equipment
4.4.5	Full body coverage dedicated protective clothing to be worn inside the containment barrier where human or zoonotic pathogens are handled.
4.4.8	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	Entry and Exit of Personnel, Animals and Materials
4.5.5	Personnel to verify correct reading of monitoring device(s) that visually demonstrate inward directional airflow, prior to entry into area where inward directional airflow is provided.
4.5.12	Personnel to doff dedicated PPE (or additional layer of PPE, when worn) when exiting animal cubicles or PM rooms, except when exiting to the dirty corridor.
4.7	Animal Work Considerations
4.7.5	Animal carcasses to be removed from cubicles/PM rooms via the dirty corridor or divided into smaller portions and placed into labelled, leakproof, and impact resistant transport containers.
4.8	Decontamination and Waste Management
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 the dirty corridor, when present, to be decontaminated when grossly contaminated and at the end of an experiment.

APPENDIX III

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 and Guidelines, so it is described in more detail in the BIOSAFETY OVERVIEW section of this manual.

- [Canadian Biosafety Standards and Guidelines \(CBSG\), 1st Edition, 2013](#) will form the basis of regulations under the Human Pathogens and Toxins Act.
 - **The CBSG 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)

- **Human Pathogens and Toxins Act (2009), (HPTA)**
 - 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)
 - The HPTA was based on the requirements of the Laboratory Biosafety Guidelines (2004), that have since been replaced by the Canadian Biosafety Standards and Guidelines 1st Edition (2013)
 - The HPTA was only partly in effect in 2013, with the regulations under the HPTA still to be published (estimated that they will be fully in force in 2015)
 - Contains a list of regulated toxins (Schedule 1).

- Human Pathogens Importation Regulations, SOR/94-558
 - Regulatory authority for the Public Health Agency of Canada with respect to the importation of human pathogens into and transportation within Canada.
 - Will be repealed when the HPTA is fully in force in 2015

- **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.
 - Issuance of import permits is one of the ways 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 labeled in a specified manner.
 - There must be a Material Safety Data Sheet (MSDS) available to accompany each hazardous substance used at the work site.
 - 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 involving biohazards and to adhere to the PHAC and the CFIA Standards and Guidelines, including but not limited to:
 - Establish an Institutional Biosafety Committee and appoint a Biosafety Officer
 - Provide appropriate training, as prescribed, prior to beginning the work, for all persons whose research may involve biohazards
 - Maintain a safe working environment by regularly inspecting and maintaining all equipment and facilities used specifically for research, storage, or disposal of biological hazards
 - Comply with all applicable federal and provincial laws
 - **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 and Guidelines; 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