



# Nipissing University Biosafety Policies and Procedures Manual

Revised: July 2024

**Emergency Contact Information:**

**In case of unauthorized individuals contact:**

Security: ext. 5555 (after hours cell phone is 705-498-7244)

**In case of spill contact:**

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## **Nipissing University Health & Safety Policy Statement**

Nipissing University recognizes the legal, social and moral responsibility to safeguard the health and safety of University staff, students and visitors by maintaining a safe, healthy environment.

In recognition of the importance of the preceding statement, the university-wide health and safety program is maintained on a continuing basis and is reviewed annually to ensure that it meets the needs of the University.

This program's priorities are to:

i) Minimize the risk of health hazards and personal injury hazards through maintaining a functional Joint Health & Safety Committee and designating the BSO as a Co-Chair. The Committee will have representatives from each employee constituent group as per the current government legislation and the Committee Terms of Reference. These committee members will then elect the other Co-Chair from its membership.

ii) Encourage in all University staff and students, positive attitudes and behavior regarding health and safety issues.

iii) Establish and practice safe procedures throughout the University.

All Employees must be dedicated to the continuing objective of reducing risk of injury at Nipissing University. Ultimately, everyone at Nipissing has a shared responsibility for their own health and safety and the safety of their fellow employees by working in compliance with the law, and with the safe work practices and procedures established by the University.

Supervisors must also take responsibility for the health and safety of employees and students under their supervision. Supervisors are responsible for ensuring that equipment used by employees and students are in safe working order, that employees and students receive adequate training in their specific work tasks, and that employees and students work in compliance with established health and safety procedures.

Commitment to health and safety is in the best interest of all and must form an integral part of the culture of Nipissing University, from the president to every staff member and student.

Original signed by: President and Vice-Chancellor  
Nipissing University

## **Introduction**

This manual was created to fulfill the requirements for working with Level 1 and 2 Pathogens as described in the Canadian Biosafety Standard 3<sup>rd</sup> Edition (2022), the Canadian Biosafety Handbook 2<sup>nd</sup> ed. (2015) and the Canadian Biosafety Guideline – Developing a Comprehensive Biosecurity Plan (2016) produced by the Public Health Agency of Canada.

Nipissing University does not currently have the infrastructure to hold or use risk group 3 or 4 pathogens. Therefore, the use of risk group 3 and 4 pathogens for any purpose is strictly prohibited.

## **Biosafety Program Components**

The Biosafety program has the following components:

1. Nipissing University Biosafety Policies and Procedures Manual;
2. Nipissing University Laboratory Safety Manual;
3. A Guideline for the Safe Use of Autoclaves;
4. Containment Level 1, 2 and 2+ inspections and permits;
5. Purchasing of biohazardous materials;
6. Training;
7. Medical surveillance;
8. Biosecurity;
9. Biohazardous waste management;
10. Biohazardous spills and incident management.

## Definitions

**Aerosol** – A suspension of fine solid particles or liquid droplets in a gaseous medium (e.g., air) that can be created by any activity that imparts energy into a liquid/semi-liquid material (e.g., liquid culture or nutrient agar plate).

**Animal Pathogen** – Any pathogen that causes disease in animals; including those derived from biotechnology. In the context of the Canadian Biosafety Standard, “animal pathogen” refers only to pathogens that cause disease in terrestrial animals, including those that infect avian and amphibian animals, but excluding those that cause disease in aquatic animals and invertebrates.

**Animal Pathogen Import Permit** – A permit issued by the Public Health Agency of Canada or the Canadian Food Inspection Agency for the importation into Canada of: animal pathogens or toxins, animal by-products, or other organisms carrying an animal pathogen or part of one; under Section 51(a) and (b) of the Health of Animals Regulations.

**Antimicrobial** – An agent that kills microorganisms or suppresses their growth and multiplication.

**Antiseptic** – A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.

**Authorized Worker** – A person named on a valid biosafety certificate that has completed the necessary training (biosafety training and lab-specific training) to safely handle biohazardous agents/materials as described within the certificate.

**Biocide** – A general term for any agent that kills organisms.

**Biological safety cabinet (BSC)** – a specially designed work enclosure that contains biohazardous material, allowing that material to be manipulated without allowing it to escape to the surrounding laboratory space.

**Biological Safety Officer (BSO)** – the person formally responsible for managing biosafety issues, and who has a working knowledge of the laboratory practices and procedures within the facility as per *Human Pathogens and Toxins Act, S36(3)*. The Biological Safety Officer may exercise the powers and shall carry out the functions set out in the regulations. *Human Pathogens and Toxins Act, S36 (1), S36 (5) and Human Pathogens and Toxins Regulations, 9(1)*. At Nipissing University, the BSO is the Laboratory Safety Coordinator.

**Biosafety** – All aspects of containment used to prevent any unintended exposure to and accidental release of pathogens.

**Biosafety Officer (BSO)** – See Biological Safety Officer (above).

**Biosafety Program** – The management of all aspects of biological safety, which includes risk assessments/certifications, medical surveillance, and containment strategies (i.e., operational practices, lab design and physical requirements, and biosecurity).

**Biosecurity** – Measures taken to prevent the theft, misuse, or intentional release of biohazardous agents/materials. *Canadian Biosafety Handbook, Chapter 6, Biosecurity*

**Chemical germicide** – A chemical or a mixture of chemicals used to kill microorganisms.

**Competent person** – A person who (a) is qualified because of knowledge, training and experience to organize the work and its performance, (b) is familiar with this Act and the regulations that apply to the work, and (c) has knowledge of any potential or actual danger to health or safety in the workplace. *Occupational Health and Safety Act S.1(1)*.

**Containment** – The combination of physical design parameters and operational practices that protect personnel, the immediate work environment, and the community from exposure to biological material.

**Containment Level** – Minimum physical containment and operational practice requirements for handling infectious material or toxins safely. There are four containment levels ranging from a basic laboratory (containment level 1 [CL1]) to the highest level of containment (containment level 4 [CL4]).

**Containment Zone** – A physical area that meets the requirements for a specified containment level.

**Controlled Activities** – Any activities referred to in Section 7(1) of the Human Pathogens and Toxins Act.

**Culture** – The *in vitro* propagation of microorganisms, tissue cells, or other living matter under controlled conditions (e.g. temperature, humidity, nutrients) to generate greater numbers or a higher concentration of the microorganisms.

**Decontamination** – Any process for removing and/or killing microorganisms. The same term is also used for removing or neutralizing hazardous chemicals and radioactive materials.

**Disease** – A disorder of structure or function in a living human or animal or one of its parts, resulting from infection or intoxication.

**Disinfectant** – A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.

**Disinfection** – A physical or chemical means of killing microorganisms, but not necessarily spores.

**Dual-use Potential** – Qualities of a pathogen or toxin that allow it to be either used for legitimate scientific applications (e.g. commercial, medical, or research purposes), or intentionally misused as a biological weapon to cause disease (e.g. bioterrorism).

**HEPA Filter** – A device capable of filtering 99.97% of airborne particles 0.3 µm in diameter, the most penetrating particle size. Due to the effects of impaction, diffusion and interception, HEPA filters are even more efficient at trapping and retaining particles that are either smaller or larger than 0.3 µm in diameter.

**HPTA** – Human Pathogens and Toxins Act.



**HPTR** – Human Pathogens and Toxins Regulations.

**Infectious Material** – Any isolate of a pathogen or any biological material that contains human or animal pathogens and, therefore, poses a risk to human or animal health.

**Inventory** – A list of biological assets associated with a containment zone, identifying pathogens, toxins and other infectious material in storage, both inside and outside the containment zone.

***In vitro*** – Latin for ‘within glass’; describes experimentation involving components of a living organism within an artificial environment (e.g., manipulation of cells in a petri dish), including activities involving cell-lines or eggs.

***In vivo*** – Latin for ‘within body’; describes experimentation conducted within the whole living organism (e.g. studying the effect of antibiotic treatment in animal models).

**Licence** – An authorization to conduct one or more controlled activities with human pathogens or toxins issued by the Public Health Agency of Canada under Section 18 of the Human Pathogens and Toxins Act.

**Local Risk Assessment** – Site-specific risk assessment used to identify hazards based on the infectious material or toxins in use and the activities being performed. This analysis provides risk mitigation and risk management strategies to be incorporated into the physical containment design and operating practices of the facility.

**Material Transfer Agreement (MTA)** – A contract that governs the transfer of tangible research materials (often biological agents/materials) between two organizations, when the recipient intends to use it for his or her own research purposes.

**Medical Surveillance** – The process of evaluating and supporting an individual’s health status as it relates to their potential occupational exposure to biohazardous agents/materials.

**Microbicide** – A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of “biocide”, “chemical germicide” or “antimicrobial”.

**Microorganism** – A cellular or non-cellular microbiological entity, capable of replication or transferring genetic material and that cannot be reasonably detected by the naked eye. Microorganisms include bacteria, fungi, viruses, and parasites, and may be pathogenic or non-pathogenic in nature.

**Overarching Risk Assessment** – A broad risk assessment that supports the biosafety program as a whole and may encompass multiple containment zones within an institution or organization. Mitigation and management strategies reflect the type of biosafety program needed to protect personnel from exposure and to prevent the release of pathogens and toxins.

**Pathogen** – A microorganism, nucleic acid, or protein capable of causing disease or infection in humans or animals.

**Pathogenicity** – The ability of a pathogen to cause disease in a human or animal host.

**Personal Protective Equipment (PPE)** – Equipment and/or clothing worn by personnel to provide a barrier against infectious material or toxins, thereby minimizing the risk of exposure.

**Primary Containment** – The first level of physical barriers designed to contain pathogens and toxins and prevent their release. Examples include biological safety cabinets, glove boxes, centrifuge rotors with sealable cups, etc.

**Prion** – Small proteinaceous infectious particle generally considered to be responsible for causing a group of neurodegenerative diseases in humans and animals known as transmissible spongiform encephalopathies.

**Principal Investigator (PI)** – is defined as a person who has any of the following: assigned space for research activity; charge over the research activity; and/or receives grants for research activity.

**Puff-back** – The reversal of airflow from the face of a Class II Type B2 biological safety cabinet due to failure of the exhaust fan.

**Release** – The discharge of infectious material or toxins from a containment system.

**Representative Load** – A simulation batch of materials of a similar type (e.g., gloves, plastics, liquids) and quantity used to validate a decontamination method for routine loads.

**Risk** – The probability of an undesirable event occurring and the consequence of that event.

**Risk Group** – The classification of biological material based on its inherent characteristics, including pathogenicity, risk of spread, and availability of effective prophylactic or therapeutic treatments, that describe the risk to the health of individuals and the public as well as the health of animals and the animal population.

**Sporicide** – A chemical or mixture of chemicals used to kill microorganisms and spores.

**Standard Operating Procedure (SOP)** – A document that standardizes safe work practices and procedures for activities with infectious material and toxins in a containment zone, as determined by a local risk assessment.

**Sterilization** – A process that kills and/or removes all classes of microorganisms and spores.

**TOR** – Terms of Reference

**(Microbial) Toxin** – A poisonous substance that is produced or derived from a microorganism and can lead to adverse health effects in humans or animals.

**Validation** – The act of confirming that a method achieves its objective by observing that specific parameters have been met (e.g. using biological indicators to confirm that a given autoclave cycle can decontaminate a representative load of waste). Validation infers that a method is suitable for its intended purpose.

**Virulence** – The degree or severity of a disease caused by a pathogen.

**Waste** – Any solid or liquid material generated by a facility for disposal.

**WHMIS 2015** – Workplace Hazardous Material Information System based on the United Nations Global Harmonized System.

**Zoonoses** – Diseases that are transmissible between living animals and humans.

**Zoonotic Pathogen** – A pathogen that causes disease in humans and animals, and that can be transmitted from animals to humans and vice versa. They are considered both human and animal pathogens.

## Licensing and Biosafety Permits

All teaching and research facilities or programs that intend to engage in the use of risk group 2 or greater, biohazardous substances must be registered with and licenced by the Public Health Agency of Canada (PHAC) prior to becoming operational.

Regardless of the risk group of the agent, a local risk assessment and possibly a permit, is required for all research or teaching activities involving the use or manipulation of potentially hazardous biological agents. This includes viruses, bacteria, fungi, parasites, recombinant DNA, prions and other microorganisms/genetic systems, human and animal tissues, cells, blood and body fluids and any materials containing such agents. Assessments are necessary when research or teaching activities are:

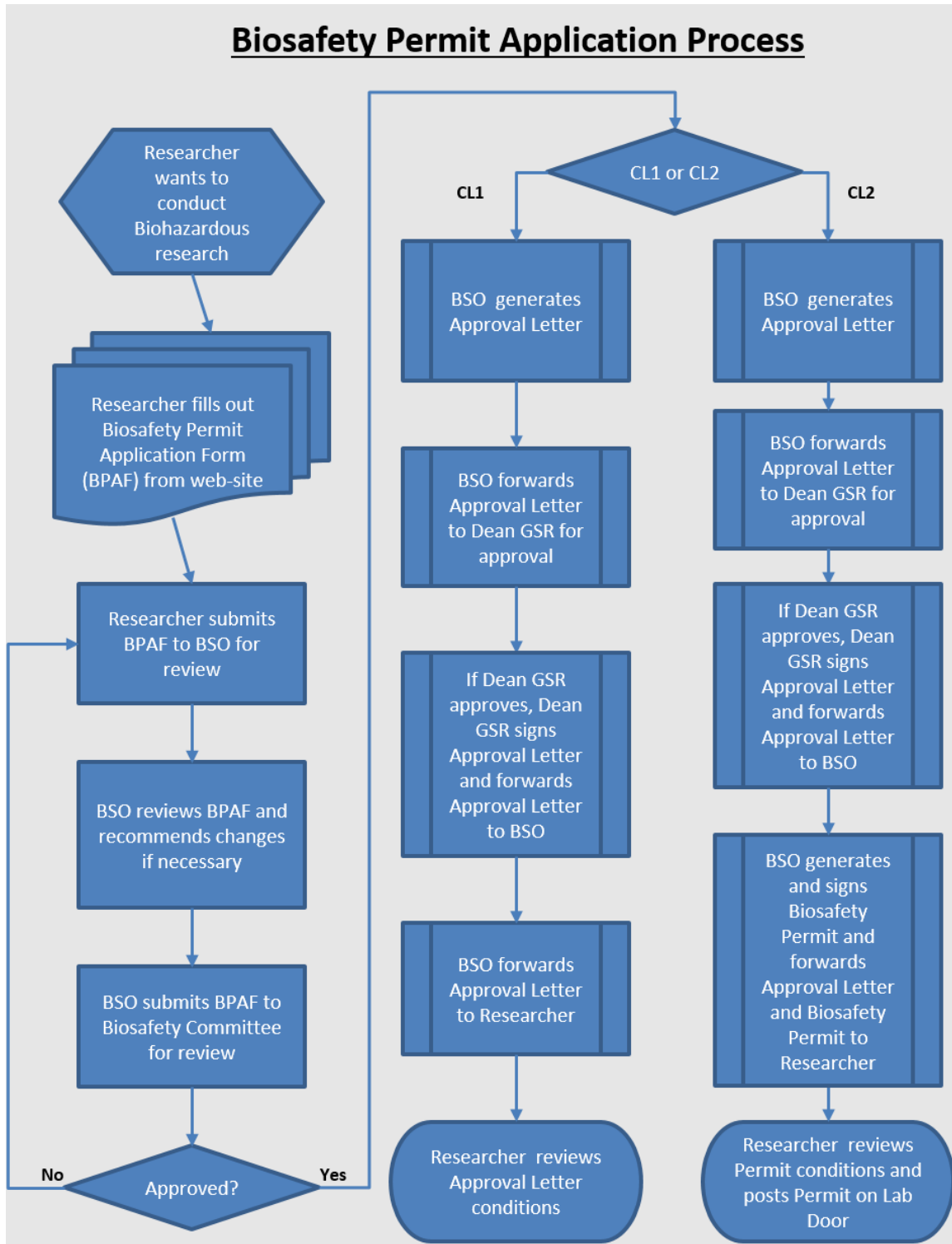
- supervised or conducted by employees or members of the University, or
- supported by funds provided by or through the University.

## Biosafety Permit Approval Process

A 'Biohazardous Materials Use Risk Assessment and Permit Application' form must be completed and submitted to the BSO. The submission of a *Biohazardous Materials Use Risk Assessment and Permit Application* form implies a willingness to allow the Biosafety Officer (BSO) to visit the laboratory where hazardous biological material will be used in order to ensure compliance with the Nipissing University Biosafety Policies and Procedures Manual and associated statutes and regulations. The biohazard permit application process is outlined in Figure 1.

Biosafety permits and letters are valid for up to 5-years, subject to an annual review and regular laboratory inspections and audits. Permits are also subject to an annual renewal process which consists of the submission of a *Biohazardous Materials Use Permit Renewal and Amendment* form to the BSO. Biosafety permits may be renewed a maximum of four times (5 years total). If a research program will continue beyond the initial 5-year term, a new *Biohazardous Materials Use Risk Assessment and Permit Application* form must be submitted prior to the date of expiration of the protocol to the BSO along with all other applicable documentation (SOPs etc.).

Minor changes or amendments to a research program (e.g., personnel changes) are reviewed by the BSO. Major changes or amendments (e.g., change of biohazardous agent, etc.) must be submitted through the BSO and vetted and approved by the Biosafety Committee and the Dean, Graduate Studies and Research. Changes must be approved prior to the continuance of the project. Once approved, a new permit or letter is issued.



**FIGURE 1.** Biosafety approval process for the approval of activities involving biohazardous material at Nipissing University. Abbreviations: CL1 (Containment Level 1); CL2 (Containment Level 2); BSO (Biological Safety Officer); Dean GSR (Dean Graduate Studies and Research).

## Legislation and Regulation

### Occupational Health and Safety Legislation

Activities involving the use of biological agents and laboratory animals, the production and disposal of waste, and the use of certain equipment are governed by various legislation, guidelines, and standards. Adherence to the requirements of this manual will ensure that work is performed safely and in compliance with the requirements of external agencies and regulatory bodies.

The Ontario Occupational Health and Safety Act and Regulations outline the rights and responsibilities of all workplace parties. All employees, contractors, volunteers, students, and visitors at Nipissing University are required to follow these general acts and regulations. For more information, please see the Ontario Ministry of Labour website at: <http://www.labour.gov.on.ca/english/hs/>

Bill C-45 is an Act to amend the Criminal Code of Canada that came into effect on March 31, 2004. This Act imposes a new legal duty on anyone who undertakes, or has the authority, to direct how work is done to ensure workplace health and safety. These changes apply to all Canadian workplaces including the administrative, teaching and research areas of Nipissing University.

### Biosecurity

Biosecurity is a set of protocols and resources that are put into place to prevent harmful biological materials from leaving the containment zone without proper authorization. Biosecurity is also meant to prevent dangerous biological agents or biotechnological applications that have the potential to be used in military applications (dual-use research) from falling into the hands of unauthorized persons or groups. As such, biosecurity requires the cooperation of senior administrators, scientists, safety personnel, technologists and students to ensure the safety and security of biological agents at Nipissing University.

### Legislation

Laboratories and facilities that handle or store human and terrestrial pathogens or toxins or other regulated infectious material are regulated by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) under the *Human Pathogens and Toxins Act* (HPTA), the *Human Pathogens and Toxins Regulations* (HPTR), the *Health of Animals Act* (HAA) and the *Health of Animals Regulations* (HAR). As such, regulated laboratories and facilities are required to develop and maintain a biosecurity plan in accordance with the requirements established in the *Canadian Biosafety Standard* (CBS) 3<sup>rd</sup> Edition (2022) and the guidance provided by the *Canadian Biosafety Handbook* (CBH), 2<sup>nd</sup> Edition (2016) and the *Developing a Comprehensive Biosecurity Plan* guideline.

## **Elements of Biosecurity Plan**

### **Roles and responsibilities**

#### ***Senior Administration***

Under the Human Pathogens and Toxins Act (HPTA) and Regulations, Nipissing University's senior management are the ultimate authority for the safety and security of a Containment Level 2 laboratory. The HPTA regulations require that senior management delegate appropriate authority to a qualified individual – the BSO – to oversee facility biosecurity for the institution. The individual identified as the license holder is ultimately accountable for activities carried out with the pathogens and toxins in a licensed facility.

#### ***Biosafety Committee***

The Biosafety Committee is mandated by the office of the Provost and Vice President, Academic and administered by the office of Graduate Studies and Research, to provide policy direction and make recommendations to the Provost and Vice President, Academic and Research for all matters pertaining to the use of biohazardous materials in research and teaching. The BSO is a member of the Biosafety Committee.

The Biosafety Committee is responsible for:

- a) Developing and maintaining University policies for handling biohazardous materials in compliance with internal and external standards including: Public Health Agency of Canada (PHAC), Canadian Food Inspection Agency (CFIA), National Institute of Health (NIH), Occupational Health and Safety Act and Regulations (OHSA) and Nipissing University's policies;
- b) Ensuring that all users, are fully aware of the guidelines and the nature of containment required for research/teaching;
- c) Reviewing and approving applications for use of biohazardous materials;
- d) Advising the Provost and Vice President, Academic on matters relating to biohazards.

#### ***Biosafety Officer***

The Biosafety Officer's (BSO) responsibilities include developing, implementing, and improving the biosecurity plan and acting as a point of contact for any biosecurity-related incidents, maintaining a list of individuals with access to pathogens, toxins, and other regulated infectious material, maintaining training records, and ensuring measures are in place to adequately protect sensitive information. The BSO is responsible for communicating biosafety information, legislative changes and biosafety issues to the license holder, deans and departmental chairs, laboratory supervisors and laboratory personnel as required to ensure continued compliance with the HPTA and regulations.

The Canadian Biosafety Standards (CBS) mandates that the BSO is responsible for communicating with the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA).

### ***Deans and Departmental Chairs***

Deans and Departmental Chairs are responsible for the broad oversight of the containment zone and are responsible for ensuring that personnel comply with the biosafety related legislation.

### ***Laboratory Supervisors***

Laboratory supervisors, including Principal Investigators and Laboratory Instructors, are responsible for the direct oversight of all personnel working in the containment zone and ensuring laboratory personnel comply with biosafety legislation.

### ***Laboratory Personnel***

Laboratory personnel include all employees, contractors, volunteers, students, and visitors who work in the containment zone. Laboratory personnel are responsible for working in a safe manner and must work in compliance with all associated legislation, regulations, and guidelines as outlined in this manual and during any training received and must report any contraventions of the above to their supervisor. In addition, Laboratory personnel must report any health and safety or biosecurity concerns to their supervisor.

### ***Security Personnel***

Security personnel play a vital role in biosecurity. Security personnel are responsible for monitoring the security of laboratory areas during evening and weekend hours. They will deal with security breaches and issues (e.g., dealing with unauthorized persons in the laboratory area). They will also assist with first responders in the event of an emergency.

### ***Maintenance Staff***

Maintenance and custodial staff are required to have basic training regarding chemical and biological safety prior to entering any containment zone and are only responsible for removing non-hazardous trash from the containment zone. If any health and safety concerns are noticed, these concerns must be relayed to their supervisor in a timely manner.

### **Personnel Suitability and Reliability**

Personnel having access to the containment zone must have the appropriate skills, training, competency, experience, and mindset in order to work within the containment zone. The University is able to deny or remove an individual who exhibits qualities or behaviors that suggest the individual is incapable of safely working with, or protecting the security of the pathogens, toxins, or other assets handled or stored within the containment zone or facility.



For the most part, Human Resources is tasked with ensuring that personnel have the necessary qualifications to work in the containment zone during the hiring process. The BSO will monitor biosecurity compliance on an as needed basis to ensure continuing personnel suitability. If an individual no longer meets the minimum suitability and reliability requirements, the BSO will report these findings to the Assistant Vice President, Human Resources, Equity, Diversity and Inclusion to decide on an appropriate action plan to resolve the issue.

### ***Visiting Scholars and Non-Student Volunteers***

The BSO, in cooperation with the laboratory supervisor, will assess the level and types of training required to perform tasks within the containment zone. The supervisor and/or the BSO will jointly assess the safety compliance of the researcher. If an individual is unable to meet or maintain the minimum suitability and reliability requirements, the BSO will discuss the situation with the laboratory supervisor to decide on an appropriate action plan to resolve the issue.

### ***Student Workers, Researchers, and Volunteers***

In the case of student workers, student researchers, or student volunteers, the BSO, in cooperation with student's supervisor, will assess the level and types of training required to perform tasks within the containment zone. The supervisor and/or the BSO will jointly assess the safety compliance of the students. If an individual is unable to meet or maintain the minimum suitability and reliability requirements, the BSO will discuss the situation with the individual's supervisor to decide on an appropriate action plan to resolve the issue.

### ***Service Animals***

If a person wishes to enter a laboratory accompanied by their service animal, the laboratory supervisor must contact the BSO, who will review the laboratory environment and determine if there is a risk to the animal or the research being conducted in the laboratory.

### ***Unauthorized Visitors or Intruders***

Visitors who are unknown, unexpected, or unwelcome are considered unauthorized and will be asked to leave the facility. Security should be immediately notified to identify and remove unauthorized persons, and to assist lost visitors.

No individual under 18 years of age is permitted to visit areas that may contain inherently or potentially hazardous conditions including chemicals, radioactive materials, biohazards, or hazardous equipment unless the individual is enrolled as a student at Nipissing University, or part of an approved tour or group, or is authorized by the BSO and the Supervisor responsible for the facility.

## **Physical Security**

Access to the containment zone must be controlled to prevent unauthorized access to Risk Group 2 pathogens and other regulated infectious materials. To that end, the main barrier to containment zone access is through locked doors with key access (see diagram). Key access should only be available to personnel who are authorized to access the containment zone. Keys should not be shared to unauthorized personnel.

Because the containment zone is a multi-use facility, all pathogenic material must be inaccessible when the containment zone is being used for other than CL2 activities. Therefore, all pathogenic material must be secured in lockable refrigerators, incubators or waste containers when not in use or the containment zone is being used for non-CL2 activities such as lectures, tutorials, or CL1 laboratory activities.

## **Pathogen and Toxin Accountability and Inventory Control**

Pathogen and toxin accountability is required in order to ensure the biohazardous material is not lost, stolen or otherwise moved out of the containment zone without proper procedures and authorization. Pathogen and toxin accountability includes the assignment of qualified authorized personnel to oversee the control of the pathogens, toxins, and regulated infectious material, the maintenance of accurate and timely records and the routine verification of materials and records. It also means that these accountable authorized personnel are answerable for their actions and decisions involving regulated infectious material to their supervisors, the license holder, or animal pathogen import permit holder and possibly PHAC and the CFIA.

### ***Inventories and Inventory Control***

Any pathogen, toxin or other regulated infectious material that will be in storage within or outside the containment zone must be inventoried with the name, risk-group, and location of the material. The inventory must be maintained by a single qualified individual, and it must be immediately updated as materials are added or removed. The inventory must also capture all regulated material including that which is stored outside of the containment zone (e.g., refrigerators, or freezers). In addition, the inventory must also reference relevant documentation such as import permits, pathogen transfer authorization, etc.).

A biennial inventory audit by the laboratory supervisor (Principal Investigator or Laboratory Instructor) is required as well as ad hoc audits by the BSO. The inventory audits are to be documented and signed off by the BSO.

### ***Dual-Use Material and Processes***

Dual-use potential is defined as a material or process that has qualities that allow it to be either used for legitimate scientific applications or intentionally misused as a biological weapon to cause disease. It is important for both the researcher/laboratory supervisor, biosafety committee, and senior administration to recognize that the possibility exists for legitimate scientific applications to be intentionally misused for

non-legitimate applications. To that end, all biohazardous materials and the processes that utilize those materials must be examined for their dual-use potential (Figure 2).

Consideration of dual-use potential includes a risk assessment that looks at the following:

- Types of pathogens, knowledge, technology, and products anticipated to be generated through research;
- Pathways by which pathogens, knowledge, technology and products resulting from research could be misused to harm public health or national security;
- Ease of obtaining materials, tools and equipment to replicate experiments if technology or products resulting from research could be misused to harm public health or national security;
- Possibility of harm to humans or animals if a pathogen is released outside of the laboratory;
- Likelihood that the knowledge, information, technology and products of research will be used to harm public health and safety, the environment, or national security.

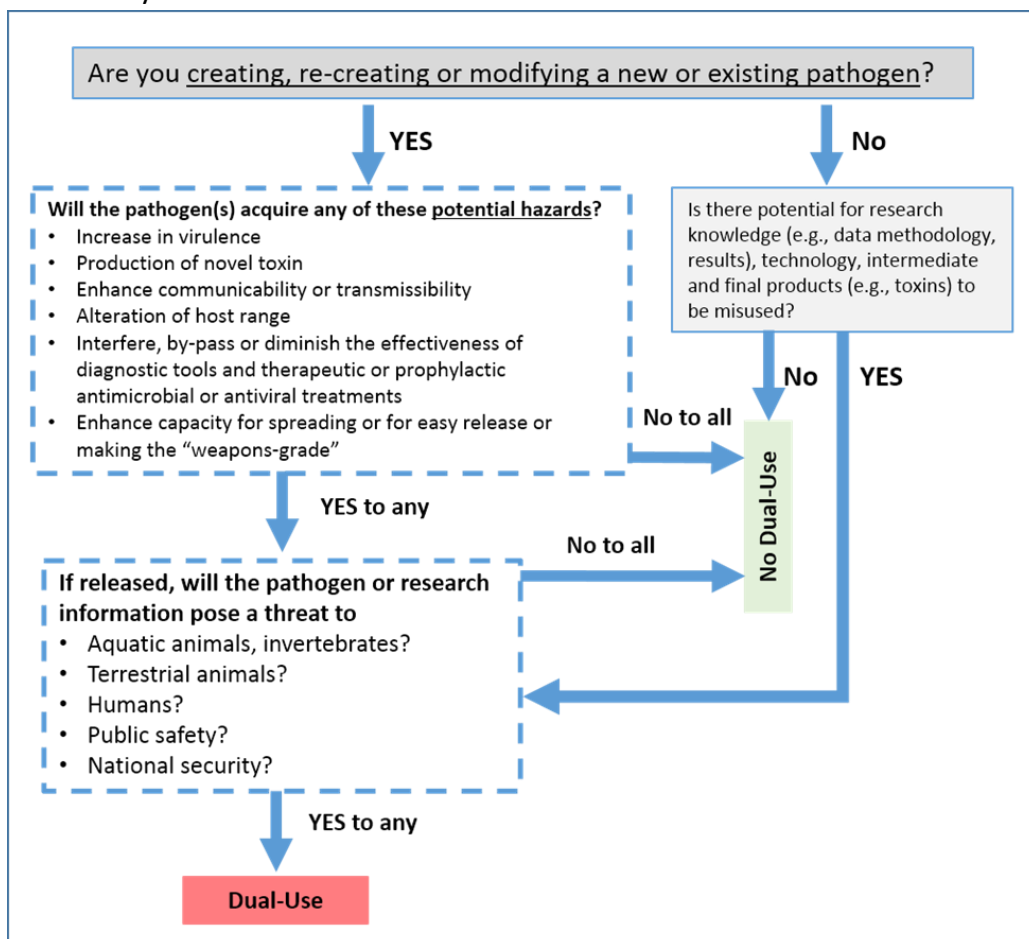


FIGURE 2. Decision tree used to decide if research material and processes can be considered as dual-use (adapted from PHAC).

### Dual-use Risk Mitigation

When it comes to assessing the dual-use potential of proposed research activities, the first step in that assessment must come from the researcher proposing the research activities. To that end, when a researcher submits a permit application, the section pertaining to possible dual-use potential must be filled out completely before it is submitted to the BSO for an initial review and evaluation for both biosafety and biosecurity issues, including dual use as outlined in Figure 2 (above). Once the BSO has reviewed and evaluated the permit application it is forwarded to the biosafety committee for further review and evaluation. Once the biosafety committee is satisfied with the permit application, the committee makes a recommendation to the Dean of Graduate Studies and Research, with or without conditions, to grant a permit for the work.

### ***Security during Movement and Transportation***

#### Receipt of regulated material from external sources.

Shipping and Receiving is the central area where packages containing regulated material are brought into the facility unopened from an external source (supply house, research institute, etc.). Upon receipt of a package containing regulated material, the receiving personnel must inspect the package for breakage and/or leaks. If found to be leaking or damaged, the hazardous spill response protocols must be immediately implemented and the BSO notified.

If the packaging is in good condition and the paperwork is in order, the BSO and the consignee should be notified as soon as possible that the package has arrived. At no time should the package be left unattended unless the area is secured and locked.

The consignee must, after receiving the package from the Shipping and Receiving Department, immediately transfer the package to the containment zone, inspect the contents and paperwork and log the contents into the inventory. Copies of the shipping paperwork (including the material transfer agreement and any other relevant documentation pertaining to the regulated material) must be forwarded to the BSO in a timely manner.

### Internal Transfers

Internal transfers of regulated material are handled differently depending on whether they are regulated under the Pathogen and Toxin Licence or under the CFIA import permit system. Regardless of which regulation system governs the material, a Biohazardous Agent Transfer Notification document must be filled out and forwarded to the BSO to be signed prior to the transfer taking place. A copy of the signed transfer document must be placed on file with the inventory and the inventory updated to reflect the movement of the regulated material to another laboratory or facility within the University.

### *Internal Transfers under CFIA Import Permit*

If regulated material that has been imported under an animal pathogen import permit issued by the CFIA is to be transferred from one laboratory to another laboratory within the University, **authorization is required from CFIA before the transfer can take place** as CFIA import permits are specific to the laboratory or room to which the regulated material has been initially imported. This will involve obtaining an amendment to the original import permit from CFIA. The BSO must be contacted in order to initiate the amendment.

### *Internal Transfers under the Pathogen and Toxin Licence*

If the transferring of regulated material has been authorized under the Pathogen and Toxin licence issued to the University by PHAC and does not fall under a CFIA import permit, there is no need to notify PHAC regarding the transfer. However, the BSO must be notified before the transfer using a Biohazardous Agent Transfer Notification document.

### *External Transfers and Exports*

Transfers of human pathogens or toxins within Canada must be done such that reasonable care has been taken to ensure that the intended recipient is licensed to work with the agent or is otherwise exempted from the requirement to hold a licence. The same is true with respect to senders who are exporting human pathogens or toxins, in that the sender must ensure that the intended recipient will follow the applicable biosafety and biosecurity standards and policies in the foreign jurisdiction. The BSO must be consulted prior to arranging for the transfer of material from this institution to another institution.

The same duty of care applies to animal and zoonotic pathogens imported under a Pathogen and Toxin licence issued by PHAC. However, if an animal pathogen was imported under an animal pathogen import permit issued by CFIA, prior authorization from CFIA is required before the transfer or export can occur.

Under HPTR, the BSO of both institutions must be notified before arrangements can be made to transfer a human pathogen or toxin. This allows for attempts to be made to locate the package if it is not received within a reasonable amount of time. In order to facilitate this requirement for transfers originating at Nipissing University, a Biohazardous Agent Transfer Notification document must be filled out and forwarded to the BSO for approval before making arrangements to transfer or export material regulated under HPTR. A copy of the completed Biohazardous Agent Transfer Notification document must accompany the shipment.

### **Cybersecurity**

Cybersecurity is paramount in safeguarding sensitive biological data and ensuring the integrity of digital infrastructure within biological research and healthcare facilities. Cybersecurity measures protect against unauthorized access, data breaches, and cyberattacks that could compromise confidential information, including pathogens,

genetic data, research findings, and patient records. Implementing robust cybersecurity protocols, such as encryption, regular software updates, secure authentication methods, and continuous monitoring for potential threats, is essential. Training staff on best practices for digital security and fostering a culture of vigilance can mitigate risks, ensuring that biological and digital assets remain secure against evolving cyber threats.

Any known or suspected cybersecurity breaches of biosafety data of any form must be reported to the BSO and Security Services must be immediately engaged.

### **Incident and Emergency Management**

Incidents can involve a major or minor spill, loss of containment, pathogen exposure, stolen or missing pathogens, and/or lost or stolen keys. Depending on the type and severity of an incident, the response to that incident will vary. In all cases, incidents need to be properly reported, documented, and investigated in order to learn from the events and to correct or address any problems or issues that may have caused the incident and to prevent any reoccurrence, and notify external authorities when necessary.

If there is a possibility that the incident was a result of a criminal act, Security Services must be immediately engaged, and local law enforcement assistance obtained.

### ***Incident Reporting***

Under the provisions of the Canadian Biosafety Standard, 3<sup>rd</sup> edition (2022), all incidents involving infectious material, including those involving inadvertent release, inadvertent production, an exposure incident, or a missing human pathogen or toxin, must immediately be reported to the containment zone supervisor, BSO and licence holder. Upon notification the containment zone manager and BSO must immediately implement hazard reduction and mitigation strategies and initiate a preliminary assessment to determine if exposure has likely occurred.

All incident reports are retained for two (2) years in hard paper format and retained indefinitely electronically.

### **Incident Reporting to the Public Health Agency of Canada**

Nipissing University is obligated to notify PHAC without delay in the event of incidents and exposures under the terms of the HPTA and HPTR. **The following scenarios require reporting without delay to your supervisor and the BSO:**

- When it is believed that a human pathogen or toxin has been released inadvertently from a facility;
- When a human pathogen or toxin that a person is not authorized to possess is inadvertently produced or otherwise comes into their possession;
- When there is reason to believe that a human pathogen or toxin has been stolen or is otherwise missing;

- When an incident involving a human pathogen or toxin has caused, or may have caused, disease in an individual (any exposure incident).

### Incident Reporting to the Canadian Food Inspection Agency

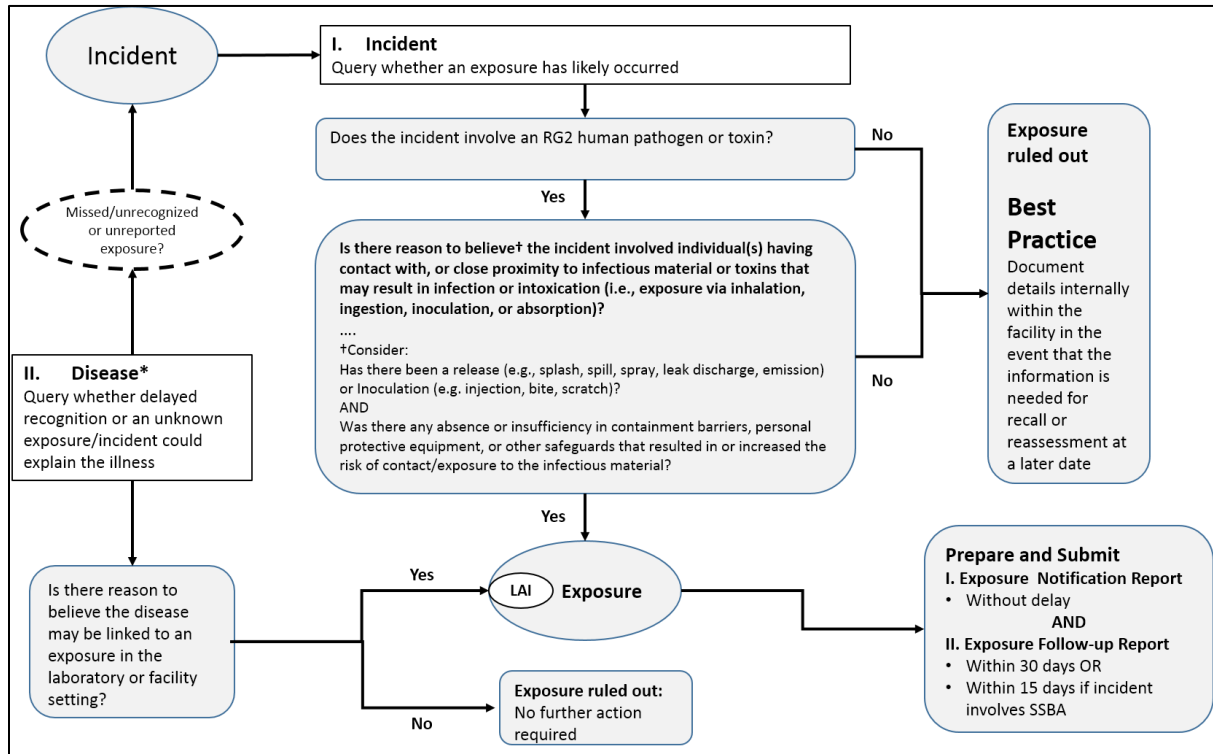


FIGURE 3. Decision tree used to decide on whether PHAC must be notified in the event of an incident or illness arising from a possible laboratory exposure.

Nipissing University is obligated to notify the CFIA without delay in the event of incidents and exposures under the terms of the Health of Animals Act (HAA) and the Health of Animals Regulations (HAR). **The following scenarios require reporting without delay to your supervisor and the BSO:**

- An animal is discovered to be infected with pathogens causing or showing signs of a reportable disease or toxic substance;
- Based on the conditions included on an animal pathogen import permit, any incident involving an animal pathogen, toxin or other regulated infectious material in the facility covered by the animal pathogen import permit.

### Incident Investigation

Following an incident, it may not be known or clear if it is a biosecurity incident. If there is a possibility of a criminal act, it is advisable that law enforcement be contacted at an early stage of the investigation.

The incident investigation process includes the following stages:

- Initial response;
- Collection of evidence and information;
- Analysis and identification of root causes;
- Development of corrective and preventative action plans; and,
- Evaluation and continual improvement.

If you have been involved in an incident, or have witnessed an incident, please contact your supervisor and the BSO as soon as possible so that the details of the incident are still fresh in memory and can be relayed accurately. If criminal activity is suspected, do not disturb the scene, and post a 'DO NOT ENTER' sign on the door of the area where the suspected activity took place.

### **Information Management and Security**

Biosecurity involves not only the protecting the physical assets (pathogens, etc.), but also the non-physical assets such as inventories and storage locations, biosafety/biosecurity risk assessments and biosecurity plans, experimental protocols and results, proprietary scientific information, building plans, personnel, and financial records.

Information can be classified into four categories based on the level of security required and the requirements of federal and provincial privacy laws.

- **Public** – information intended for the general public, upon appropriate approval (published on publicly accessible webpages for example).
- **Internal** – information not intended for the general population. For example, data analysis and draft documents circulated for review.
- **Limited or Restricted Access** – information only intended for authorized individuals. Release of information may negatively impact the organization. For example, pathogen inventories, SOPs, study data, inspection reports, etc.
- **Confidential** – information only intended for a small number of authorized individuals with a need to know. Examples include incident investigation reports, gain of function research, critical proprietary information, security plan details, personnel records, etc.

Inventory information should only be stored on a secure server or secure desktop drive. The inventory should only be available to authorized personnel such as the laboratory technologist, laboratory instructor, research students and supervisors engaged in research activities using pathogens. If the inventory needs to be in hard copy format, it should be placed in a locked desk drawer or filing cabinet to prevent unauthorized access.

### **Health and Medical Surveillance**

The objective of a health and medical surveillance program is to monitor laboratory personnel for occupationally-acquired infections or diseases. All personnel must



understand the hazards and risks associated with their specific work or classroom activities. Prior to beginning work with infectious materials, all individuals who may be exposed to such material must be informed about any risks associated with the material. They must also be informed about any preventable measures that are available against the infectious material and the risks and benefits of those preventative measures.

The risk information provided must include the following:

- A wallet sized card outlining the pathogens that could be encountered and their risk group to be provided for the purpose of informing their physician about any possible pathogen exposures that could occur during the course of their activities with infectious material;
- Public Health Agency of Canada (PHAC) Pathogen Safety Data Sheets (PSDS's) and/or fact sheets outlining the early signs and symptoms for each pathogen;
- A containment level – pathogen – disease cross reference chart.

### **Immunocompromised and Pregnant Individuals**

Immunocompromised (IC) and pregnant persons are subject to elevated risks associated with exposure to pathogenic organisms. In addition, if a persons' household member(s) belong to an at-risk group (e.g., children, elderly, pregnant or IC individuals), these individuals are also subject to an elevated risk associated with exposures from lab acquired pathogens.

Immunocompromised (IC) and pregnant persons (or those residing with at-risk individuals) must have the option of taking extra care and/or not working with certain biologically hazardous materials or organisms.

The need to balance the protection of people with a persons' right to privacy is imperative. To that end, Nipissing University has developed the following protocol:

- Persons should inform their course instructor or supervisor, respectively, that they may have a medical condition that could prevent them from taking part in activities involving biohazardous materials. **At no time are details of the medical condition to be shared with the course instructor or supervisor – this information is confidential and does not need to be shared.**
- The laboratory instructor or supervisor will discuss the type and nature of the accommodations necessary to protect the person from harm. If necessary, Student Development Services or Human Resources may need to be involved in order to meet the accommodation requirements.

### **Immunoprophylaxis**

Laboratory personnel should be protected against laboratory-acquired infections by appropriate immunization with relevant, licenced vaccines unless they already have

documented protective levels of pre-existing immunity. Hepatitis B immunization is strongly recommended for all persons who routinely handle or have occupational exposure to human blood, body fluids, organs or tissues. Other immunizations may be recommended as circumstances dictate.

## **Post-Exposure Plan**

Laboratory acquired infections (LAI's) can occur through various routes, including ingestion, inhalation, puncture, or absorption. The types of laboratory events that can lead to an infection include exposure to infectious aerosols, spills and splashes, accidental needle sticks, cuts from sharps, bites and scratches from animals, centrifuge accidents and secondary spread of biologically hazardous materials to non-laboratory areas.

All exposures/injuries must be reported to the laboratory supervisor immediately after the initial emergency response. An incident report must also be filled out and submitted to the BSO within 24 hours.

Any incident where an exposure occurred may be referred for medical attention to the Campus Health Centre, North Bay Regional Health Centre emergency, or family physician. Provide as much relevant information to the healthcare provider as possible, including the type of exposure, circumstances related to the incident and the route of exposure.

Suspected exposures and/or illnesses arising from a laboratory incident must be reported to the BSO in order to be reported to the WSIB and the Public Health Agency of Canada's Pathogen Safety Directorate. The information required must include the type of exposure, circumstances related to the incident and the route of exposure and any other information as prescribed under the OHS Act and HPTA and their associated regulations (see Incident Reporting above).

## **Confidentiality of Information**

Personal information in connection to any incident or suspected exposure is collected under the authority of The Nipissing University Act, 1992 and in accordance with the *Freedom of Information and Protection of Privacy Act (FIPPA)* and the *Personal Health Information Protection Act (PHIPA)*. It will only be used for the purpose of occupational health and medical surveillance. Direct questions about its collection, use and disclosure to the Access and Privacy Officer at 705-474-3450 extension 4307.

Any known breach of medical confidentiality must be reported immediately to the Access and Privacy Officer.

## **Use and Disclosure of Medical Information**

Medical information may be reviewed by a medical consultant for purposes of providing adequate occupational health and medical surveillance services to Nipissing University. Medical information shall be disclosed in accordance with the *Freedom of Information*

*and Protection of Privacy Act (FIPPA) and the Personal Health Information Protection Act (PHIPA). Medical information may be disclosed to third parties with a person's written consent or when required by law (e.g., when properly subpoenaed, or in accordance with the Workplace Safety and Insurance Act, or Occupational Health and Safety Act). Where any occupational health and medical surveillance is established for an occupational hazard, individual test results may be disclosed to the individual person.*

## **Practices and Procedures**

Individuals who work in a laboratory that handles infectious substances are at risk of exposure to the substances they handle. Laboratory acquired infections (LAIs) are not uncommon. There are a number of ways in which infectious substances can enter the body and cause infection, including ingestion, inhalation, contact with mucous membranes, including conjunctivae (transfer of microorganisms to the eyes by contaminated hands) or through open cuts or sores.

The types of events that can lead to an infection include the following: exposure to infectious aerosols; spills and splashes; accidental needle stick injuries; cuts from sharp objects and broken glass; bites and scratches from animals or ectoparasites; oral pipetting (a prohibited activity); centrifuge accidents; secondary spread of infectious materials to non-laboratory areas. The greatest risk of infection comes from aerosols, which can enter the body through inhalation, ingestion, mucous membrane contact, etc. Operational practices and techniques must be used to minimize the creation of aerosols associated with common laboratory procedures and developed for the containment level of each infectious agent that will be utilized.

## **Training**

Laboratory Safety training, Biosafety training and WHMIS 2015 training is mandatory for all employees, contractors, volunteers, students, and visitors who work with microorganisms, cell cultures, human blood and body fluids, or any other potentially infectious material regardless of the risk group of the material. On completion of Biosafety training, the participant will:

- understand the process of risk assessment for work with microorganisms and cell lines;
- understand the concept of containment level as it applies to biohazard laboratories;
- understand how a biological safety cabinet works and its role in a biohazard laboratory;
- know the procedures for accidental exposure or spills of biohazardous materials;
- understand the risks associated with human blood and body fluids;
- know how to apply precautions when working with human blood and body fluids.

In addition to initial training, it is essential that a continuous, on-the-job safety training programme be in place to maintain safety awareness among laboratory and support staff. Under the HPTA and HPTR, a training needs assessment must be conducted annually, and refresher training provided as determined by that review or when warranted by a change in the biosafety program. Laboratory supervisors, with the assistance of the BSO and other resource persons, play a key role in staff training and safety awareness.

For comments or information on training schedules, please contact the BSO at 705-474-3450 extension 4811.

### **General Laboratory Safety Practices and Containment Level 1**

The following general practices are required by Health Canada for all laboratories handling infectious substances (CL1). There are more rigorous guidelines for laboratories handling Containment Level 2 and 2+ biohazardous agents following this section.

1. Good microbiological laboratory practices intended to avoid the release of infectious materials are to be employed.
2. A documented procedural (biosafety) manual must be available to all staff, students and faculty and its requirements must be followed. This manual must be reviewed and updated regularly.
3. All personnel working with infectious or potentially infectious substances 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; all personnel who receive the training must show evidence that they understand the training provided; an annual refresher training program is also to be implemented to ensure that personnel have the most up-to-date information. Retraining may also be triggered by less than satisfactory laboratory audits.
4. All trainees must be supervised by authorized personnel when engaging in activities with infectious material and toxins until they have fulfilled the training requirements and are deemed competent by their supervisor.
5. Eating, drinking, smoking, storing of food, personal belongings or utensils, and applying cosmetics, is not permitted in any laboratory.
6. Contact lenses are permitted to be worn in the laboratory as long as safety eyewear is also worn. Removing or manipulating contact lenses within the laboratory environment is prohibited and should only be done outside of the laboratory after washing hands.
7. Wearing of jewellery in a laboratory is not recommended. All rings should be removed prior to working in the laboratory.
8. Oral pipetting of any substance is prohibited.
9. Long hair and clothing are to be tied back or restrained so that they cannot come into contact with gloved hands, specimens, containers or equipment.

10. Access to laboratory and support areas is restricted to authorized personnel only.
11. Doors to laboratories must not be left open (this does not apply to an open area of the laboratory).
12. Open wounds, cuts, scratches and grazes should be covered with a waterproof dressing prior to entering the laboratory.
13. Laboratories are to be kept clean and tidy. Storage of materials not pertinent to the work (e.g. journals, books, computers) should be minimized and kept in an area that is separate from biohazardous work areas. Paperwork and report writing should be kept separate from biohazardous work areas.
14. Suitable PPE, properly fastened, must be worn by all personnel, including visitors, trainees and others entering the work area or handling RG1 and RG2 biological material. Suitable footwear with full foot coverage and low profile heels must be worn in all laboratory areas.
15. Protective laboratory clothing must not be worn in non-laboratory areas. PPE must be exclusively worn and stored in the containment area. Laboratory clothing must not be stored in contact with street clothing.
16. If a known or suspected exposure occurs, contaminated clothing must be decontaminated prior to laundering, preferably by autoclave.
17. Where there is a known or potential risk of exposure to splashes or flying objects, whether during routine operations or under unusual circumstances, eye and face protection must be used. Careful consideration should be given to the identification of procedures requiring eye and face protection.
18. Regardless of the type of infectious material, gloves should be worn when performing potentially hazardous procedures (e.g., slide agglutination) in which there is a risk of splashing or skin contamination or when the laboratory worker has cuts or broken skin on his or her hands. The cuff of the glove must cover the sleeve of the laboratory coat (i.e. the sleeve of the lab coat is tucked into the cuff of the glove). Gloves must be removed when contaminated by splashing or spills or when work with infectious materials is completed. Gloves should not be worn outside the laboratory. Personnel should not use phones or open doors with gloves that have been used in laboratory procedures. All used gloves should be disposed of by discarding them with other disposable materials and autoclaving. **Hands should be washed immediately after removing gloves and at any time after handling materials known or suspected to be contaminated (see 'Hand-washing/hand decontamination')**.
19. The use of needles, syringes, and other sharp objects should be strictly limited; needles and syringes should be used only for parenteral 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 done in a BSC; needles should not be bent, sheared, recapped or removed from the syringe;

they should be promptly placed in an approved puncture-resistant sharps container before disposal.

20. Work surfaces must be cleaned and decontaminated with a suitable disinfectant (*see 'Disinfection and Sterilization' below*) at the end of the day and after any spill of potentially biohazardous material. Work surfaces that have become permeable (i.e., cracked, chipped or loose) to biohazardous material must be replaced or repaired immediately.
21. Contaminated materials and equipment leaving the laboratory for servicing or disposal must be decontaminated and labelled or tagged as such.
22. Efficacy monitoring of autoclaves used for decontamination with biological indicators must be done regularly and records of these results and cycle logs must be kept on file (*see autoclave instructions below*).
23. 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 contaminated contents during removal. Centralized autoclaving facilities are to follow applicable Containment Level 2 requirements.
24. Disinfectants effective against the agents in use must be available at all times within the areas where the biohazardous material is handled or stored.
25. Leak-proof containers must be used for the transport of infectious materials between laboratories within the same facility.
26. 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 any investigations used for continuing education.
27. An effective rodent and insect control must be maintained.

### **Hand Washing and Decontamination**

Frequent hand washing is essential for preventing laboratory acquired infections (LAI's). Hands should be washed immediately after removing PPE (gloves, lab jackets, eye protection) and at any time after handling materials known or suspected to be contaminated. Hands should only be washed at a dedicated hand-washing sink using

plenty of water and a non-bactericidal liquid soap. The protocol for washing hands is outlined in Figure 4 (below).

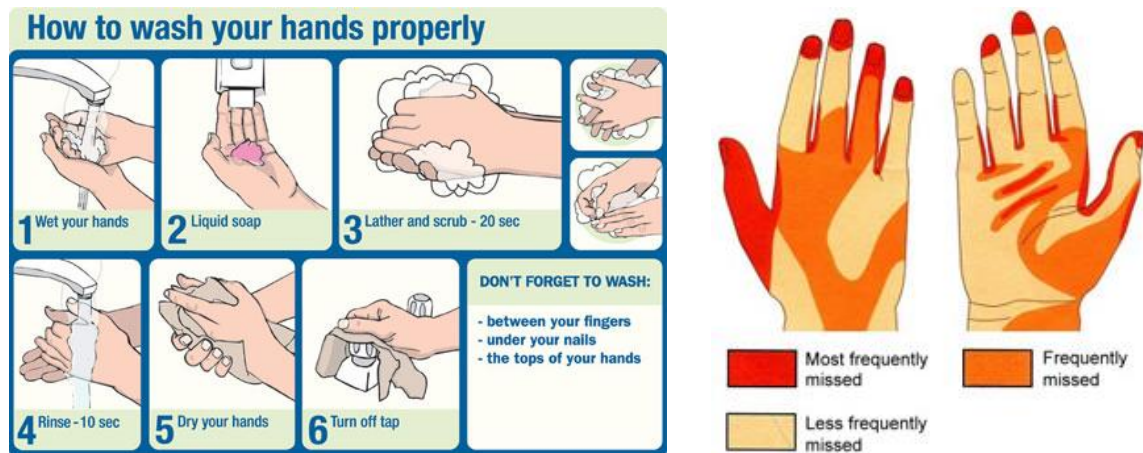


FIGURE 4. Handwashing protocol to be followed after removing gloves or after handling potentially contaminated materials. Be sure to pay attention to those areas of the hands that are frequently missed.

## Guidelines for Containment Level 2 and 2+ Laboratories

In addition to the general practices outlined above, the following are the minimum operational practices required for Containment Level 2.

1. Good microbiological laboratory practices intended to avoid the release of infectious agents are to be employed.
2. Double gloving is required when manipulating RG2 or above organisms or when handling clinical specimens, body fluids, and tissues from humans and animals. These tissues should be assumed to be positive for hepatitis B virus, human immunodeficiency virus (HIV), or other blood borne pathogens.
3. The use of non-dedicated laboratory writing implements is prohibited.
  - a. The laboratory should ensure a supply of writing implements for persons to use if written notes are required.
  - b. A dedicated pathogen-free writing area within the laboratory must be provided.
4. Personal communication devices, *if allowed*, must be placed within an impermeable plastic zip-lock bag before being brought into the laboratory.
  - a. Prior to removing the communication device from the bag and leaving the laboratory, the bag must be disinfected with an appropriate disinfectant.
  - b. Headphones or earbud use in the laboratory is prohibited.**

5. Biological Safety Cabinets (BSCs) must be used for procedures that may produce infectious aerosols and that involve high concentrations or large volumes of biohazardous material. Laboratory supervisors, in consultation with the Biosafety Officer/Biosafety Committee, should perform a risk assessment to determine which procedures and what concentrations and volumes necessitate the use of a BSC.
6. Appropriate signage indicating the nature of the hazard being used (e.g., Biohazard sign, containment level) must be posted outside each laboratory; if infectious agents used in the laboratory require special provisions, the relevant information must be included on the sign; the contact information of the laboratory supervisor and/or other responsible person(s) must also be listed.
7. Entry must be restricted to properly trained and authorized persons only.
8. All people working in the containment area must be trained in and follow the operational protocols for the project in progress. Trainees must be accompanied by a trained staff member. Employees, contractors, volunteers, students, and visitors and others, as deemed appropriate, must be provided with training and/or supervision commensurate with their anticipated activities in the containment area.
9. Emergency procedures for spill clean-up, BSC failure, fire, animal escape, and other emergencies must be written, easily accessible and followed. A record must be made of other people entering the facility during an emergency.

### **Specific Hazard Safety Practices**

The following safety practices must be followed in addition to the general practices that are outlined above. For the most part, the following hazards are not covered by the CBS and CBG and therefore do not fall under Containment Level 2 practices. However, there may be instances where Containment Level 2 practices are required for handling these hazards.

#### **Human Pathogens**

Some microorganisms (viruses, bacteria, fungi, etc.) are species specific, selectively infecting and causing disease in a limited number of, or only one, host species. Unrelated and distantly related species may not be similarly affected by the same infectious microorganism due to differences in physiology, metabolism, biochemistry, and other factors. In general, the risk to a laboratory technician working with a virus that only infects and causes disease in rodents is lower than the risk to a laboratory technician working with tissues and cells from humans or other primates. If the human material contains a viable pathogen, it will likely be a human pathogen, with the potential to infect and cause disease in another human. Although a single mode of transmission may predominate, disease causing micro-organisms can be spread or transmitted from one host to the next, directly or indirectly, by a number of methods. Transmission methods include aerosol generation and inhalation, ingestion of contaminated food and water, skin and mucous membrane contact with contaminated surfaces, contact contamination of an open wound or lesion, and autoinoculation via a



cut, and laceration or puncture with a contaminated instrument. **Any work with materials having the potential to transmit RG2 or higher pathogens must be done at containment level 2.**

### ***Human and Animal Cells or Cell Lines***

Cells or cell lines are derived from human or animal sources and include all primary, secondary, and immortalized cells and cell lines, as well as hybridomas that are being stored, maintained and/or cultured. Under most circumstances cells or cell lines are not regulated under HPTA. However, if the cell or cell line contains an intact RG2, RG3, or RG4 human pathogen or toxin, then it is regulated under HPTA. In addition, if the cell or cell line contains a viral fragment(s) of an animal pathogen, it is regulated under the CFIA.

The potential hazards associated with human cells include blood borne pathogens HBV, HIV, HCV, HTLV, EBV, HPV, and CMV. Other primate cells and tissues also pose risks. Immortalized cells carrying the viral agents SV-40, EBV adenovirus or HPV are also potentially hazardous. Tumorigenic human cells can also pose a risk as a result of a self-inoculation.

A full risk assessment, that includes the origin of the cells as well as the source, must be conducted before beginning any work with human or animal cell lines. **Human and primate cell lines must be handled at containment level 2 with all work being done in a biological safety cabinet (BSC).** All material must be decontaminated by autoclaving or disinfection before being discarded. All personnel working with human cell lines must be offered a hepatitis vaccine prior to beginning work on the cell lines and be evaluated by a health care professional following an exposure incident.

### ***Human Blood Borne Pathogens***

Human blood is recognized as a potential source of pathogenic microorganisms that may present a risk to workers who are exposed during the performance of their duties. Although the hepatitis B virus (HBV) and the human immunodeficiency virus (HIV) are often cited as examples, a “blood borne pathogen” is any pathogenic microorganism that is present in human blood or other potentially infectious materials and that can infect and cause disease in persons who are exposed to blood containing this pathogen. “Other potentially infectious materials” means material that has the potential to transmit blood borne pathogens. This includes infected human tissues and the following body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, amniotic fluid, saliva in dental procedures, and any other body fluid that is visibly contaminated with blood. **Any work with materials having the potential to transmit blood borne pathogens must be done at containment level 2.**

### ***Routine Practices***

In 1999, Health Canada published the 'Routine Practices' to protect workers from the transmission of infectious micro-organisms contained in blood and bodily fluids. In 2012, the Provincial Infectious Diseases Advisory Committee of Ontario and Public Health Ontario published the Routine Practices and Additional Precautions in All Health Care Settings, 3<sup>rd</sup> edition.

Routine Practices are a combination of universal precautions and body substance isolation and have a much bigger scope than the WHO Universal Precautions published in 1988 and aim to protect against the transmission of all microorganisms through contact with all body fluids, excretions, mucous membranes, non-intact skin and soiled items in addition to blood. All human blood, human body fluids, and other human materials are to be considered potentially infectious for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and other blood borne pathogens. Hepatitis B immunization is highly recommended as an adjunct to Routine Practices for workers who have occupational exposure to human blood or other potentially infectious materials. The following is an adaptation of Routine Practices to protect laboratory workers.

### **Routine Practices**

- 1) All workers should routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with human blood or other body fluids is anticipated.
- 2) Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited.
- 3) Double gloves should be worn when touching blood and body fluids, mucous membranes, or non-intact skin, for handling items or surfaces soiled with blood or body fluids. If a glove is torn or damaged during use, it should be removed, and a new glove should be used as promptly as safety permits. Disposable gloves should not be washed or disinfected for reuse. Washing with surfactants may enhance penetration of liquids through undetected holes in the glove. Disinfecting agents may cause deterioration of the glove material.
- 4) Where possible, all procedures that are likely to generate droplets of blood or other bodily fluid should be performed in a biosafety cabinet. If the use of a biosafety cabinet is not available or cannot be accommodated, N95 masks and protective eyewear or face shields should be worn during those procedures to prevent exposure of mucous membranes of the mouth, nose, and eyes.
- 5) Gowns or aprons should be worn during procedures that are likely to generate splashes of blood or other body fluids. Protective clothing should be removed before leaving the area.

- 6) Hands and other skin surfaces should be washed immediately and thoroughly if contaminated with blood or other body fluids. Hands should be washed immediately after gloves are removed since no barrier is 100% effective.
- 7) Workers should take precautions to prevent injuries caused by needles, scalpels, and other sharp instruments or devices during procedures, when cleaning used instruments, during disposal of used needles, and when handling sharp instruments after procedures. Needles and syringes should be used only in those situations when there is no alternative. To prevent needle stick injuries, needles should not be recapped, purposely bent or broken by hand, removed from disposable syringes, or otherwise manipulated by hand. After they are used, disposable syringes and needles, scalpel blades, and other sharp items should be placed in puncture-resistant containers for disposal. The puncture-resistant container should be located as close to the use area as practical. Contaminated reusable pointed and sharp objects such as large bore needles and scalpels should be placed in a puncture resistant container for transport to the reprocessing area.
- 8) Workers who have exudative lesions, weeping dermatitis, cuts, open wounds or other breaks in the skin should either refrain from all direct contact with blood and other body fluids until the condition resolves or utilize protective barriers to reduce the risk of exposure.
- 9) Pregnant workers should be especially familiar with and strictly adhere to precautions to minimize the risk of prenatal transmission of blood borne pathogens.

### **Working with Laboratory Animals in a Containment Zone**

Containment zone(s) housing research animals that are being used for *in vivo* pathogen or toxin experiments must be approved and licensed by PHAC and/or the CFIA. Animal facilities must be designed and operated in accordance with the *Canadian Biosafety Standard 3<sup>rd</sup> Edition (2022)*, published by the Public Health Agency of Canada and the *Containment Standards for Aquatic Facilities*, published by the Canadian Food Inspection Agency. Environment and Climate Change Canada (ECCC) also regulates animals (both transgenic and exotic) through the New Substances Notification Regulations (Organisms) under the Canadian Environmental Protection Act (CEPA) 1999. In addition to these two standards, the *Guide to the Care and Use of Experimental Animals*, published by the Canadian Council on Animal Care and other CCAC guidelines and policies (as revised from time to time) must also be followed to ensure that every care is taken to avoid unnecessary pain or suffering and to provide the animals with the highest possible care.

Working with animals poses a variety of risks and hazards including exposure to infectious agents (naturally occurring or experimentally produced), animal bites, scratches, allergies, kicks, crushing injuries and physical hazards. Animals (including insects) can harbour infectious organisms, which might be shed intermittently or can give rise to a chronic carrier state. Animals should be kept in the containment level appropriate to the risk presented if there is a possibility that such an agent can be

excreted, secreted, exhaled or shed by the animal during the course of an experiment. Animals may also be intentionally inoculated with viruses or other organisms in any of the four Risk Groups or with viable materials (e.g., transformed cells) suspected of containing these agents. Under these circumstances, the animals should be kept at the containment level appropriate for the risk of the agent.

In addition to keeping infectious agents from spreading to laboratory workers, there is a need to address, in the equipment and practices, the issues of cross contamination between animals and of keeping adventitious agents from inadvertently infecting experimental animals. As such, the following precautions should be followed:

1. Infected animals and insects should be segregated from uninfected animals wherever possible, and it is preferable to separate any handling area from the holding area.
2. Animals or insects in use in an experiment must be maintained at a level of containment that is at least equivalent to the containment level for the biological agent with which it has been infected or treated.
3. Provision must be made to ensure that inoculated animals or insects cannot escape
4. Dead animals or insects and the refuse (e.g., bedding, feces, and food) from the animal room and cages must be placed in a leak proof container and autoclaved or incinerated, if potentially infected.
5. All cages must be properly labelled, and procedures in the holding area must minimise the dispersal of dander and dust from the animals and cage refuse.
6. Lab coats, gloves, and safety eyeglasses must be worn by animal care providers while feeding and watering animals or cleaning cages. Lab coats, gloves and safety eyeglasses are also to be worn by animal care providers while caring for aquatic animals. Other personal protective equipment may be required based on risk assessments by the Animal Care Committee (ACC) or Biosafety Committee.
7. Reusable gloves, boots, floors, walls and cage racks should be disinfected frequently.
8. All aspects of the proposed use of animals in research must meet the current veterinary standards and regulations for the care and use of animals in experimental programs.
9. The appropriate species must be selected for animal experiments

10. The investigator and/or person(s) responsible for the animal experiment must ensure that all those having contact with the animals and waste materials are familiar with and aware of any special precautions and procedures that may be required. Where possible, personnel should be protected by immunization with appropriate vaccines.
11. All incidents, including animal bites and scratches or cuts from cages or other equipment must be documented and reported to the BSO via the "Injury, Incident Reporting and Investigation" e-form. All persons suffering from an animal bite, scratch or a cut from cages or other equipment must report to their health care provider for medical assessments and follow-up.

### ***Animal Cells, Blood and Fluids and Fixed Tissues***

The biological hazards of animal cells, tissues, blood, and body fluids arise from the possibility that they might contain or transmit infectious agents. It is prudent to consider all cell lines to be potentially infectious. Cells known or suspected to contain such agents, or primary cultures from animals and humans known or reasonably suspected to be infected, should be assigned to the risk group for the suspected agent. Primate cell lines, all samples of human tissues and fluids, all primate tissues, and all cell lines new to the laboratory should be handled at Containment Level 2. Factors such as the particular source of the material, the volume and concentration of the agent, the extent of culturing and incubation, the types of manipulations to be conducted, and the use of additional precautions could influence the containment level required.

#### *Animal Cells*

##### *Primary cell cultures and animal tissues*

The following containment requirements apply to primary cell cultures and tissues from human, non-human, primate, and non-primate animal sources when handled in the laboratory or used for animal passage. Cells and tissues known or suspected to be contaminated or infected with biohazardous agents must be handled at the containment level appropriate to those agents.

Human and non-human primate material: Containment Level 2 (or higher)

Non-primate animal material: Containment Level 1 unless otherwise indicated by a local risk assessment.

##### *Established cell lines*

Human or other animal cell lines known to be uncontaminated or uninfected with biohazardous agents may be handled at Containment Level 1. Cultures known or suspected to be contaminated or infected with any of the agents must be handled at the containment level appropriate to those agents.

## Blood and Bodily Fluids

The need for precautionary measures extends to situations in which human blood, saliva, urine, feces, and other body fluids must be handled. The precautions required may be more stringent when the specimens are used for culturing purposes, but initially, their handling should be consistent with Containment Level 2, especially if they will be used to culture cells or organisms.

Reduction of the containment level may be acceptable if potential hazards associated with the material are expected to be diminished because of dilution, use of chemical or other treatments or additional protective measures and practices.

### *1) Culturing of specimens in research laboratory*

Blood or blood fractions and other body fluid specimens of human or animal origin that are known or suspected to contain any biohazardous agents must be handled at the containment level appropriate to those agents when these specimens are cultured in volumes greater than that which is necessary for routine diagnostic work.

### *2) Clinical diagnostic work in laboratory*

For clinical diagnostic work with specimens of human blood, serum and other body fluids (urine, cerebrospinal fluid, etc.) from the general population, Containment Level 2 and Routine Practices apply. For routine clinical diagnostic work with specimens that are known to be from infected individuals, the containment level appropriate to the agent must be maintained.

## Fixed Tissues and Tissue Sections

Tissues and tissue sections from human and animal sources are routinely fixed by treatment with chemical agents, such as formaldehyde to preserve structures for later examination and study. Generally, these chemical treatments inhibit all biological activity.

In general, fixed tissues and tissue specimens should be handled under at least Containment Level 1 conditions. A higher level of containment may be required depending on the source of the material, the nature of the agent and whether it is inactivated (e.g. prions in central nervous system tissues). Where a biological agent, usually requiring a higher level of containment, is present in the tissue, the laboratory Principal Investigator must provide documentation to the Biosafety Committee which supports a request for a lower level of containment.

## **Recombinant DNA and Genetic Manipulations**

For the purposes of this document, recombinant DNA includes:

- DNA molecules produced outside living cells by joining natural or synthetic DNA segments to DNA molecules capable of replication in living cells,
- DNA molecules produced in living cells by joining enriched or natural segments to intracellular DNA, and,

- DNA molecules resulting from replication of such recombinant molecules.

Guidance in assessing potential risks in recombinant DNA research can only be very general; each case requires an individual risk assessment. It is unrealistic to define all of the genetically engineered organisms that might be created or used in the laboratory. The majority of recombinant DNA research involves only a very low possibility of creating a hazard because the source of the DNA being transferred, the vector and the host are all innocuous or have low risk characteristics.

However, some genetic manipulation does raise a significant possibility of risk. In any research with genes coding for hazardous products, host vector systems with limited ability to survive outside the laboratory should be used. As such, each case needs to have a risk assessment prior to beginning any experiments. Some examples of genetic manipulations and the precautions that should be followed are outlined below:

### ***Risk assessments for genetically modified organisms (GMOs)***

Risk assessments for work with GMOs should consider the characteristics of the donor and recipient organisms. Examples of characteristics for consideration include the following:

#### Hazards arising directly from the inserted gene

Assessment is necessary in situations where the product of the inserted gene has known biologically or pharmacologically active properties that may give rise to harm. The consideration of such cases should include an estimation of the level of expression required to achieve biological or pharmacological activity. Examples of such properties are:

- 1) Toxins
- 2) Cytokinins
- 3) Hormones
- 4) Gene expression regulators
- 5) Virulence factors or enhancers
- 6) Oncogenic gene sequences
- 7) Antibiotic resistance
- 8) Allergens

Other considerations to consider are the hazards associated with the foreign gene on the recipient and host such as the following:

- 1) Susceptibility of the host.
- 2) Pathogenicity of the host strain, including virulence, infectivity and toxin production.
- 3) Modification of the host range.
- 4) Recipient immune status.
- 5) Consequences of exposure.
- 6) Increased fitness over wild-type (possible genetic pollution).

### Hazards arising from the alteration of existing pathogenic traits

Many modifications do not involve genes whose products are inherently harmful, but adverse effects may arise as the result of alteration of existing non-pathogenic or pathogenic traits. Modification of normal genes may alter pathogenicity. In an attempt to identify these potential hazards, the following points may be considered (the list is not exhaustive).

- 1) Is there an increase in infectivity or pathogenicity?
- 2) Could any disabling mutation within the recipient be overcome as a result of the insertion of the foreign gene?
- 3) Does the foreign gene encode a pathogenicity determinant from another organism?
- 4) If the foreign DNA does include a pathogenicity determinant, is it foreseeable that this gene could contribute to the pathogenicity of the GMO?
- 5) Is treatment available?
- 6) Will the susceptibility of the GMO to antibiotics or other forms of therapy be affected as a consequence of the genetic modification?
- 7) Is eradication of the GMO achievable?

### ***Biological expression systems***

Biological expression systems consist of both a host cell and vectors. For an expression system to be effective and safe, it must be non-pathogenic. An example of this type of system is the pUC18 plasmid in combination with the *Escherichia coli* K12 strain. The pUC18 has been entirely sequenced and all genes required for expression in other bacteria deleted from its precursor plasmid pBR322. *E. coli* strain K12 is a non-pathogenic strain that is incapable of colonizing the gut of healthy humans or animals. Routine genetic manipulations with this combination can be safely performed at containment level 1, provided the inserted foreign DNA expression products do not require higher biosafety levels.

### Biosafety and biosecurity considerations for expression vectors

- 1) The expression of DNA sequences derived from pathogenic organisms may increase the virulence of the GMO.
- 2) Inserted DNA sequences are not well characterised, e.g. during preparation of genomic DNA libraries from pathogenic organisms.
- 3) Gene products have potential pharmacological activity.
- 4) Gene products code for toxins.

### ***Transgenic and “knock-out” organisms***

Animals carrying foreign genetic material (transgenic animals) should be handled in containment levels appropriate to the characteristics of the products of the foreign genes. Animals with targeted deletions of specific genes (“knock-out” animals) do not generally present particular biological hazards.



### ***Transgenic Plants***

Transgenic plants expressing genes that confer tolerance to herbicides or resistance to insects are currently a matter of considerable controversy in many parts of the world. Many discussions focus on the food-safety of such plants and the long-term ecological consequences of their cultivation.

Transgenic plants expressing genes of animal or human origin are used to develop medicinal and nutritional products. A risk assessment should determine the appropriate biosafety level for the production of these plants based on the criteria above.

## **Laboratory Equipment**

Whenever lab equipment is purchased, preference should be given to equipment that:

- Limits contact between the operator and the infectious agent.
- Is corrosion-resistant, easy to decontaminate and impermeable to liquids.
- Has no sharp edges or burrs.
- Every effort should be made to prevent equipment from becoming contaminated. To reduce the likelihood of equipment malfunction that could result in leakage, spill or unnecessary generation of aerosolized pathogens:
  - Review the manufacturer's documentation. Keep for future reference.
  - Use and service equipment according to the manufacturer's instructions.
  - Ensure that anyone who uses a specific instrument or piece of equipment is properly trained in setup, use and cleaning of the item.
  - Decontaminate equipment before it is sent out for repairs or discarded.

The following sections outline some of the precautions and procedures to be observed with some commonly used laboratory equipment.

### **Blenders, Sonicators, Homogenizers, Shaking Incubators, and Vortex Mixers**

The operation of blenders, sonicators, homogenizers, mixers, and other similar equipment can generate aerosols. As such, the following requirements and recommendations should be followed:

- Read and understand the operations manual and/or ask your supervisor for instructions prior to using any equipment.
- If the equipment generates sound, be sure to use adequate hearing protection.
- Laboratory equipment and associated accessories specially designed to contain infectious aerosols should be used for manipulations of pathogens and toxins.
- When equipment designed to contain infectious aerosols is not available, the equipment should be operated in a BSC or other primary containment device.
- Only use screw top tubes for holding samples. Snap caps can generate aerosols when they are opened.

Allow time for aerosols to settle before opening or removing covers or caps.

## Centrifuges

Centrifuges are a source of potential biological contamination due to the rapid speeds and relatively high pressure exerted by such devices. The following safety measures should be used when using any centrifuge:

- Read and understand the operations manual and/or ask your supervisor for instructions prior to using the equipment.
- Only use a centrifuge that has a rotor cover or uses sealable rotor cups.
- Prior to starting, make sure the centrifuge is clean. Do not operate with any material spills in either the body or the rotor.
- Make sure the centrifuge is level. If a portable model, make sure it is secure on the bench top before starting.
- Inspect all equipment to be placed in centrifuge for cracks or weak areas.
- Use the lowest speed and time setting that will accomplish the job.
- Load the rotor from within a BSC. Balance all loads.
- Do not open the lid until it comes to a complete stop.
- Wait for at least one minute before opening the lid to remove the rotor. Should a spill occur, please follow the directions below under the heading “Spill within a centrifuge – Level I response”.
- Only open the rotor inside a BSC.
- Periodically inspect the centrifuge. Check the seal around top, baskets, rotors and wiring.
- Avoid use of volatile materials when possible.
- Plastic centrifuge tubes with seal-forming screw tops should be used whenever possible.
- Centrifuges should not be placed or run in a biological safety cabinet.

## Lyophilizers

Lyophilizers are used to remove liquid by a process commonly referred to as freeze drying. Because the removal of liquid is complete, the chance of generating aerosol contamination can be quite high if the appropriate safety procedures are not followed. The following are guidelines for using biological or potential biological materials in a lyophilizer:

- Read and understand the operations manual and/or ask your supervisor for instructions prior to using the equipment.
- Ensure equipment is clean and sanitized before using.
- Ensure appropriate filters are attached to vacuum and exhaust lines.
- Do not remove samples before the cycle is complete. Do not attempt to break the vacuum.
- Periodically inspect the equipment.

- Where possible cap all material before removal from the unit.

## **Vacuum**

If there is a vacuum system serving multiple areas, care should be taken that there are filters in the system, and that there is an overflow trap containing an appropriate disinfectant to prevent entry of contaminated material into the piping system and pumps (see Appendix 2). It is often best to use either a stand-alone pump-type vacuum system, or to use a water siphon vacuum system that is attached to a faucet (provided that measures are taken to prevent back-flow).

## **Bunsen Burners**

Bunsen burners provide two types of hazards: fire on the body and pathogen aerosolization.

### ***Fire hazard***

Bunsen burners should only be used with gloves made of chloroprene (neoprene, polychloroprene). Other material types such as nitrile or latex are extremely flammable and can easily catch fire if exposed to direct flame.

### ***Aerosolization hazard***

Bunsen burners should only be used to provide a zone of sterility on an open bench. Aerosolization of infectious material can occur when using a Bunsen burner to sterilize an inoculation loop and should be avoided. A microincinerator or disposable inoculation loops should be used as an alternative to an open flame.

## **Autoclaves**

An autoclave is a specialized piece of equipment designed to deliver heat under pressure to a chamber, with the goal of decontaminating or sterilizing the contents of the chamber. Because there are many variables associated with ensuring total decontamination of an autoclave load and hazards associated with the operation of an autoclave, *training is required prior to a user being allowed to operate the autoclave*. For more information please see the 'NU Autoclave Guidelines' available on the 'Nipissing University Laboratory Safety' webpage.

Please contact the BSO at extension 4811 for information on training schedules.

## **Biological Safety Cabinets**

### **Class II biological safety cabinets**

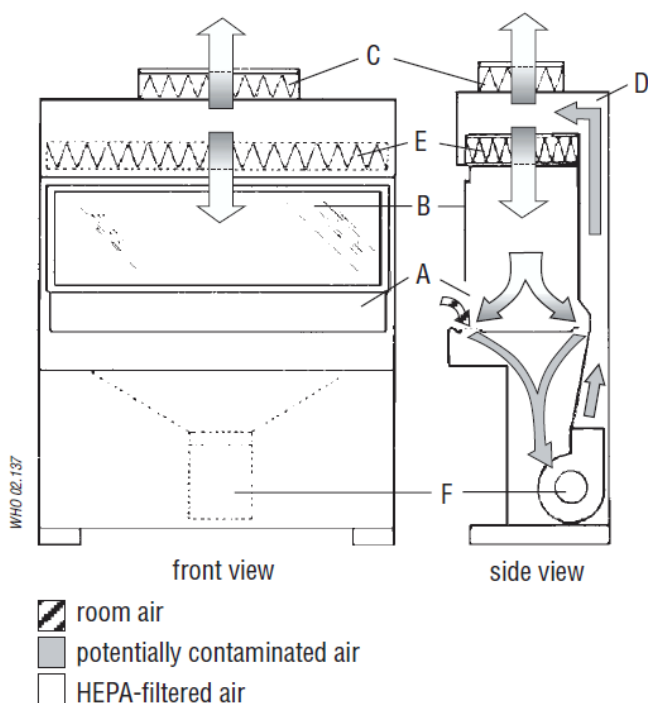
As the use of cell and tissue cultures for the propagation of viruses and other purposes grew, it was no longer considered satisfactory for unsterilized room air to pass over the work surface. The Class II BSC was designed not only to provide personnel protection but also to protect work surface materials from contaminated room air. Class II BSCs, of which there are four types (A1, A2, B1 and B2), differ from Class I BSCs by allowing only

air from a HEPA-filtered (sterile) supply to flow over the work surface. The Class II BSC can be used for working with infectious agents in Risk Groups 2 and 3. Class II BSCs can be used for working with infectious agents in Risk Group 4 when positive-pressure suits are used. Nipissing University uses Class II A2 biological safety cabinets in the laboratories. *Under the provisions of the Canadian Biosafety Standard 3<sup>rd</sup> Edition, 2022 and the Canadian Biosafety Handbook, 2<sup>nd</sup> ed., 2016, anyone using a BSC must first be trained in the use of and be knowledgeable about the operation of the BSC they will be using.*

Please contact the BSO at extension 4811 for information on training schedules.

### ***Class II type A2 biological safety cabinet***

The Class II type A2 BSC is shown in Figure 1. An internal fan draws room air (supply air) into the cabinet through the front opening and into the front intake grill. The inflow velocity of this air should be at least 0.38 m/s at the face of the front opening. The supply air then passes through a supply HEPA filter before flowing downwards over the work surface. As the air flows downwards it “splits” about 6–18 cm from the work surface, one half of the downwards flowing air passing through the front exhaust grill, and the other half passing through the rear exhaust grill. Any aerosol particles generated at the work surface are immediately captured in this downward airflow and passed through the front or rear exhaust grills, thereby providing the highest level of product protection. The air is then discharged through the rear plenum into the space between the supply and exhaust filters located at the top of the cabinet. Owing to the relative size of these filters, about 70% of the air recirculates through the supply HEPA filter back into the work zone; the remaining 30% passes through the exhaust filter into the room or to the outside.



**Figure 5.** Schematic diagram of a Class II A2 biological safety cabinet. A, front opening; B, sash; C, exhaust HEPA filter; D, rear plenum; E, supply HEPA filter; F, blower (from Laboratory Biosafety Manual, 3<sup>rd</sup> Edition, WHO 2004).

Air from the Class II A2 BSC exhaust can be recirculated to the room or discharged to the outside of the building through a thimble connection to a dedicated duct or through the building exhaust system. Recirculating the exhaust air to the room has the advantage of lowering building fuel costs because heated and/or cooled air is not being passed to the outside environment.

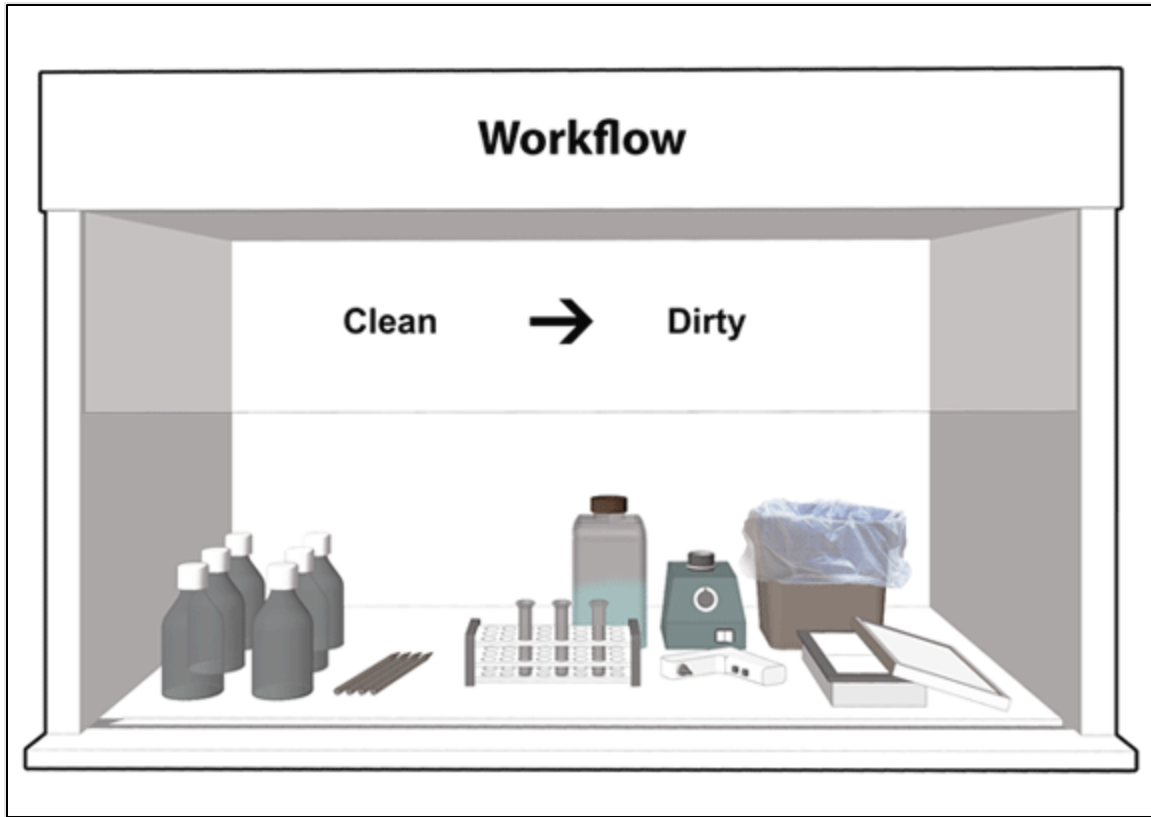
The velocity of air flowing through the front opening into a BSC is about 0.45 m/s. At this velocity the integrity of the directional air flow is fragile and can easily be disrupted. Ideally, the biological safety cabinet should be placed away from disruptive air currents caused by excessive personnel traffic, air-conditioning or heating vents, or laboratory windows and doors. Whenever possible a 30 cm clearance should be provided behind and on each side of the cabinet to allow for easy access for maintenance. A minimum 30 – 35 cm clearance above the cabinet is required to provide for accurate air velocity measurement across the exhaust filter and for exhaust filter changes.

### ***Precautions when using biological safety cabinets***

Prior to any personnel using any biological safety cabinet, it is mandatory that they are familiar with how the biological safety cabinet works, the precautions that are required for its use and how to work safely within its confines. All of this information can be found in the SOP or BSC's operating manual, which can be found attached to the side of the BSC, online at the Nipissing University Laboratory Safety website or on the

manufacturer's website. In general, the following precautions should be followed when using a BSC:

1. Ultraviolet lights are not required in BSCs. It is advisable not to rely on UV lamps for disinfecting a BSC as they are not as effective at sterilizing as chemical-based surface decontamination (e.g. bleach or alcohol). Ultraviolet lights must be turned off while the room is occupied to protect eyes and skin from inadvertent exposure.
2. **Open flames must never be used in a biological safety cabinet.** If heat sterilization is required, use a microbead sterilizer for sterilizing small instruments or an electric furnace loop sterilizer.
3. When starting the cabinet for the day's first use, be sure to leave it run for 5 minutes prior to placing any items inside the cabinet.
4. During this time, wash hands and arms thoroughly with germicidal soap.
5. Wear a long-sleeved lab coat with knit cuffs (if available) and over-the-cuff laboratory gloves. Use protective eyewear. Wear a protective mask if appropriate.
6. When using the cabinets, maintain the integrity of the front opening air flow when moving arms in and out of the cabinets. Arms should be moved in and out slowly, perpendicular to the front opening. Manipulations of materials within the cabinet should be delayed for 1 minute following placement of the hands and arms inside the cabinet to allow the cabinet to adjust and 'air-sweep' the surface of the hands and arms. Minimize the movements across the front of the opening by placing all necessary items in the cabinet prior to beginning manipulations.
7. Keep the front grill of the cabinet free of paper, equipment, or other items. Surface decontaminate all items with 70% alcohol or other appropriate disinfectant when placing them inside the cabinet. Place all materials as far back in the cabinet towards the edge of the work surface without blocking the rear grill. Aerosol-generating equipment (e.g. mixers, etc.) should be placed to the rear of the cabinet.
8. Keep bulky items such as biohazard bags, discard pipette trays, and suction collection flasks to one side of the interior of the cabinet. Active work should flow from clean to contaminated areas across the work surface (Figure 6). **Do not place autoclavable biohazard bags or pipette collection trays outside the cabinet.**



**Figure 6.** Workflow pattern in a biological safety cabinet.

9. All items within the BSC should be surface decontaminated and removed from the BSC when work is completed to avoid the opportunity for microbial growth.
10. The interior surfaces of the BSC must be decontaminated before and after each use. The interior walls must be wiped down with a disinfectant that will kill all microorganisms that might be found inside the cabinet. At the end of the workday, the final decontamination should include a wipe-down of the work surface, the sides, back and interior of the glass. A solution of bleach or 70% alcohol should be used where effective for target organisms (refer to PSDS for pathogens used). A second wiping with sterile water is needed when a corrosive substance such as bleach is used.
11. If a spill occurs inside the cabinet, please see the section on biohazardous spills below.
12. The cabinet should be left running if possible. If the cabinet is to be turned off for the night, be sure to let the empty cabinet run for 5 minutes before turning it off.

## **Disinfection and Sterilization**

A basic knowledge of disinfection and sterilization is crucial for biosafety in the laboratory. Since heavily soiled items cannot be properly disinfected or sterilized, it is equally important to understand the fundamentals of cleaning prior to disinfection

(precleaning). In this regard, the following general principles apply to all known classes of microbial pathogens.

Decontamination can be achieved by:

1. Physical methods
  - a. Heat which includes autoclaving (most practical and recommended) and incineration (for disposal of sharps and tissues)
  - b. Irradiation which includes UV light ( $\lambda$  253 nm is germicidal) and gamma radiation (which disrupts DNA and RNA)
  - c. Filtration which includes HEPA 0.2 micron (for biological safety cabinets and ventilation).
2. Chemical methods
  - a. Generally used for disinfection rather than sterilization.

Specific decontamination requirements will depend on the type of experimental work and the nature of the infectious agent(s) being handled. The generic information given here can be used to develop both standardised and more specific procedures to deal with biohazard(s) involved in a particular laboratory.

Contact times for disinfectants are specific for each material and manufacturer. Therefore, all recommendations for use of disinfectants should follow manufacturer specifications.

### **Pre-Cleaning Laboratory Materials**

Cleaning is the removal of dirt, organic matter, and stains. Cleaning includes brushing, vacuuming, dry dusting, washing or damp mopping with water containing a soap or detergent. Dirt, soil, and organic matter can shield micro-organisms and interferes with the killing action of decontaminants.

Precleaning is essential to achieve proper disinfection or sterilization. Many germicidal products claim activity only on precleaned items. Precleaning must be carried out with care to avoid exposure to infectious agents. Materials chemically compatible with the germicides to be used later must be used. It is common to use the same chemical germicide for precleaning and disinfection.

### **Chemical Germicides**

Chemical disinfectants are used for the decontamination of surfaces and equipment that cannot be autoclaved and for clean-up of spills of infectious materials, rooms and animal cubicles and a variety of other items for which heat treatment is not feasible. Chemical germicides are not normally required for regular cleaning of floors, walls, equipment and furniture, however there may be occasions such as in cases of outbreak control when their use is warranted.

Many germicides can be harmful to humans or the environment. They should be selected, stored, handled, used and disposed of with care, following the manufacturer's recommendations. Many disinfectants can be almost completely deactivated by using



cotton and microfibre towels (e.g. ammonia products) further demonstrating the need to read manufacturer’s instructions carefully.

For personal safety, gloves, aprons and eye protection are recommended when preparing dilutions of chemical germicides. The germicidal activity of many chemicals is faster and better at higher temperatures. However, higher temperatures can accelerate their evaporation rate and/or degrade them faster.

The proper use of chemical germicides will contribute to workplace safety while reducing the risk from infectious agents. As far as possible, the number and quantity of germicidal chemicals used and stored should be limited for economic reasons, inventory control, and to limit environmental pollution.

Selection of an appropriate disinfectant depends upon the resistance of the microorganisms of concern (Table 1). Refer to PSDS for the specific pathogens being used for the appropriate disinfectants.

**Table 1.** Commonly used disinfectant methods for use with different microorganism types.

<b>Class of Organism</b>	<b>Disinfectant methods</b>
Vegetative bacteria ( <i>E. coli</i> , etc.)	1% domestic bleach 70% alcohol 6% formulated hydrogen peroxide
Mycobacteria and fungi	1% domestic bleach 70% alcohol 6% formulated hydrogen peroxide
Spore forming bacteria ( <i>Bacillus sp.</i> ) Note: autoclaving is the best method to use for disinfection. Otherwise, leave disinfectant in contact for several hours to ensure sporicidal activity	10% domestic bleach 6% formulated hydrogen peroxide
Enveloped viruses (HIV, Herpes)	1% domestic bleach 70% alcohol 6% formulated hydrogen peroxide
Non-enveloped viruses (Hepatitis, Adenovirus)	6% formulated hydrogen peroxide
Prions	Autoclave at 131 – 136°C for 60 minutes, or Autoclave at 121°C for 4.5 hours, or Soak in 1N NaOH for 1 hour, then autoclave at 121 °C for 1 hour. Treat work surfaces with 10% bleach for at least 30 minutes 2N NaOH may also be used to treat surfaces.

	If skin becomes contaminated, treat for 5 – 10 minutes with 1N NaOH followed by extensive washing with water.
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Commonly used classes of chemical germicides are described below, with generic information on their applications and safety profiles. *As a general rule, alcohols, bleaches and formulated hydrogen peroxides are the preferred disinfectants to be used in the laboratory.* Unless otherwise indicated, the germicide concentrations are given in weight/volume (w/v).

### ***Alcohols***

Ethanol (ethyl alcohol, C<sub>2</sub>H<sub>5</sub>OH) and 2-propanol (isopropyl alcohol, (CH<sub>3</sub>)<sub>2</sub>CHOH) have similar disinfectant properties. They are active against vegetative bacteria, fungi and lipid-containing viruses *but not against spores*. Their action on nonlipid viruses is variable. **For highest effectiveness they should be used at concentrations of approximately 70% (v/v) in water: higher or lower concentrations are not as germicidal.**

A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items. *Mixtures with other agents are more effective than alcohol alone, e.g. 70% (v/v) alcohol containing 2 g/l available chlorine.* A 70% (v/v) aqueous solution of ethanol can be used on skin, work surfaces of laboratory benches and biosafety cabinets, and to soak surgical instruments. Since ethanol can dry the skin, it is often mixed with emollients. Alcohol-based hand-rubs are recommended for the decontamination of lightly soiled hands in situations where proper handwashing is inconvenient or not possible. *However, it must be remembered that ethanol is ineffective against spores and may not kill all types of nonlipid viruses.*

Alcohols are volatile and flammable and must not be used near open flames. Working solutions should be stored in proper containers to avoid the evaporation of alcohols. Alcohols may harden rubber and dissolve certain types of glue. Proper inventory and storage of ethanol in the laboratory is very important to avoid its use for purposes other than disinfection. Bottles with alcohol-containing solutions must be clearly labelled to avoid autoclaving.

### ***Chlorine (sodium hypochlorite or bleach)***

Chlorine, a fast-acting oxidant, is a widely available and broad-spectrum chemical germicide. It is normally sold as bleach, an aqueous solution of sodium hypochlorite (NaOCl), which can be diluted with water to provide various concentrations of available chlorine. *To ensure the full-strength bleach has enough chlorine, it should be discarded 6 months after purchase and replaced with a new bottle.*

Chlorine, especially as bleach, is highly alkaline and can be corrosive to metal. Its activity is considerably reduced by organic matter (protein). Storage of stock or working solutions of bleach in open containers, particularly at high temperatures, releases chlorine gas thus weakening their germicidal potential. The frequency with which

working solutions of bleach should be changed depends on their starting strength, the type (e.g. with or without a lid) and size of their containers, the frequency and nature of use, and ambient conditions. As a general guide, solutions receiving materials with high levels of organic matter several times a day should be changed at least daily, while those with less frequent use may last for as long as a week.

A general all-purpose laboratory disinfectant should have a concentration of 1 g/l available chlorine. A stronger solution, containing 5 g/l available chlorine, is recommended for dealing with biohazardous spillage and in the presence of large amounts of organic matter. Sodium hypochlorite solutions, as domestic bleach, contain 50 g/l available chlorine and should therefore be diluted 1:50 or 1:10 to obtain final concentrations of 1 g/l and 5 g/l, respectively (see Table 2). Industrial solutions of bleach have a sodium hypochlorite concentration of nearly 120 g/l and must be diluted accordingly to obtain the levels indicated above.

**Table 2.** Recommended dilutions of chlorine-releasing compounds.

Available chlorine required	Clean Conditions <sup>a</sup>	Dirty Conditions <sup>b</sup>
	0.1% (1 g/l)	0.5% (5 g/l)
Sodium hypochlorite solution (bleach) (not older than 12 months from date of manufacture) <sup>c</sup> (5% available chlorine)	20 ml/l (2% v/v)	100 ml/l (10% v/v)

<sup>a</sup> After removal of bulk material.

<sup>b</sup> For flooding (e.g. on blood or before removal of bulk material).

<sup>c</sup> Bleach is dated with the manufacture date in Julian date format (see below for an explanation of date codes for bleach).

Bleach is not recommended as an antiseptic but may be used as a general-purpose disinfectant and for soaking contaminated, metal-free materials.

Chlorine gas is highly toxic. Bleach must therefore be stored and used in well ventilated areas only. Also, bleach must never be mixed with acids to prevent the rapid release of chlorine gas. Many by-products of chlorine can be harmful to humans and the environment, so indiscriminate use of bleach should be avoided.

Bleach/liquid chlorine is dated with the manufacture date in Julian date format. Bleach has a *maximum shelf life of 12 months from the date of manufacture*. To determine the date of manufacture please see Table 3 and Appendix 3. A good practice is to dispose of unused bleach 6 months after purchase or check the date codes.

**Table 3.** Bleach/liquid chlorine date code decoder (Year/Day of Year). Manufacture year may be designated by a single digit code or a two-digit code.

Manufacturer	Example code	Manufacture year	Manufacture day	Expiry day (12 months from manufacture day)
Chlorox	A53183TX-1 08:41	2013	183 = July 2	Jul 2, 2014
Walmart Great Value	14183 11:03 B1	2014	183 = July 2	Jul 2, 2015
Walmart White Cloud	14144 12L59B2 Tx-01	2014	144 = May 24	May 24, 2015
Smart (Home Depot)	34713FL07:30	2013	347 = Dec 13	Dec 13, 2014
HTH Liquid Chlorinator	14JA0366B 11:15	2014	036 = Feb 5	Feb 5, 2015

***Formulated hydrogen peroxide and peracids***

Like chlorine, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and peracids are strong oxidants and can be potent broad-spectrum germicides. They are also safer than chlorine to humans, the environment and most surfaces.

Formulated hydrogen peroxide (also known as accelerated hydrogen peroxide or AHP) products have other ingredients that stabilize the hydrogen peroxide content, accelerate its germicidal action and make it less corrosive. Examples of these products include: Diversey Oxivir™; Virox Preempt™; Contec Accel TB™; Chlorox hydrogen peroxide cleaners.

Formulated hydrogen peroxide can be used for the decontamination of work surfaces of laboratory benches and biosafety cabinets, as well as soft surfaces. Stronger solutions may be suitable for disinfecting heat-sensitive medical/dental devices. The use of vaporized hydrogen peroxide or peracetic acid (CH<sub>3</sub>COOOH) for the decontamination of heat-sensitive medical/surgical devices requires specialized equipment and specially trained personnel.

***Formaldehyde***

Tissues that are preserved with formaldehyde containing solutions (e.g. formalin) are considered pathogen free. However, it should be noted that formaldehyde is a suspected carcinogen. It is a dangerous, irritant gas that has a pungent smell, and its fumes can irritate eyes and mucous membranes. It must therefore be stored and used in a fume-hood or well-ventilated area and should not be used for routine sterilization. National chemical safety regulations must be followed.

**Local environmental decontamination**

Decontamination of the laboratory space, its furniture and its equipment require a combination of liquid and gaseous disinfectants. Surfaces can be decontaminated using a solution of sodium hypochlorite (NaOCl); a solution containing 1 g/l available chlorine may be suitable for general environmental sanitation, but stronger solutions (5 g/l) are

recommended when dealing with high-risk situations. Formulated solutions containing 3 - 6% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) make suitable substitutes for bleach solutions for environmental decontamination.

After fumigation the area must be ventilated thoroughly before personnel are allowed to enter. Appropriate respirators must be worn by anyone entering the room before it has been ventilated. Gaseous ammonium bicarbonate can be used to neutralize the formaldehyde.

Fumigation of smaller spaces with hydrogen peroxide vapour is also effective but should only be done by specially trained personnel using specialized equipment to generate the vapour.

### **Handwashing/hand decontamination**

Suitable gloves should be worn when handling biohazardous materials. However, this does not replace the need for regular and proper handwashing by laboratory personnel. Hands must be washed after handling biohazardous materials and animals, and before leaving the laboratory.

In most situations, thorough washing of hands with ordinary soap and water is sufficient to decontaminate them, but the use of germicidal soaps is recommended in high-risk situations (spills on the body, etc.). Hands should be thoroughly lathered with soap, using friction, for at least 10 s, rinsed in clean water and dried using a clean paper or cloth towel (if available, warm-air hand-dryers may be used).

Foot- or elbow-operated faucets are recommended. Where not fitted, a paper towel should be used to turn off the faucet handles to avoid re-contaminating washed hands.

### **Waste Management**

Biohazardous waste can take the form of contaminated air escaping to the outside of an enclosure, liquid waste or solid waste, such as contaminated glassware, contaminated clothing and gloves. In order to reduce the likelihood of environmental contamination by biohazardous waste it is crucial that all biohazardous waste be treated prior to release or disposal. In Ontario, biohazardous waste is regulated under the Part V of the Environmental Protection Act, Regulation 347 and Guideline C-4: The Management of Biomedical Waste in Ontario, 2009. As such, the following guidelines for the containment and treatment of biohazardous wastes must be followed.

All biohazardous waste must be segregated from all other waste and handled in accordance with the containment, labeling and storage requirements in Table 4. In addition, the containers must conform to the minimum standards described below.

## **Biomedical Waste Containers (non-sharps waste)**

The following are the minimum standards for a single use and reusable biohazardous/biomedical waste container, other than for sharps waste (See Table 4).

### ***Single use biohazard waste container***

The container must be:

- a) an unlined rigid and leak proof plastic drum or pail, or;
- b) an outer cardboard container that can be sealed and is lined with a liner made of a leak proof plastic film that can be securely tied
- c) must be capable of withstanding the weight of the biohazardous waste without tearing, cracking, crushing, breaking or otherwise allowing the accidental release or discharge of the waste;
- d) must be colour coded and clearly marked as specified in Table 4.

### ***Reusable biohazardous waste container***

A container that is:

- a) fabricated of a puncture resistant and leak proof material that can be cleaned and disinfected prior to use;
- b) capable of withstanding the weight of the biohazardous waste without tearing cracking, crushing, breaking or otherwise allowing the accidental release or discharge of the waste;
- c) is visually inspected for tears, cracks or leaks every time it is emptied;
- d) must not be used for biohazardous waste destined to be incinerated;
- e) must not be used for sharps waste.

## **Biohazardous waste containers (sharps waste)**

Biohazardous sharps waste requires the use of a single use container that is made of puncture and leak resistant materials and have a lid which cannot be removed after the container is sealed. Reusable sharps waste containers are not to be used.

## **Treatment and Disposal of Biohazardous/Biomedical Waste**

Biohazardous waste, other than microbiological and other animal waste must be disposed of by a licenced disposal company. In Ontario, Stericycle Canada is the only waste disposal company that is licenced to remove, transport and process anatomical, blood, and sharps waste (Table 4). All other types of waste, can be collected, transported and processed as chemical waste (Photech Environmental Solutions Inc. is Nipissing University's current chemical waste disposal company) with the exception of microbiological waste, which is processed locally.

### ***Biohazardous/Biomedical Waste Storage and Disposal***

Biohazardous waste must be stored in a secure area, not accessible to the public and not adjacent to supply storage or areas used for food preparation and consumption.

Waste must not be allowed to accumulate but must be processed as soon as possible after generation. Where required, a refrigerator designated only for biohazardous waste, should be available for waste in accordance to Table 4. All waste storage areas, cabinets and refrigerators must be labelled with the universal biohazard symbol.

### ***Air***

The purpose of an air exhaust system is to remove contaminated air from a work area, conveying it through a decontaminating system if necessary and discharging it to the outside. In a level 2+ facility, it is essential that any aerosolizable level 2 or 2+ pathogen be handled in a certified class II A2 biological safety cabinet (BSC) (see section on biological safety cabinets for more information).

### ***Microbiological waste***

All solid waste including nutrient plates, plasticware, paper, clothing, or any other solid item that has come in contact with biohazardous material, must be placed into bags clearly marked with the biological hazard symbol. The waste will then be processed by autoclave treatment (following the protocols outlined in the autoclave operation section below) and then discarded in the regular garbage. Autoclave waste must be processed in a timely manner and must not be allowed to accumulate.

Used enterotubes should never be placed into the autoclave waste stream due to heat shrinkage during the autoclave cycle (see Figure 3). They should be disposed of as sharps waste and placed into a solid-sided sharps container.



**Figure 7.** Un-autoclaved enterotube (top) and autoclaved enterotube (bottom). The enterotube innoculating needle protrudes from the media holder after autoclaving resulting in a sharps hazard.

Liquid waste, depending on the culture and organism (see Table 1 above for appropriate disinfection methods), may be disposed of by adding bleach to the appropriate concentration followed by disposal down the sink. **Please note: the preferred method for disposing of microbiological waste is by autoclave treatment.**

Under no circumstances should materials that have come in contact with a disinfectant agent containing chlorine (e.g. bleach) or formaldehyde be processed in an autoclave. These substances will cause corrosion and pitting of the pressure chamber.

### **Autoclaving biohazardous waste**

All autoclaved waste must have at least one Biological Indicator (BI) test vial added to the waste bags prior to autoclaving. The test vials must indicate a killed spore count of  $1 \times 10^6$  upon processing. No autoclaved waste must leave the premises until the test vials are processed and indicate the required killed spore count. Once the BI test indicates all microbial organisms have been killed, the waste can then be disposed of as regular garbage.

Testing using a biological indicator (BI) must be undertaken at least every six days of operation or once every two weeks, whichever time is less. The testing regime should alternate between solid load verification and liquid load verification.

Please see 'A Guideline for the Safe Use of Autoclaves' for information on autoclaving biohazardous waste.



Table 4. Containment and labelling requirements for biohazardous waste, including sharps.

Waste Category (Disposal Method)	Containment		Container Label		Refrigeration Storage at or below 4°C	
	Single-use container	Reusable container	Label Colour	Label (see Appendix 1)	Waste to be refrigerated at all times	Waste to be refrigerated if held more than 4 days
Human anatomical waste (Stericycle)	X		Red	Anatomical Symbol	X	
Animal anatomical waste (Stericycle)	X		Red	Anatomical symbol	X	
Human or animal anatomical waste (fixed in formaldehyde or other preservative) (Photech) <sup>1</sup>	X		Red			
Human blood waste (Stericycle)	X		Yellow	Universal biohazard symbol		X
Animal blood waste (Stericycle)	X		Yellow	Universal biohazard symbol		X
Microbiology laboratory waste (On-site)	X	X <sup>2</sup>	Yellow	Universal biohazard symbol		X
Cytotoxic waste (not co-mingled with sharps waste) (Photech)	X		Red	Cytotoxic symbol		
Waste that has come in contact with human blood waste that is infected or suspected of being infected with any infectious substance (human) (Stericycle)	X		Red	Universal biohazard symbol	X	
Sharps waste (Stericycle)	X		Yellow	Universal biohazard symbol		
Cytotoxic sharps waste (Stericycle)	X		Red	Cytotoxic symbol		
Other animal waste (bedding, food, feces, etc.) (On-site)	X	X	N/A	N/A		

<sup>1</sup> Waste fixed in formaldehyde or other preservative should be decanted prior to disposal.

<sup>2</sup> Initial waste holding container only (e.g. bench-top waste bucket or large hinged garbage can)

## Accidental Release

A biohazardous spill occurs anytime there is an unplanned release of potentially infectious material into the environment. Proper response to these incidents can ensure personnel and community safety while eliminating environmental contamination. A biohazardous spill can range from something as innocuous as a simple nosebleed or someone vomiting, to a discarded hypodermic needle, to an unintentional release of a laboratory microbiological specimen. As such, a spill response which takes place within a laboratory setting will be different from one that takes place outside of the laboratory.

In order for a biohazardous spill response to be effective and safe for the campus community, affected work groups must:

- Refer the biological spill procedure for their work environment;
- Assure that spill clean-up materials are available for use; and
- Assure that all personnel are trained in the provisions of the spill response procedure.

Spills, accidents, exposures to infectious materials, and loss of containment must be reported immediately to the Laboratory Supervisor and an incident/injury report submitted to the BSO, within 24 hours. Written records of such incidents must be maintained, and the results of incident investigations should be used for continuing education.

## Risk Assessment/Spill Criteria - Laboratories

Infectious micro-organisms have traditionally been categorized into four different risk groups based on the relative hazards they pose. The factors used to categorize a particular organism are: pathogenicity, infectious dose, mode of transmission, host range, availability of effective preventative measures, and availability of effective treatment. These classifications assume that an organism will be grown in small volumes in a laboratory for research or diagnostic purposes. The four levels of risk identified by Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) are as follows:

- **Risk Group 1** (*low individual and community risk*) – any biological agent that is unlikely to cause disease in healthy workers or animals.
- **Risk Group 2** (*moderate individual risk, low community risk*) – any pathogen that can cause human disease but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventative measures are available, and the risk of spread is limited.

- **Risk Group 3** (*high individual risk, low community risk*) – any pathogen that usually causes serious human disease or can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that causes diseases treatable by antimicrobial or antiparasitic agents.
- **Risk Group 4** (*high individual risk, high community risk*) – any pathogen that usually produces very serious human disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice-versa, directly or indirectly, or by casual contact.

It is of utmost importance to know the agents you are working with. Suppliers and/or PSDS's can provide detailed information on the characteristics of the agent as well as effective containment and clean-up procedures. Section VIII of PHAC Pathogen Safety Data Sheets<sup>3</sup> addresses the specific spill requirements of each agent and should be consulted prior to any spill clean-up. When dealing with any biological spill, the degree of risk and subsequent spill response are dependent on the following:

- What organism was spilled? What are the physical characteristics and potential hazards of that particular organism? What risk group does it belong to?
- How much was spilled? What is the volume and concentration of the organism?
- Where was the spill? In the biological safety cabinet (BSC), in the lab, in a centrifuge, outside the lab?
- What is the potential for release to the environment? Were aerosols or droplets generated?

**Minor Biohazardous Spill** (Level 1 response) – is one that can be handled safely by laboratory personnel without the assistance of safety and emergency personnel. Minor spills include:

- The release of RG-1 organisms without splashing or agitation
- The release of a small volume of RG-2 organisms without splashing or agitation.

**Major Biohazardous Spill** (Level 2 response) – is one that may require outside assistance. These include:

- Any spill involving a biological agent that the individual does not feel confident in their ability to effectively mitigate.
- The release of RG1 or RG2 organisms resulting in excessive splashing and agitation (e.g., aerosolization).

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<sup>3</sup> PHAC pathogen safety data sheets can be found here: <http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php>

- The release of a large volume of RG-1 or RG2 organisms (I.e., enough present to seek its own level or run to a low point).
- Exposure by worker to potentially biohazardous agent via needle stick, cut, animal bite or scratch, via mucous membrane contact, or via non-intact skin contact.

***Biohazard Spill Response Kit:***

- A. Each facility or Department that uses, handles, or stores biohazardous materials will make a determination, with the assistance of the BSO, on the need and quantity of stocked biohazardous spill kits. It is the fiscal responsibility of each facility or Department to procure and maintain biohazardous spill kits.
- B. All potentially affected laboratory personnel, including employees, contractors, volunteers, students, and visitors must be properly trained in the proper use of these biohazardous spill clean-up kits.
- C. The kit should be maintained in a 5-gallon leak-proof bucket clearly indicating that it contains a biohazard spill kit and contain the following:
  1. Concentrated household bleach – check expiry date or stabilized accelerated hydrogen peroxide (AHP);
  2. Spray bottle for making 10% bleach solution;
  3. Forceps or tongs for handling sharps;
  4. Paper towel or other suitable absorbent;
  5. Biohazard bags of various sizes;
  6. Disposable gloves;
  7. Safety glasses;
  8. Laboratory coat;
  9. Spill signs (x2) to post at entrances to spill area.

**Biohazardous Spill Response - Laboratories**

In general, for all biohazardous spills, observe the following protocols:

- Notify others in the area of a spill immediately to limit potential of further contamination to additional personnel or the environment.
- Assess the situation and determine the classification of the spill – either minor or major based on risk assessment, agent and Pathogen Safety Data Sheet information.

- If a large spill or spill from height, evacuate the laboratory for at least 30 minutes to allow aerosols to settle before allowing entry to the laboratory for spill clean-up procedures.

***Minor Biohazardous Spills (Level I Response):***

A minor spill is one that poses minimal risk to personnel and can be easily and safely cleaned up. A spill that does not involve aerosolization, involves an RG1 pathogen, or a small spill (10 ml or less) of RG2 pathogen would be considered a minor spill. If, based on the outcome of the spill evaluation process, you believe that it is safe to clean-up a spilled biohazardous spill, follow these steps:

- Remove any contaminated clothing and lab coats. Wash exposed skin with antiseptic soap and water. Get the biohazard spill kit and review spill clean-up procedure before proceeding with clean-up.
- Remove spill supplies from kit and line bucket/container with biohazard bag. Retrieve a sharps container for disposal of sharps, if required.
- Don two pairs of disposable gloves and safety eye wear.
- If applicable, the supplied tongs are to be used to pick-up any contaminated sharp items (needles, broken glass, etc.) and place them in an approved sharps container for disposal.
- If a solid agar plate has spilled or dropped, do the following first:
  - Irrespective of orientation, determine if any of the agar has ruptured and dislodged from the force of the impact.
  - Mark the cast-off zone around the spill with paper towel.
  - Using tongs and/or dustpan, recover the plate, cover and agar and place all into a biohazardous waste bag (this will be autoclaved, so do not put any disinfectant into this bag).
- Cover the spill with paper towels and carefully pour decontamination solution around the spill (see recommendations on the PSDS for appropriate solution to use), allowing it to mix with the material. Spray decontamination solution directly on top of the absorbent material, ensuring that it is well soaked.
- Add a second layer of dry towelling. This will wick the disinfectant and microbes into the dry towelling.
- **Allow a contact time of 20 minutes.**
- Remove the absorbent material using the supplied tongs and deposit it into a biohazard bag.

- Remove residual disinfectant with paper towels. Dispose of paper towels into the biohazard bag.
- Repeat above steps for sufficient disinfection of contaminated surfaces as required.
- Close the bag and dispose of as treated biohazardous waste (place bag into a black bag and put into the regular waste stream). **Do not autoclave – treated waste is not considered biohazardous**
- Allow the surface to dry. Wipe up the bleach residue with water.
- Remove the outer pair of gloves and place them into a second biohazard bag (this bag will be autoclaved).
- With the inner gloves still on, remove the safety eyeglasses and laboratory coat and decontaminate by autoclaving.
- Remove the inner pair of gloves and place them in the biohazard bag.
- Close the bag and dispose of as biohazardous waste (autoclave).
- Wash your hands with soap and water. Dry your hands with clean paper towel.
- Determine what spill response materials have been used during the spill clean-up and arrange to have them replaced.
- Submit a fully completed “Injury/Incident Report” to the BSO as soon as possible.

***Spill Inside a Biosafety Cabinet (BSC) (Level I Response):***

**If a major spill occurs within the BSC (a spill that is not contained by the work surface) then this spill must be elevated to a Level II response.** If the spill is contained within the work surface, follow the directions below:

- Allow BSC to operate unattended for five (5) minutes to facilitate aerosol purification. BSC must run during cleanup to provide personnel and environmental protection.
- Call for assistance if needed. It is useful to have a second person with “clean” hands to get materials for clean-up.
- Put on appropriate personal protective equipment (eye protection, lab coat, two pairs of gloves).
- Cover spill inside the BSC with absorbent material (e.g. paper towels).
- Carefully soak the paper towels with an appropriate disinfectant, working from the outside of the spill to the inside.
  - i. The agent spilled must not be resistant to the disinfectant selected for cleanup.

- ii. If bleach is used as a decontaminant, be sure to wipe up any traces of the bleach after the appropriate contact time, followed by a thorough rinse of the area with 70% ethanol or water and wiping dry.

**Note: Bleach can cause discoloration and/or pitting of the stainless-steel surface providing a refuge for bacteria to live and grow.**

- If the catch basin below the work surface has become contaminated:
  - i. Close the drain valve;
  - ii. Flood the drain pan with disinfectant;
  - iii. Empty the drain pan into a container with disinfectant.
- Avoid generating aerosols.
- Allow a 20-minute disinfectant contact time.
- Remove paper towels with tongs or forceps.
- Remove broken glass or other sharps with tongs, or forceps.
- Place contaminated sharps in a puncture-resistant biohazard sharps container.
- Use paper towels to wipe up any residual treated material, then dispose of towels with infectious waste.
- Wipe down all surfaces or items inside the BSC once more with towels and disinfectant.
  - Place all contaminated disposable materials (no sharps) in a biohazard bag and dispose as treated contaminated waste. **Do not autoclave – treated waste is not considered biohazardous.**
- Place all contaminated re-usable items in biohazard bag, then sterilize by autoclaving.
- Remove gloves and other protective equipment.
- Thoroughly wash hands with soap and water.
- BSC must run for at least 10 minutes after cleanup before being used for experiments.
- Report the spill incident to your supervisor or Principal Investigator.
- Submit a fully completed “Injury/Incident Report” to the BSO within 24 hours.
- Contact the BSO if the spill involved a large volume or agent spread by aerosols; the BSO will provide guidance to determine if BSC requires formaldehyde decontamination.

***Spill Within a Centrifuge (Level I Response):***

Hazards associated with centrifuging include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users should be properly trained and operating instructions that include safety precautions should be prominently posted on the unit.

Infectious aerosols are created by practices such as filling centrifuge tubes, removing plugs or caps from tubes after centrifugation, removing supernatant, and resuspending sedimented pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation. To minimize the generation of aerosols when centrifuging biohazardous material, follow the procedures below:

- Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Fill and open centrifuge tubes, rotors and accessories in a BSC. Avoid overfilling of centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
- Always balance buckets, tubes and rotors properly before centrifugation.
- Do not decant or pour off supernatant. Use a vacuum system with appropriate in-line reservoirs and filters (See Appendix 2 for diagram of apparatus set-up).
- Work in a BSC when re-suspending pelleted material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes to permit the aerosol to settle before opening the tube.

At the end of a centrifuge run, wait five (5) minutes before opening the centrifuge. This will allow any aerosols to settle. If a tube breaks within the centrifuge bucket and the containment has not been breached, open the centrifuge bucket in a BSC and proceed to decontaminate the spill as outlined above.

If there is no containment of the spill or the containment has been breached into the centrifuge rotor cavity, carefully close the lid and allow the aerosols to settle for at least 30 minutes and follow the protocols outlined below:

- Remove any contaminated protective clothing and place in a biohazard bag. Wash hands and any exposed skin surfaces with soap and water.
- Don lab coat, two pairs of gloves, N95 respirator and eye protection prior to opening the centrifuge and then open carefully to assess the situation.
- Attempt to determine if the spill is contained in a closed cup, bucket or tray carrier, or within a closed rotor.



- If the spill is contained, spray the exterior of the carrier or rotor with disinfectant and allow adequate contact time. Take the carrier or rotor to the nearest BSC approved for use with this agent.
  - i. **NOTE:** If a BSC is not available or if the rotor cannot be removed, the centrifuge should remain closed.
  - ii. Post a sign indicating "**contaminated-do not use**". Notify the laboratory supervisor and the BSO for assistance.
- Obtain and place containers, suitable for holding tubes, broken glass or other containers while cleaning centrifuge components, into the BSC.
- Carefully retrieve unbroken tubes, wipe outside with disinfectant, and place them into the other empty container in the BSC, out of the way. The broken glass tube(s) must be removed with forceps or similar instrument and immersed in a beaker of disinfectant solution for a time appropriate to achieve disinfection. The pieces can then be disposed of in a sharps container.
- After proper decontamination, carriers, rotors etc. can be washed with a mild detergent according to the manufacturer's instructions.
- Thoroughly wipe the inside of the centrifuge chamber with disinfectant saturated towels. Allow for adequate contact time before wiping up excess liquid.
  - i. If using bleach, be sure to thoroughly rinse all surfaces with water to ensure that no surface corrosion will occur.
- Remove PPE as described above and wash hands with soap and water. Dry your hands with clean paper towel and then use an alcohol-based hand sanitizer.
- Submit a fully filled out incident/injury report and forward it to the BSO within 24 hours.

### ***Biohazardous Spill on Body (Level I Response)***

If a biohazardous substance is spilled on body, the following protocol should be followed:

- Immediately remove contaminated clothing and place in a suitable biohazard bag. All contaminated materials must be treated as biohazardous.
- Vigorously wash exposed areas with soap and water for at least 10 minutes. Alternatively, a hand sanitizer containing at least 65% isopropanol (rubbing alcohol) can be used.
- If eye exposure occurs, use eyewash per instructions (a minimum 15-minute flush time per eye).
- Obtain medical attention as soon as possible.

- Submit a fully filled out incident/injury report and forward it to the BSO within 24 hours (may require reporting to PHAC).

***Major Biohazardous Spill (Level II Response)***

- Review definition of a Major Biohazardous Spill (above).
- If a spill results from large quantities of any RG-1 biological agent (500 mL +) or RG-2 in any quantity larger than 10 ml or a spill with aerosolization, emergency procedures must be included as part of the Standard Operating Procedures for that agent. The SOP must be reviewed by the University Biosafety Committee prior to implementation.
- Employees should only attempt to clean up large or major spills if they have received Hazardous Materials Spills Response training and when appropriate spill clean-up materials and appropriate PPE are readily available and are properly utilized.
- Otherwise, in the event of a major spill for which personnel are not properly prepared, particularly if any person has been significantly exposed, contaminated, or injured to such an extent that medical or other outside assistance is needed, follow the following steps:
  - Evacuate the affected areas and secure these areas (e.g. close the doors).
  - Alert campus security by calling Ext. 5555 during daytime hours or (705) 498-7244 after hours or weekends. Do not attempt to call from within the affected area. Be sure to call from a safe distance from the contaminated site.
  - Remain close to the phone, if requested to do so, until contacted by emergency responders.
  - Stand-by to provide more information about the spill, including organism name, quantity, hazards and any other relevant information. Have a copy of the PSDS on hand if available. Assist emergency personnel upon arrival.
  - For any biohazardous spill that occurs outside the building, with potential for adversely affecting the environment, contact campus security. Campus security may initiate the Nipissing University Emergency Management Plan process by contacting the plan activation authority (PAA).
- Submit a fully filled out injury and incident reporting e-form and forward it to the BSO within 24 hours.

***Worker Exposure to Potentially Biohazardous Agent (Level II Response)***

The following emergency response procedures shall be followed when a worker has been potentially exposed to a biohazardous agent(s) via a needle stick, cut, animal bite or scratch, via mucous membrane contact, or via non-intact skin contact.

- 1) The exposed site must be washed immediately.

- i. In case of a needle stick, cut, animal bite or scratch, wash with soap and water after allowing the wound to bleed freely.
  - ii. If mucous membrane (eyes, nose, mouth) or non-intact skin (cuts, rash, eczema or dermatitis) contact, flush with water at the nearest faucet or eye wash station for a minimum of 15 minutes.
- 2) The worker must seek prompt medical attention from the nearest hospital emergency department or emergency clinic, or a Medical Practitioner of their choosing. Any information including the Pathogen Safety Data Sheet or equivalent for the biohazardous agent must also be taken to the care provider.
- 3) The worker must inform the Supervisor/Principal Investigator as well as the Bio Laboratory Safety Officer of the exposure incident as soon as possible.
- 4) The worker must provide information for an injury and incident reporting e-form describing the incident in detail, including the route of exposure and the emergency actions taken, and a description of the worker's duties as they relate to the exposure incident.
- 5) As soon as possible the Supervisor to submit fully completed injury and incident reporting e-form found here: <https://www.nipissingu.ca/departments/human-resources/health-safety/risk-management/injury-incident-reporting-and-investigation>.

## **Power Outage**

A power outage represents a potential for the release of biohazardous organisms if certain precautions are not taken either before a power outage or immediately following a power outage.

### ***Refrigerators and Incubators***

All fridges containing biological agents will be identified as top priority for backup power in the event of a power outage. These fridges will not be opened until power has been restored. If the biological agent is in an incubator, it will be left until power is restored.

### ***Biological safety cabinet power failure***

If the airflow in a biological safety cabinet changes abruptly or the power fails more than momentarily while an experiment is underway within the cabinet, there is a potential for a loss of containment and exposure of the worker to a biohazardous agent. As such, the following procedures must be followed immediately upon loss of power to the BSC:

- Stop work, secure and seal any biohazardous material, taking care not to create aerosols.
- Close the sash.
- The operator must notify the BSO and/or the Manager of Environmental Health and Safety and under no circumstances attempt to repair or dismantle the cabinet.

- If workers have been exposed to infectious material due to cabinet failure, the worker must seek appropriate first aid or medical treatment and then promptly notify the supervisor who will ensure an accident/incident report is completed and forwarded to the BSO as soon as possible.
- A respirator, half face with HEPA filters, must be available in Containment Level 2 or 2+ areas.

## Appendix 1

Symbols to be affixed to biohazardous waste containers<sup>4</sup>.

Universal Biohazard Symbol



Anatomical Symbol



Cytotoxic Symbol

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<sup>4</sup> Guideline C-4: The Management of Biomedical Waste in Ontario, November 2009.

## Appendix 2

Vacuum Aspirator Set-up to be used when removing supernatant from pelleted biohazardous sample. Be sure to place the appropriate disinfectant into the two trap flasks. This set-up can also be used to safeguard a vacuum set-up with multiple points of access.

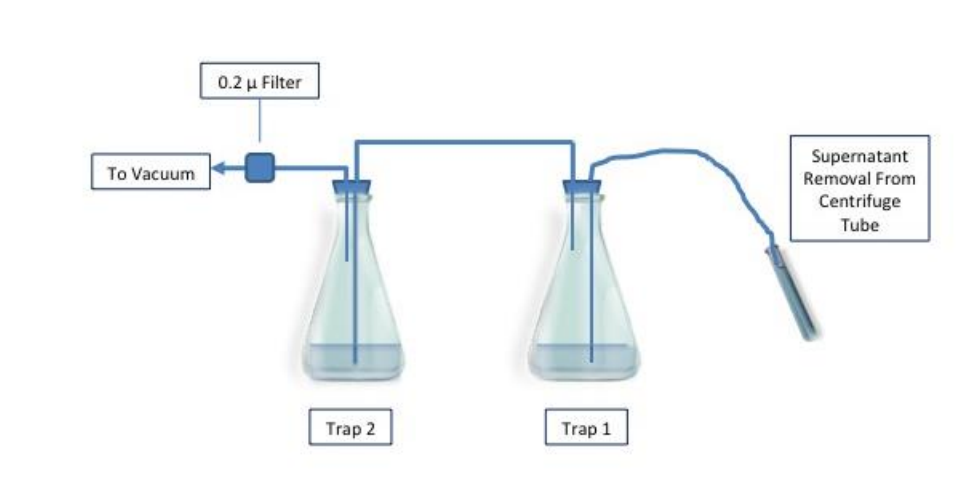


Figure 1. Vacuum aspirator set-up using regular Erlenmeyer flasks.

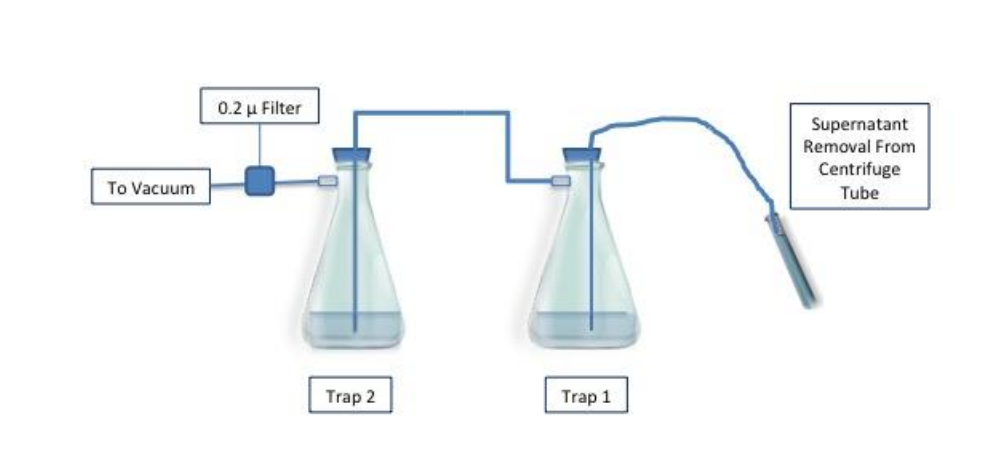


Figure 2. Vacuum aspirator set-up using side-arm flasks.

### Appendix 3. Julian Day Calendar

**Leap years:**

(2000, 2004, 2008, 2012, 2016, 2020...)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1	1	32	61	92	122	153	183	214	245	275	306	336
2	2	33	62	93	123	154	184	215	246	276	307	337
3	3	34	63	94	124	155	185	216	247	277	308	338
4	4	35	64	95	125	156	186	217	248	278	309	339
5	5	36	65	96	126	157	187	218	249	279	310	340
6	6	37	66	97	127	158	188	219	250	280	311	341
7	7	38	67	98	128	159	189	220	251	281	312	342
8	8	39	68	99	129	160	190	221	252	282	313	343
9	9	40	69	100	130	161	191	222	253	283	314	344
10	10	41	70	101	131	162	192	223	254	284	315	345
11	11	42	71	102	132	163	193	224	255	285	316	346
12	12	43	72	103	133	164	194	225	256	286	317	347
13	13	44	73	104	134	165	195	226	257	287	318	348
14	14	45	74	105	135	166	196	227	258	288	319	349
15	15	46	75	106	136	167	197	228	259	289	320	350
16	16	47	76	107	137	168	198	229	260	290	321	351
17	17	48	77	108	138	169	199	230	261	291	322	352
18	18	49	78	109	139	170	200	231	262	292	323	353
19	19	50	79	110	140	171	201	232	263	293	324	354
20	20	51	80	111	141	172	202	233	264	294	325	355
21	21	52	81	112	142	173	203	234	265	295	326	356
22	22	53	82	113	143	174	204	235	266	296	327	357
23	23	54	83	114	144	175	205	236	267	297	328	358
24	24	55	84	115	145	176	206	237	268	298	329	359
25	25	56	85	116	146	177	207	238	269	299	330	360
26	26	57	86	117	147	178	208	239	270	300	331	361
27	27	58	87	118	148	179	209	240	271	301	332	362
28	28	59	88	119	149	180	210	241	272	302	333	363
29	29	60	89	120	150	181	211	242	273	303	334	364
30	30		90	121	151	182	212	243	274	304	335	365
31	31		91		152		213	244		305		366

**Regular years:**

(2001-2003, 2005-2007, 2009-2011, 2013-2015...)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1	1	32	60	91	121	152	182	213	244	274	305	335
2	2	33	61	92	122	153	183	214	245	275	306	336
3	3	34	62	93	123	154	184	215	246	276	307	337
4	4	35	63	94	124	155	185	216	247	277	308	338
5	5	36	64	95	125	156	186	217	248	278	309	339
6	6	37	65	96	126	157	187	218	249	279	310	340
7	7	38	66	97	127	158	188	219	250	280	311	341
8	8	39	67	98	128	159	189	220	251	281	312	342
9	9	40	68	99	129	160	190	221	252	282	313	343
10	10	41	69	100	130	161	191	222	253	283	314	344
11	11	42	70	101	131	162	192	223	254	284	315	345
12	12	43	71	102	132	163	193	224	255	285	316	346
13	13	44	72	103	133	164	194	225	256	286	317	347
14	14	45	73	104	134	165	195	226	257	287	318	348
15	15	46	74	105	135	166	196	227	258	288	319	349
16	16	47	75	106	136	167	197	228	259	289	320	350
17	17	48	76	107	137	168	198	229	260	290	321	351
18	18	49	77	108	138	169	199	230	261	291	322	352
19	19	50	78	109	139	170	200	231	262	292	323	353
20	20	51	79	110	140	171	201	232	263	293	324	354
21	21	52	80	111	141	172	202	233	264	294	325	355
22	22	53	81	112	142	173	203	234	265	295	326	356
23	23	54	82	113	143	174	204	235	266	296	327	357
24	24	55	83	114	144	175	205	236	267	297	328	358
25	25	56	84	115	145	176	206	237	268	298	329	359
26	26	57	85	116	146	177	207	238	269	299	330	360
27	27	58	86	117	147	178	208	239	270	300	331	361
28	28	59	87	118	148	179	209	240	271	301	332	362
29	29		88	119	149	180	210	241	272	302	333	363
30	30		89	120	150	181	211	242	273	303	334	364
31	31		90		151		212	243		304		365

## Document Revision History

Date	Author	Revision
23 June 2011	D Vadnais	New Document
31 August 2011	D Vadnais	Minor revisions
October 2013	D Vadnais	Revisions to reflect regulatory changes required under the Canadian Biosafety Standards and Guidelines 1 <sup>st</sup> ed.
January 2016	D Vadnais	Revisions to reflect regulatory changes required under the Canadian Biosafety Standards 2 <sup>nd</sup> ed. and Human Pathogens and Toxins Regulations.
January 2016	D Vadnais	Added section on medical surveillance.
May 2017	D Vadnais	Updated the Nipissing University Safety Policy.
May 2017	D Vadnais	Revised and updated the Glossary
August 2017	D Vadnais	Revised sections dealing with Biosecurity
August 2017	D Vadnais	Revised section dealing with decontamination
August 2017	D Vadnais	Added Appendix 3 – Julian Day Calendar converter
August 2017	D Vadnais	Revised spill response procedure for RG2 pathogens
Nov 2017	D Vadnais	Added handwashing procedures
Nov 2017	D Vadnais	Minor revisions throughout document
April 2019	D Vadnais	Added section on enterotube waste
July 2019	D Vadnais	Added section on human and animal cell lines.
March 2022	A. Weeks, A. Stillar, C. Lalonde, K O'Reilly, S. Minnery & M. Banks	Updated Nipissing University Health and Safety Policy Updated Biosafety Committee Responsibilities Other minor revisions throughout document
July 2022	M. Banks	Minor revisions as a result of PHAC inspection deficiencies and recommendations
October 2022	A. Stillar	Changed job title of HR Generalist: Health Safety and Wellness to more generic Biological Safety Officer (BSO). Fixed Figure numbers.
June 2023	A. Stillar	Updated section on Biosafety Permit Approval Process to include annual renewals.
July 2024	A. Stillar & M. Banks	Revisions to reflect regulatory changes required under the Canadian Biosafety Standards 3 <sup>rd</sup> ed. (2022). Added Cybersecurity section. Other minor revisions.



