Guideline
Operational Practices
Level 2 Biosafety Permits

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Introduction

This document is meant as a guideline for the good microbiological practices that laboratories working with Risk Group 2 (RG2) biological agents must follow, as outlined in the Canadian Biosafety Standard (CBS) and detailed in the Canadian Biosafety Handbook (CBH). The CBS not only specifies the physical containment requirements of laboratories but also specifies the good operating practices that must be adhered to when handling biological agents. These good laboratory practices are important for reducing the risk of laboratory acquired infections, reducing the risk of your samples/cultures of being contaminated and preventing the inadvertent release of biologicals into the environment.

Good microbiological practices include the use of Personal Protective Equipment (PPE), hand washing, disinfecting work areas, procedures that minimize the creation of aerosols, and proper decontamination and disposal of materials. They also mandate that personnel are notified of potential risks, receive safety training, that lab-specific procedures are written and provided and that emergency response procedures are available.

Supervisors must be familiar with these operational requirements and ensure they are followed in their laboratories.


Responsibilities

Principal Investigator/Permit Holder

The list of responsibilities of the Principal Investigator/Biosafety Permit Holder can be found here: https://ehs.utoronto.ca/our-services/biosafety/biosafety-manual/biosafety-permit-holder/

Laboratory Users

Laboratory users are personnel who work with, have access to, or may be exposed to biological material, their responsibilities can be found here: https://ehs.utoronto.ca/our-services/biosafety/biosafety-manual/laboratory-users/

Operational Practice Requirements - CBS Chapter 4

The operational requirements specified in Chapter 4 of the CBS are designed to reduce the risks associated with handling or storing pathogens, toxins and other infectious material including infectious animals that are Risk Group 2 to 4. In this guideline the requirements for work with RG2 materials will be discussed and summarized.
Biosafety Program Management CBS 4.1

Administrative Controls
Institutions and organizations that handle or store biologicals of RG2 or higher must have a biosafety program in place for the oversight of safety and containment practices and designate a biosafety representative(s). Here at the University of Toronto, the overall control of the program is through the University’s Institutional Biosafety Committee (IBC) with the administrative and technical support provided by the Biosafety team in the Office of Environmental Health and Safety. (CBS 4.1.1, 4.1.2, 4.1.3, 4.1.4)

Risk Assessments and Planning
An overarching risk assessment must be conducted and documented to identify the hazards and appropriate mitigation strategies for activities involving infectious material or toxins. An overarching risk assessment is defined as a broad risk assessment that supports the organization’s biosafety program. The IBC oversees the research conducted at the University of Toronto involving biological agents and ensures that this research is in conformity with the national, provincial, local and university regulations and standards. The IBC is also responsible for setting and enforcing appropriate safety standards through the issuance of biosafety permits for all work with biological material. The permit and permit amendment review process is done by members of Local Biosafety Committees (LBC) and relevant subject matter specialists when required.

However, the primary responsibility for the regulatory compliance, safety and security of staff, students and the public lies with the permit holder/principal investigator in charge of the research. See the section on responsibilities above for a list of permit holder obligations. (CBS 4.1.6)

A biosecurity risk assessment must be conducted and documented. Biosecurity measures are designed to prevent the loss, theft, misuse, diversion, or intentional release of pathogens, toxins and other related assets (i.e. equipment, animals). The biosecurity measures outlined in the operational practices includes biological inventories, regulations on lab access and the storage of biologicals. (CBS 4.1.7)

Site-specific local risk assessments (LRAs) must be conducted to examine each task involving infectious material or toxins so that risks are identified and safe work practices developed and documented. LRAs are the responsibility of permit holders. (CBS 4.1.8) For more details on LRAs see CBH 4.4.1 and Canadian Biosafety Guideline – Local Risk Assessment: [https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance/canadian-biosafety-guidelines.html](https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance/canadian-biosafety-guidelines.html)

A training needs assessment must be conducted. This is an evaluation used to identify the current and future training needs of the facility (organization, containment zone (lab)) and identify gaps in the current training program. The biosafety office provides training courses that all personnel working with biologicals must complete. Permit holders must provide in-lab training and conduct regular training needs assessments. Further details on this can be found below in the training section. (CBS 4.1.9, 4.1.14)
**Biosafety Program Elements**

A biosafety manual must be developed, implemented, kept up to date and made available to all personnel. This manual must include the institution’s biosafety program and policies, an emergency response plan, biosecurity measures (i.e. accountability of biologicals, inventories, incident response etc.), site-specific Standard Operating Procedures (SOPs) based on LRAs, training program, housekeeping and equipment maintenance and a medical surveillance program (if appropriate). (CBS 4.1.10, 4.1.11, 4.1.12, 4.1.15, 4.1.16) For details on how to provide your lab-specific biosafety manual see this document:


A respiratory protection program must be available when respirators are in use. (CBS 4.1.13) The University of Toronto’s respiratory protection program can be found here:

https://ehs.utoronto.ca/training/respiratory-protection-training-fit-testing/

**Medical Surveillance Program CBS 4.2**

Personnel working with human fluids, tissues and primary cells, may need to take part in the medical surveillance program available at the University of Toronto. This program provides health surveillance, testing and immunization with relevant, licensed vaccines for university employees and students.

These personnel must also take the online EHS course on Bloodborne Pathogens, EHS603.

For information about the university's medical surveillance program your staff/students should contact the Occupational Health Nurse at ehs.occhealth@utoronto.ca.

Further information on the medical surveillance program can be found at: https://ehs.utoronto.ca/our-services/occupational-health/ and on the biosafety webpage: https://ehs.utoronto.ca/our-services/biosafety/biosafety-manual/medical-surveillance-immunoprophylaxis/

**Training Program CBS 4.3, CBH Chapter 8**

**Online EHS Training**

Before working with any biologicals, personnel must first take the required Environmental Health & Safety (EHS) courses. To determine which courses need to be taken, see the training matrix for laboratory personnel (list of available online courses) which can be found here:

https://ehs.utoronto.ca/training/laboratory-personnel/

Required online training for all personnel working/in contact with biologicals:

- **EHS 601 Laboratory Biosafety**: all personnel attached to a biosafety permit must complete this course prior to starting work in the laboratory.
University of Toronto Environmental Health and Safety

- **EHS 602 Biosafety Refresher**: must be taken annually starting one year after completing EHS 601.
- **EHS 101 WHMIS and Lab Safety**: all personnel attached to a biosafety permit must complete this course prior to starting work in the laboratory.
- **EHS 112 WHMIS Refresher**: must be taken annually starting one year after completing EHS 101.

**Training requirements for Permit Holders:**

- **EHS 601 Laboratory Biosafety**: please note that EHS 601 and the no longer available EHS 014 (PI online biosafety quiz) are equivalent and if you have taken EHS 014 in the past you do not have to take EHS 601.
- **EHS 621 PI Biosafety Refresher**: must be taken annually starting one year after completing EHS 601.
- **EHS 101 WHMIS and Lab Safety**.
- **EHS 112 WHMIS Refresher**: must be taken annually starting one year after completing EHS 101.

**Examples of other training available (for full list see training matrix):**

- **EHS 603 Bloodborne Pathogens**: any personnel that work with primary human tissues/cells/fluids must take this course prior to starting work with these biological agents.
- **EHS 532: Respiratory Protection**: all personnel requiring a respirator must take this course. It includes an on-line course and an in-person fit test every 2 years.
- **EHS 113 Compressed Gas Safety for Lab Users**: labs that use compressed gas can have their personnel take this course in tandem with their lab’s in-house training.
- **EHS 803 Hazardous Waste Management at UofT**: summary and guideline about hazardous waste disposal.
- **EHS 820 Response to Small Laboratory Spills**: how to deal with small chemical spills.

Any personnel that have not fully completed all required training (trainees) must be directly supervised by trained personnel when working in the lab until they have completed their training (CBS 4.3.8).

If anyone has questions regarding EHS training, they can check the Training FAQ page at: https://ehs.utoronto.ca/training-faq/ or contact the course coordinator at ehs.courses@utoronto.ca

Personnel may check their EHS training history by logging into the EHS portal here: https://ehs.utoronto.ca/training/my-ehs-training/

**In-House Lab-Specific Training**

Personnel must know and understand the potential hazards of their work, and follow all operational practices and procedures. The fundamentals of safe biological work are discussed in the Laboratory
Biosafety course (EHS601). Supervisors must also ensure that all personnel, have successfully completed in-house training on their lab’s SOPs including equipment use, waste procedures and emergency response. Personnel must show understanding and competence in those procedures prior to working in the lab (CBS 4.3.7). This requirement is applicable to both new and experienced personnel. All in-house training must be documented, dated and signed by both the trainee and trainer, and available upon request by EHS personnel and external regulators. Documentation of all in-house training should be kept by the supervisor for a minimum of 5 years after the personnel has left the lab (CBS 4.10.1).

In-house training must include the following:

- Personnel must be trained on the relevant components of the lab-specific Biosafety Manual and SOPs as determined by a training needs assessment (CBS 4.3.1).
- Personnel must be trained on the potential hazards associated with the work involved, including the signs and symptoms of disease(s) caused by the infectious material or toxins in use and the necessary precautions to prevent exposure to, or release of, pathogens or toxins (CBS 4.3.2). This should include safe work practices and physical control measures such as decontamination and waste management, and the correct use of PPE. Information on relevant safety information e.g. Pathogen Safety Data Sheets (PSDSs) and how to find and use these materials should be provided.
- Personnel must be trained on the relevant (to their activities) physical design and operation of the containment zone and containment systems (CBS 4.3.3) and on the correct use and operation of laboratory equipment (CBS 4.3.4). This should include how to determine if the equipment is functioning properly and how to protect against a release or exposure. A review of any secondary containment systems should also be included. Some examples of equipment and systems are Biological Safety Cabinets (BSCs), HVAC control systems, autoclaves, primary containment caging systems, centrifuges, incubators, fume hoods and any other laboratory equipment/apparatus used when handling biologicals or toxins.
- Personnel working with animals must be trained in restraint and handling techniques (CBS 4.3.5).

**Training Needs Assessments**

Training needs assessments must be undertaken by supervisors on a regular basis, at minimum annually, or more often if there is a change in program intent, new research protocols/practices or following any incidents that may have occurred in the lab (CBS 4.3.9, 4.10.1). These assessments are necessary to determine if all personnel are up to date on their required training (both EHS courses and in-house training).

Retraining or training reviews may have to be undertaken when SOPs are amended with changes to equipment, projects or procedures. Any in-house retraining or reviews must also be documented.

**Annual In-House Emergency Response Plan (ERP) Review**
All personnel listed on a biosafety level 2 permit must attend an annual in-house emergency response review, and their attendance at this review must be documented. Your ERP review should include (but is not limited to) spill procedures, ocular exposure, spill on body, needle puncture, BSC alarm, evacuation, fire, location of spill kits and safety equipment (showers, eyewashes, fire extinguishers, first aid kit), emergency contact #s, incident reporting, etc. Documentation of these reviews should be readily available upon request by EHS personnel and external regulators (CBS 4.3.10).

**Biosafety Memorandum of Understanding and Agreement (MOU)**

It is the biosafety permit holder’s responsibility to have on file signed MOU forms for everyone listed on their permit. These forms are to be kept for at least 5 years from the last date of their personnel’s appointment and in perpetuity if work with lentivirus is undertaken. The MOU file is to be available for review upon request by EHS personnel. The biosafety MOU can be found here:


**Personal Protective Equipment (PPE) CBS 4.4, CBH Chapter 9**

The PPE to be worn when working in any lab should be in accordance with the highest risk or possible hazard for the equipment, biological agents, material or chemical used in the procedure. The supervisor must advise personnel on what PPE is required based on a LRA to identify potential risks in any lab procedure.

PPE must be worn at all times when working with biological agents and stored within the containment zone (lab) (CBS 4.4.1). Check the Safety Data Sheet(s) (SDSs) for the chemicals that will be used in the procedure, to ascertain if any additional PPE is required.

- Lab coats must be long sleeved and knee length (CBS 4.4.1)
- Long pants or skirt (the entirety of the legs must be covered)
- Shoes are to be closed toe and heel, low heeled (or no heeled) and have non slip soles (CBS 4.6.3)
- Gloves must be worn when handling biologicals, toxins or infected animals (CBS 4.4.4). Specific glove types may have to be specified for different procedures (based on your LRA). Some examples include, nitrile/latex laboratory gloves for handling specimens, and insulated utility gloves for handling freezing materials. Ensure gloves are compatible with possible hazards
- Safety goggles or face shield if there is a possibility of ocular splash, or flying debris (CBS 4.4.2)
- Respirators may be required due to the potential exposure to aerosols outside of a primary containment device based on your LRA. Those personnel that must wear respirators must be fit tested every 2 years, see: [https://ehs.utoronto.ca/training/respiratory-protection-training-fit-testing/](https://ehs.utoronto.ca/training/respiratory-protection-training-fit-testing/)
Personnel to remove PPE carefully to minimize possible contamination of their skin, hair or clothing when leaving the containment zone (lab) (CBS 4.5.14)

Potentially contaminated clothing articles and PPE should be decontaminated prior to washing (CBS 4.8.5, 4.8.6): https://ehs.utoronto.ca/wp-content/uploads/2015/10/Lab-Coat-Washing-Guidelines.pdf

Additional information and resources on PPE are provided below:

General Laboratory PPE Assessment tool: https://ehs.utoronto.ca/wp-content/uploads/2016/06/Laboratory-PPE-Assessment-Tool.pdf

General information on PPE: https://ehs.utoronto.ca/resources/personal-protective-equipment-ppe/


**Entry and Exit of Personnel, Animals and Materials CBS 4.5**

*Laboratory doors are to be kept closed*, do not prop them open. Labs are to be locked if unoccupied. Unlocked and unoccupied labs are considered a serious biosecurity concern. Not only is there a possibility of biological agents or hazardous chemicals being removed from the lab but personal items and lab equipment could be stolen. (CBS 4.5.1)

*Access to the laboratory must be limited to authorized personnel* and authorized visitors only. Authorized personnel are the fully trained personnel listed on your permit. Visitors are to be accompanied at all times. (CBS 4.5.2)

*Appropriate and current biohazard signage* must be posted at the entrance(s) of all CL2 labs. Any specific entry requirements to a lab must also be posted at the entrance(s) to the lab. (CBS 4.5.8)

*Personal clothing must be stored separately from PPE*. Lab coats are not to be left on chairs or other surfaces, the coat hooks should be used or clean lab coats could be stored until use. Ensuring that PPE do not come into contact with personal clothing prevents cross-contamination and the spread of contamination outside of the containment zone. (CBS 4.5.10)

*Personal belongings must be kept away from laboratory work areas*. Ensure personal belongings (including electronic devices) of lab personnel are not located where they may come into contact with biological agents (the student office or a designated drawer would be good alternatives). (CBS 4.5.11)
When leaving the lab, remove PPE in a manner that minimizes the possibility of contamination of the skin, hair or clothing. Do not wear PPE in public areas i.e. washrooms, cafeterias. Personnel must remove gloves and wash their hands before exiting the laboratory. (CBS 4.5.14, 4.5.15)

**Work Practices CBS 4.6**

**General**

Contact with the face or mucous membranes with items contaminated or potentially contaminated with pathogens or toxins is prohibited. Prohibiting activities such as eating, drinking, chewing gum, applying cosmetics, inserting ear buds, or inserting/removing contact lenses reduces the risk of exposure through the contact of mucous membranes of the eyes, nose, ears and mouth with contaminated or potentially contaminated items. Food containers and utensils are not allowed in any bio-permitted labs. (CBS 4.6.1)

Hair that may become contaminated when working in the containment zone must be restrained or covered. Long hair (may include beards) should be tied back, restrained or covered (to prevent contact with specimens, containers, or equipment) while working. (CBS 4.6.2)

Footwear worn in the lab must be selected to prevent injuries and incidents. Shoes are to be closed toe and heel, low heeled (or no heeled) and have non slip soles. (CBS 4.6.3)

Jewellery that may come in contact with biologicals, caught in equipment (rings, long necklaces) or that may puncture gloves must not be worn. (CBS 4.6.4)

Oral pipetting of any substance is prohibited, therefore mechanical devices must be used (CBS 4.6.5).

Open wounds, cuts, scratches and grazes must be covered with waterproof dressings before starting work. Labs must have waterproof dressings (waterproof adhesive bandages) available for personnel. Instructions on the requirement to cover cuts or scrapes on hands with waterproof dressings before work with biological agents should be included in relevant SOPs and as part of the PPE section of your permit-specific biosafety manual. (CBS 4.6.6)

Traffic flow patterns for the movement of personnel, equipment, samples, and animals from areas of least contamination (clean) to areas of greatest contamination (dirty) limits the spread of cross contamination. Labs should consider this when locating (placement of) equipment and work areas. Some areas of the laboratory could be segregated for specific uses. (CBS 4.6.7)

Dedicated paper/computer work areas that are kept apart from laboratory work areas are to be utilized for paperwork and report writing. Separating spaces dedicated to report writing and other paperwork from areas where infectious material or toxins are actively handled protects against contamination of these areas and any associated materials and supplies that cannot be easily decontaminated. (CBS 4.6.8)

Use of needles, syringes, and other sharp objects to be strictly limited and avoided when suitable alternatives are available. Needles, syringes, and other sharp objects can cause punctures or needle stick injuries, and potentially result in the injection or inoculation of personnel. Examples of suitable
alternatives include safety-engineered needles and plastic ware. Bending, shearing, re-capping, or removing needles from syringes is to be avoided, and, when necessary, performed only as specified in SOPs. Dispose of sharps in approved sharps waste containers directly after use without further manipulation. See the “Safe Work Practices – Safe Sharps Use” document available on the biosafety website: https://ehs.utoronto.ca/wp-content/uploads/2020/09/Safe-Work-Practices-Safe-Sharps-Use.pdf for further information. (CBS 4.6.9, 4.6.10)

Work surfaces must be cleaned and decontaminated with a disinfectant effective against the pathogen(s) in use, or a neutralizing chemical effective against the toxin(s) in use, at a frequency to minimize the potential of exposure to infectious material or toxins. Work stations (benchtops) should be kept clean and uncluttered to allow for cleaning/disinfecting. Work areas should be cleaned/disinfected when work is done/at end of the day. The regular decontamination of work surfaces with an appropriate disinfectant or neutralizing chemical minimizes the spread of contamination and protects personnel from inadvertent exposure to pathogens and toxins. (CBS 4.6.11)

If using bench coat/bench protector paper, regular change-outs should occur as part of housekeeping and if spills occur. The covering of work surfaces with bench coat must not interfere with the required decontamination procedures.

Verification of the integrity of primary containment devices must be performed routinely, as described in SOPs. The integrity of primary containment devices (examples include biological safety cabinets, glove boxes and animal micro isolators) is verified to confirm that they are functioning as intended and to prevent personnel exposure to pathogens or toxins resulting from a breach of containment. BSCs, when present, must be certified upon initial installation, annually, and after any repairs, modification, or relocation (even within the same lab). (CBS 4.6.14, 4.6.15)

**Handling Infectious Material and Toxins**

Good microbiological laboratory practices must be employed. Laboratory practices (i.e., including the use of PPE, handwashing, disinfecting work areas, use of procedures that minimize the creation of aerosols, and proper decontamination and disposal of materials) protect containment zone (lab) personnel from exposure by reducing the risk of cross-contamination and the spread of contamination. (CBS 4.6.18)

Samples of pathogens, toxins, or other regulated infectious material must be opened only in containment zones (labs) that meet the containment level requirements to which that infectious material or toxin has been assigned. Handling pathogens, toxins, and other regulated infectious material in containment zones that do not meet the minimum requirements can result in personnel exposure incidents or the accidental release of the pathogens and toxins. (CBS 4.6.19)

Containers of pathogens, toxins, or other regulated infectious material stored outside the containment zone must be labelled, leak-proof, impact resistant, and kept either in locked storage equipment or within an area with limited access (locked room). If you have a freezer or a liquid nitrogen tank, in which you store Risk Group 2 biologicals (even temporarily) outside of a CL2 lab (i.e. in a CL1
storage only room), then a RG2 storage sign (provided by the biosafety office) needs to be posted on your storage unit. Inform the biosafety office of the storage unit’s location, make, model and serial number by email at ehs.biosafety@utoronto.ca with the permit holder’s name and the phrase “storage sign” in the subject line. The biosafety office will then generate the sign and send it to the permit holder.

Certain higher risk materials (i.e. prions, Security Sensitive Biological Agents (SSBAs)) may require more stringent storage and security procedures. (CBS 4.6.20, 4.6.21, 4.6.22)

A certified BSC must be used for procedures involving open containers of infectious material or toxins that may produce aerosols or when aerosol generation cannot be contained by other methods. A certified BSC must also be used if working with high concentrations or large volumes of infectious material. (CBS 4.6.24)

When removing items from a BSC, procedures must be followed that prevents the spread of contamination. Remove gloves before exiting the BSC. This and other procedures should be followed to prevent the inadvertent spread of contamination from items removed from the BSC after handling infectious material or toxins. Removing gloves before exiting the BSC is an example of a procedure that protects personnel from exposure and prevents the spread of contamination by preventing the inadvertent contamination of surfaces, objects or other items that may be touched after exiting the BSC. In containment zones where two pairs of gloves are worn when handling infectious material or toxins, the outer pair is removed before exit from the BSC to avoid the spread of contamination. If working with higher risk RG2 agents or those that are potentially aerosolisable, then all contaminated material (both liquid and solid (ex pipet tips, tubes, flasks, plates etc.)) must be fully decontaminated within the BSC prior to removal using the appropriate disinfectant. (CBS 4.6.26)

Personnel must wash their hands after completing tasks that involve the handling of infectious material or toxins and before undertaking other tasks in the containment zone. Handwashing is one of the most effective ways to prevent the spread of contamination by many types of pathogens and toxins and protects personnel from potential exposure (e.g. touching eyes or mouth), by preventing the inadvertent contamination of surfaces, objects, or other items that may be touched; this includes activities such as paperwork or computer work that does not directly involve the manipulation of infectious material. In containment zones where two pairs of gloves are worn when handling infectious material or toxins, the outer pair is removed before exit from the BSC, and hands are washed immediately after the inner pair is removed. (CBS 4.6.27)

Centrifugation of infectious material where inhalation is the primary route of infection must be carried out in sealed safety cups (or rotors) that are loaded and unloaded in a BSC. Sealed safety cups and rotors are unloaded in a BSC to protect individuals from exposure to any aerosolized material and prevent the spread of contamination. Additionally, a maintenance plan would be required that ensures that you are evaluating the integrity of both the buckets and O-rings prior to use and that this is documented. See the “Safe Work Practices – Centrifuge Use” document available on the biosafety webpage: https://ehs.utoronto.ca/wp-content/uploads/2020/10/Safe-Work-Practices-Centrifuge-Use.pdf for further information. (CBS 4.6.28)
Use of on-demand open flames in a BSC must be strictly limited and avoided when suitable alternatives are available; sustained open flames are prohibited in a BSC. Open flames (e.g., Bunsen burner) and on-demand open flames (e.g., touch-plate micro burner) can damage the BSC HEPA filter and can create turbulence that disrupts airflow patterns. Examples of suitable non-flame alternatives include micro incinerators or sterile disposable inoculation loops. (CBS 4.6.30)

Procedures, as determined by an LRA, must 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 (CBS 4.6.31).

A). For movement within the containment zone (lab), ensure that all precautions are taken to avoid spills and the release of biologicals. The precautions taken to prevent mishaps should correspond to the inherent risk associated with the bioagent being moved. In other words, greater care should be taken with bioagents with higher inherent risk (CBH 2.1.1). Bioagents should be moved in closed containers (primary containment) which are leak-proof and impact-resistant. Screw top containers should be used rather than snap-cap tubes.

B). For movement outside of the containment zone (lab) but still within the same building, ensure that all biologicals are secured in labelled, closed and leak-proof secondary containers. The surfaces of all transportation containers must be decontaminated prior to use (CBS 4.8.8). Movement of biologicals out of the containment zone must only be done when transporting to a decontamination area, another appropriate containment zone or storage area (CBS 4.6.19, 4.8.8). Use a cart with raised edges and guard rails when moving heavy containers or a large number of samples (CBH 2.1.2). Avoid using passenger elevators, where possible use freight elevators instead. See CBH Chapter 20 for more information.


Experimentally infecting cells or other specimens derived from the person (self experimentation) conducting the experiment is prohibited. Since autologous cells (i.e., cells derived from an individual's own body) are not recognized as foreign by the immune system, the experimental infection of one's own cells may place the individual at a greater risk of infection in the event of an exposure. (CBS 4.6.34)

All level 2 permits must have a biological spill kit available to their personnel (University of Toronto biosafety policy). For information on what should be included in your lab’s spill kit: [https://ehs.utoronto.ca/our-services/biosafety/biological-spill-kit-2/](https://ehs.utoronto.ca/our-services/biosafety/biological-spill-kit-2/)

**Housekeeping and General Maintenance**
Containment zone (including floors) must be kept clean, free from obstructions, and free from materials that are in excess, not required, or that cannot be easily decontaminated. A clean, uncluttered work environment allows appropriate decontamination of the laboratory. It also minimizes slipping, tripping, fall, and collision hazards that could potentially lead to spills or injury. Storing excess materials outside the laboratory also protects this material from becoming contaminated. Containers holding chemicals or bioagents must be labelled so that people handling them are aware of any hazards and would know how to handle any spills. Keep containers off the floor or in secondary containment to minimize the chance of spills. Materials that are difficult to decontaminate (i.e. cardboard, Styrofoam) should be avoided in labs. If they can not be avoided, items that are difficult to decontaminate should not be kept on or near the floor. Either put these up on a shelf or if storage is a problem they could be placed in plastic storage bins. (CBS 4.6.35)

Housekeeping should also include items like ensuring liquid hand soap and paper towels are always available at your hand washing sink, and waterproof bandages and other First Aid supplies are checked and replaced when needed.

Lab equipment also requires regular cleaning, decontamination and other maintenance. Your lab’s housekeeping schedule/plan should also contain equipment maintenance i.e. cleaning and lubrication of centrifuge rotor/bucket seals, vacuum in-line filter change-outs etc. The lab’s maintenance program should also include any testing/validation of primary containment devices.

Weekly flushing of emergency eyewashes must be done in all labs and this flushing needs to be documented. See the UofT Safety Eyewash and Shower Standard for further information and testing advice: https://ehs.utoronto.ca/wp-content/uploads/2015/10/Emergency-Eyewash-and-Shower-Std.pdf

An effective rodent and insect control program must be maintained. If pests are observed in your building contact your building’s facility management. (CBS 4.6.37)

An acceptable mechanism must be utilized for the safe removal of high efficiency particulate air (HEPA) filters. Decontamination of HEPA filters through in situ fumigation with formaldehyde or vaporized hydrogen peroxide allows for the decontamination of HEPA filters prior to their removal. Other suitable alternatives include using HEPA filters with a bag-in/bag-out capability or using procedures to contain the HEPA filter for removal followed by its subsequent decontamination. (CBS 4.6.39) The company your lab uses to certify your BSC(s) annually will have methods to remove/replace the HEPA filter when necessary.

**Animal Work Considerations CBS 4.7**

*In vivo* work with pathogens and toxins increases the risk that personnel may be exposed. In addition, large volumes of contaminated waste (e.g. animal bedding) may be generated. Special considerations and handling techniques will reduce the risk of exposure or release of pathogens or toxins. Since the operational practices required are limited to the animal facilities, the specific CBS regulations pertaining to animal work are not discussed in this document. Specialized and specific training in handling, restraint and primary containment caging must be completed before personnel start this work.
Decontamination and Waste Management CBS 4.8, CBH Chapter 16

Gross contamination should be removed prior to decontamination of surfaces and equipment, and disposed of accordingly. Gross contamination is defined as organic material such as bedding, feed, excrement, blood and tissues on a surface that can be removed by physical means such as brushing, scraping and wiping. Removing and separately treating gross contamination, especially organic material, is essential to allow the effective decontamination of surfaces and equipment. Remove any large/concentrated items of organic matter then disinfect the contaminated surfaces. (CBS 4.8.1)

Disinfectants effective against the pathogen(s) in use and neutralizing chemicals effective against the toxin(s) in use must be available and used in the containment zone. Ensure that all SOPs and emergency response procedures (spill cleanup instructions) contain information on what disinfectants are to be used with every bioagent, include their final dilution, contact time and mixing instructions. (CBS 4.8.2)

Sharps must be discarded in containers that are leak-proof, puncture-resistant, and fitted with lids, or specially constructed for the disposal of sharps waste. Use only approved sharps waste containers. (CBS 4.8.3) For more information regarding sharp waste management, refer to the following document: www.ehs.utoronto.ca/laboratory-hazardous-waste-management-and-disposal-manual/5-5-sharp-waste-management

Primary containment devices must be decontaminated prior to maintenance. Decontamination of BSCs and other primary containment devices prior to maintenance protects individuals from exposure when working to maintain these devices. It also prevents the release of pathogens and toxins. (CBS 4.8.4)


Contaminated equipment, materials and waste must be either decontaminated and labelled as decontaminated before leaving the containment zone or placed in closed, labelled and leak-proof waste containers which have been surface decontaminated prior to removal from the containment zone for the safe transport to a designated decontamination area or storage outside the containment zone. For an overview of waste procedures in bio labs see the link provided above. (CBS 4.8.8)

Contaminated bedding must be removed at a ventilated cage changing station or within a certified BSC prior to decontamination; or decontaminated within containment cages. (CBS 4.8.13)

Permit Specific Biosafety (SOP) Manual 4.1.10, Emergency Response Plan (ERP) CBS 4.9; CBH Chapter 17 and Incident Investigation and Reporting CBS 4.9; CBH Chapter 18
Records and Documentation CBS 4.10; CBH Chapter 19

**Biological Inventory CBS 4.10.2**

Labs must have an inventory of the RG2 biologicals that are in their possession for 30 days or longer. The inventory must include what you have (i.e. name/description/identifier), state the biological’s risk group and location (i.e. room #, storage unit). It is recommended that information necessary to track material i.e. disposal of samples; transfers; inactivation, should be included so that samples are not deemed missing or lost. If any RG2 biologicals are considered missing or presumed stolen the permit holder must notify the biosafety office.

Inventory information may be accessed in response to an emergency situation where samples need to be relocated (e.g., power outage) or in response to requests from Public Health Agency of Canada (PHAC) or the Canadian Food Inspection Agency (CFIA) (e.g., targeted request for specific pathogens). Inventories can exist in many different forms. The specific format (e.g., electronic or paper inventory, a list, a logbook of samples) for establishing and maintaining inventories must be determined by the permit holder on a containment zone level. **Have a written SOP** on when and how your inventory needs to be updated.

Permit holders working with Security Sensitive Bio Agents (SSBAs) e.g. Cholera toxin, need to keep a logbook detailing the usage of these agents. (CBS 4.10.3)

If any of your biological agents requires a CFIA import permit, then more information for these agents is needed, including: Dates of importation; dates of any transfers; import permit/transfer approval permits; conditions placed on import/transfer; when and where material is used; dates of disposal/complete transfer/inactivation.

Any records for imported animal pathogens need to be kept for a minimum of 2 years after final disposal, transfer or inactivation of the imported material (CBS 4.10.10).

See Chapter 19 in the Canadian Biosafety Handbook (CBH) for detailed instructions:


**In-House Inspections CBS 4.10.5, 5.1.2**

Records of regular inspections of the containment zone and corrective actions taken must be kept on file. All labs should conduct regular in-house inspections at minimum annually (once per term or every 4 months is recommended). Records of all inspections and any needed follow up information or corrective actions must be kept by the permit holder for a minimum of 5 years. A general lab checklist
can be downloaded here, add items for checking that are consistent with your work and permit requirements:


**Equipment Maintenance Records CBS 4.10.6**

Records of building and equipment maintenance, repair, inspection, testing, or certification, including performance verification and testing records, in accordance with containment zone function, to be kept on file. Keep records of equipment maintenance, testing and certifications including BSC certification for at least 5 years. Ensure that the serial # on certification documents match the numbers on your equipment. These documents are evidence that internal inspections, repairs and testing have been performed.

Your BSC(s) certification documentation must be in your possession and available for viewing by both internal (i.e. EHS) and external (i.e. Ministry of Labour, PHAC, CFIA) inspectors.

**Resources**

Lakehead University, Human Resources, Biosafety- General Lab Practices Containment Level 1 & 2: https://www.lakeheadu.ca/faculty-and-staff/departments/services/hr/health-safety/tb/biosafety/biosafety-generallabprocedures-containment/node/29731

McMaster University, Biosafety, Training Materials: Operational Practices, Biohazard Laboratory Requirements: https://biosafety.mcmaster.ca/training_materials/6_operational_practices_inventory.pdf


The University of Western Ontario, Occupational Health and Safety, Containment Level 2 – Laboratory Biosafety Practices: https://www.uwo.ca/hr/form_doc/health_safety/doc/procedures/containment_level_2.pdf

University of Regina, Human Resources, Health, Safety & Environment, Public Health Agency of Canada
Containment Level 2 Laboratory Guide: https://www.uregina.ca/hr/hsw/assets/docs/pdf/Laboratory-Safety/Containment-Level-2-Orientation-Guide.pdf

University of Toronto, Environmental Health & Safety, Biosafety: https://ehs.utoronto.ca/our-services/biosafety/


University of Toronto, Environmental Health & Safety, Resources: https://ehs.utoronto.ca/resources/


For all additional safety and contact information, please visit our website www.ehs.utoronto.ca