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| Trent University |
| Biosafety Program: Users Manual |
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| **TrentEmployee** |
| **8/1/2015** |

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Glossary:

ARG: Animal Pathogen Risk Group

BSC: Biosafety Cabinet

BSO: Biosafety Officer

CBS: Canadian Biosafety Standards 2015

CBG: Canadian Biosafety Guidelines 2015

CFIA: Canadian Food Inspection Agency

CL2: Containment Level 2 lab

CL3: Containment Level 3 lab

CSFHAAP: Containement Standards for Facilities Handling Aquatic Animal Pathogens

CSFHPP: Containment Standards for Facilities Handling Plant Pests

HAA: Health of Animals Act

HAR: Health of Animals Regulations

HPTA: Human Pathogens and Toxins Act

IBC: Institutional Biosafety Committee

LRA: Local Risk Assessment

NAAHP: National Aquatic Animal Health Program

P.I. : Principle Investigator

PHAC: Public Health Agency of Canada

pH: scale of acidity/alkalinity 0-14, 7 is neutral, 1 very acidic, 14 very alkaline

PRG: Plant Pathogen Risk Group

RG: Risk Group

RH: Relative Humidity

SB: Synthetic Biology

TDG: Transportation Dangerous Goods

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1. **Introduction**

The use of biological organisms which may be pathogenic or toxic to humans, animals, or plants is a common practice in scientific research, but can have unexpected consequences. Working with biohazardous materials or materials which may contain biohazardous microorganisms have resulted in over 3500 cases of laboratory acquired infections and 160 deaths prior to 1997 world wide (Wedum, 1997)[[1]](#footnote-1). Of the incidents that were examined, the causes of these incidents ranged from un-determinable to equipment failure, poor lab technique and human error. Several laboratory and non laboratory incidents with Biohazardous material or their toxins have emphasized for governments the need to ensure that safe work practices and security plans are in place when using or storing biohazardous agents.

In 2009, the Government of Canada, passed into law the Human Pathogens and Toxins Act. This legislation is intended to “establish a safety and security regime to protect the health and safety of the public against the risks posed by human pathogens and toxins”. In 2012 the public health agency of Canada introduced the Canadian Biosafety Standards and Guidelines which has attempted to integrate a number of separate policies and regulations between the Public Health Agency of Canada and the Canadian Food Inspection Agency as it relates to Human and Terrestrial Animal Pathogens.

There are several other pieces of legislation and regulations that regulate work with Biohazardous material which are to protect agricultural and aquacultural industries.

They are:

Human Pathogens and Toxins Act

Canadian Biosafety Standards

Canadian Biosafety Guidelines

Containment Standards for Facilities Handling of Aquatic Animal Pathogens

Containment Standards for Facilities Handling of Plant Pests

Health of Animals Act

Plant Protection Act

Fisheries Act

National Aquatic Animal Health Program

There are three primary standards and guidelines currently in use with respect to regulation of the use of Biohazardous material. They are the Canadian Biosafety Standards, 2nd ed. (referred to as the CBS), The Containment Standards for Facilities Handling Plant Pests, 1st ed. (CSFHPP), and the Containment Standards for Facilities Handling Aquatic Animal Pathogens, 1st ed. (CSFHAAP). These three documents outline the standards of practice and guidelines for the use all biohazardous material in Canada.

You should become familiar with the appropriate Standard for the work you wish to perform.

* Work with human pathogens and toxins and terrestrial animal pathogens (including birds and amphibians) are covered by the Canadian Biosafety Standards 2nd edition.
* Work with imported pathogens in fish and crustaceans must comply with the CFIA’s CFSHAAP.
* Work with pathogens that affect aquatic organisms and human or terrestrial animals must comply with both the CFSHAAP and the CBS.
* Work with imported plant pests are handled or stored must comply with the CFIA’S CSFHPP.
* Work with plant pests that is also capable of causing disease in humans or terrestrial animals must comply with both the CSFHPP and the CBS.

**2.0 Managing Work with Biohazardous Material**

How is Work with Biohardous Material Managed at Trent?

Currently, the management system for work with biohazardous materials at Trent is managed through a Work Permit system. Researchers and Principle Investigators apply to the Office of Research for a Biosafety Work Permit. They submit the application and relevant information. The Biosafety Officer and the Science Safety Advisory Committee (which acts as the institutional biosafety committee (IBC) , while working in co-operation with the applicant, assess the risk of exposure (known as an Local Risk Assessment) and then based on the assessed risk, assign a containment level and issue a Biosafety Work Permit.

**2.1 When Do You Need a Biosafety Work Permit?**

In order to ensure that the university is meeting its obligations under all of these regulations and legislation it has chosen to manage the process by issuing Biosafety Work Permits. Biosafety work permits are required for anyone using biohazardous material or material which may contain biohazardous microorganisms of Risk Group 1, 2 and 3 (humans and terrestrial animals), ARG 1, 2 and 3 (aquatic pathogens) and PPRG 1, 2 and 3 (plant pests and pathogens). Classification of pathogens according to Risk Groups is an internationally accepted practice in the biosafety field and is used to categorize the relative hazard associated with a particular pathogen.

**2.2 How to Apply for a Permit**

A Biosafety Permit application is available on line through the ROMEO research Portal and a guidance copy is available in Appendix 2 and on the Science Safety Program, Biosafety webpages. The application must be completed and submitted to the Research compliance officer who will then forward to the Biosafety officer. The principle investigator must acknowledge the submission through the ROMEO system.

It is important to note, that some work with Biohazardous agents (plant pest or aquatic pathogens) may require additional permits/regulatory approval, import permits for the material or other legal paperwork.

**2.3 Biosafety Work Permit Training Requirements**

To work with any material classified as biohazardous will require that all individuals who will actively work with material be formally trained as per the table below.

|  |  |
| --- | --- |
| Risk Group of Biohazardous Material | Biosafety Training Level |
| RG 1, ARG 1, PPRG 1 | 1. (on line) |
| RG 2, ARG 2, PPRG 2 | 2. (in class 2 hr) |
| RG 3, ARG 3, PPRG 3 | IPCL 3 specific training plus 1 year working in a CL 2 lab. |

The biosafety level 1 course will consist of an online course offered through the university’s online training program (at the time of this printing it is called Blackboard), participants will need to complete the online test and ascertain a minimum of 70% on the test. The biosafety level 2 course will be a 2 hour in class session that will build upon the material covered in the biosafety level 1 course and will be focussed on the requirements of working in a containment level 2 lab. Participants will complete the online test and will need to ascertain a minimum of 75 % on the test before working with the material. Training to work in the IPCL 3 will follow the SOP as written for the IPCL 3. In all cases the courses will conform to the Canadian Biosafety Guidelines.

**3.0 Risk Groups, Containment levels and Local Risk Assessments (see CBS Chapt. 2)**

**3.1 Risk Groups**

While not all Biological material is pathogenic, it is fair to say that all pathogenic material is biological. 90% of all the microorganisms (some would claim more) are harmless and indeed necessary to survive. The remaining 10% is considered pathogenic to one extent or another or in other words has a negative impact on living organisms .

In order to scientifically manage the risk(s) posed by working with microorganisms that are considered pathogenic a rating system known as Risk Groups was created.

Risk Groups define the pathogenicity, infectiousness, mode of transmission, host range and the availability of preventative measures or treatment, and its natural distribution. Essentially it is attempting to characterize the consequences and likelihood of exposure to infectious material. There are four categories of Risk Groups.

Risk Group 1.

* A microorganism, nucleic acid or protein that is a) not capable of causing human or animal disease or b) capable of causing disease but unlikely to so
* May pose a threat to immunocompromised individual
* Not particularly regulated but due care should be exercised.

Risk Group 2.

* A pathogen that poses a moderate risk to the health of individuals and/or animals and a low risk to public health, livestock or poultry.
* These pathogens are able to cause serious disease in a human or animal but are unlikely to do so.
* Effective treatment and preventative measures are available, and the risk of spread of diseases caused by these pathogens is low.

Risk Group 3.

* A pathogen that poses a high risk to the health of individuals and/or animals and a low risk to public health.
* These pathogens are likely to cause serious disease in a human or animal.
* Effective treatment and preventative measures are usually available and the risk of spread of disease caused by these pathogens is low for the public.
* The risk of spread to livestock or pourltry, however can range from low to high depending on the pathogen.

Risk Group 4.

* A pathogen that poses a high risk to the health of individuals and/or animals and a high risk to public health.
* These pathogens are likely to cause serious disease in a human or animal which can often lead to death.
* Effective treatment and preventative measures are not usually available and the risk of spread of disease caused by these pathogens is high for the public.
* The risk of spread of disease to livestock or poultry, however, ranges from low to high depending on the pathogen

(For pathogens other than human and terrestrial animals, please refer to the correct Containment Standards document for more accurate definitions of the Risk Groups which would be applicable to Aquatic organisms and/or Plant Pests.)

**3.2 Other Issues of Concern**

Toxins

Biological toxins are poisonous substances that are a natural product of the metabolic activities of certain microorganisms, plants and animal species. Toxins that are regulated by the HPTA can be found in Appendix 5 of the Human Pathogens and Toxins Act.

Tissue and Cell Cultures

Cell lines or cultures are commonly used in diagnostic, research and industrial microbiology labs. Many cell lines do not inherently pose a risk however they have the potential to contain pathogenic organisms such as bacteria, fungi, mycoplasmas, viruses, prions or recombinant virions. This can occur naturally or through contamination by adventitious organisms, transformation or recombination. There have been documented LAI’s associated with the manipulation of primary cell lines.

Prions

Prions are small proteinaceous infectious particles that are generally accepted to be the cause of a group of progressive neurodegenerative diseases in humans and animals known as transmissible spongiform encephalopathies (TSEs). These particles induce the normally folded prion protein to convert to the disease-associated, misfolding prion isoforms. The isoform acts as a template that guides the misfolding of more prion proteins which eventually leads to an accumulation of large amounts of the extremely stable, misfolded protein in infected tissue causing tissue loss and cell death. The most likely transmission route is through accidental inoculation or ingestion of infected tissues. There are no treatments and no vaccines available for disease caused by TSEs such as Chronic Wasting disease (CWD) or Creutzfeltd-Jakob disease (vCJD). Work with prions is generally performed in a CL3 with additional work procedures and specialized decontamination procedures.

Genetically Modified Organisms (GMO)

Different methods can be used to alter the genetic material of a biological species. A very efficient technique for this which is routinely used now to create GMOs is by the insertion or deletion of genes and gene segments. The best method known for creating GMOs is through the application of rDNA technologies.

Recombinant DNA (rDNA)

Genetic material from more than one source, either natural or synthetic, can be combined to construct novel rDNA. rDNA technologies are widely used in modern day research and have many applications, including the production of transgenic animals, the cloning of microbial toxin genes or other genes in expression vectors, and the production of full-length infectious viral clones.

Viral Vectors

Viral vectors are vehicles that are used to deliver genetic material in host cells for subsequent gene expression. Viral vector systems used for recombinant gene transfer are usually based on viruses present in the human population such as adenoviruses, herpesviruses and retroviruses. Genetic modifications are typically made to these vectors to improve gene delivery efficiency and to enhance the safety of the system. Retroviral vector systems derived from HIV 1, are competent gene transfer vehicles which are widely used for their stable integration into the chromosome of non-dividing cells and for their long-term transgene expression.

Synthetic DNA and Synthetic Biology

Synthetic biology is a field of research that focuses on the design and fabrication of novel biological components and systems as well as the redesign and fabrication of existing biological systems. SB makes use of rDNA or synthetic DNA (sDNA) to design or construct new biological parts, devices and systems.

All of the above systems, techniques or material pose biological pathogenic hazards and each should be evaluated on a project by project basis for their potential hazards and to determine the containment level, engineering and procedural controls.

**3.3 Containment Levels**

Containment Levels are a way of defining the engineering and operational practice requirements ,administrative controls, selected procedures and personal protective equipment (PPE) required for a specific project based upon a Local Risk Assessment (LRA) of the project. Each containment level has specific minimum engineering requirements, administrative controls, work procedures and PPE. More information on Containment Levels will be provided for in Section 11.

**3.4 Local Risk Assessments**

The LRA defines the potential hazards and controls required to work with a pathogen(s) or biological hazard, in a safe and contained way. An LRA usually includes the Risk group generally associated with the pathogen(s), whether it is in vitro, in vivo or in situ work, the risk of aerosol production, the infectiousness of the pathogen(s) and the availability of vaccines or treatment for a disease caused by the pathogen.

Not all research falls neatly into each category so the LRA will determine the precautions which will need to be taken. It is not necessary that the containment level match the risk group categorization.

Due to the different requirements for working with human and terrestrial pathogens, aquatic animal pathogens and plant pests it is not possible to summarize in a single document the requirements for a specific containment level lab. The descriptions of what is required for a specific containment level can be found in the appropriate guideline (CBS, CSFFHAAP, CSFFHPP). Every PI should be review the appropriate guideline under which their work is covered for the requirements for the lab. The federal departments responsible for these guidelines have made these guidelines available on line. The CBS can be found at http://canadianbiosafetystandards.collaboration.gc.ca/index-eng.php, the CSFFHAAP can be found at <http://www.inspection.gc.ca/animals/aquatic-animals/imports/pathogens/facilities/eng/1377962925061/1377963021283>, and the CSFFHHP can be found at <http://www.inspection.gc.ca/english/sci/bio/plaveg/placone.shtml> . PI’s should be familiar with the requirements listed for the containment level of the lab they are working in.

**4.0 Permit Process**

As alluded to above, Local Risk Assessments (LRA) are the tool used to determine what the appropriate containment level a specific project should be working at when working with biohazardous or potentially biohazardous material. The LRA looks at the various risk factors including the Risk group of the material being used, the procedures to be employed, the potential for aerosol production and a variety of other factors to ascertain the containment level conditions the work should be performed under.

Risk is a function of the probability of an event occurring and the severity of the consequences of that event. When LRA’s are performed they look at a variety of factors. The goal of an LRA is to determine the containment level at which, the risk of an event which may cause harm to a lab worker, general public or the environment is acceptably low. These are by their very nature rare events but the consequences can be severe. Factors which would be considered with a LRA include animal work, in vitro/in vivo/ in situ work, work procedures which produce significant levels of aerosols, the use of certain types of equipment such as mixers, centrifuges or sonicators, the volume of material used, experience of personnel involved, infectious dose, availability of vaccines. (see Appendix 3 for template of an LRA). In order to perform an LRA a project permit application with all the applicable information for an assessment is required.

**4.1 Permit Process:**

The university maintains a license with the Public Health Agency as part of the Human Pathogens and Toxins Act (HPTA) for the possession and use of pathogenic (to humans) materials. Under this license system, the university is responsible for issuing Biosafety Work Permits which authorize individual research groups to use specified pathogenic organisms. The agreement between the university and PHAC is that the university will manage the compliance of the use of biohazardous material with the appropriate legislation and regulations. In addition the university maintains an agreement with three major research funding organizations (known by the umbrella organization as the Tri-Council Federal funding organizations) that it will follow the established regulations and guidelines produced for work with pathogenic organisms. This means that the university agrees to ensure that all work with Biohazardous material will follow the CBS, CSFHAAP or the CSFHPP.

The Biosafety Work Permit (Appendix 5) is the method chosen by the university as managing this work to ensure that research and teaching with biohazardous materials meets these guidelines. Permits for work with pathogens will be assigned upon submission of the Biosafety Project Application and a review of the project via the local risk assessment. The Biosafety Work Permit application can be completed online through the ROMEO research portal. Once completed the application is reviewed by the Biosafety Officer and the IBC and assigned a containment level. Other conditions for the work may also be assigned to the work permit. A Biosafety work permit is required for all work with RG 1-3 agents.

**4.2 Biosecurity Plan**

Biosecurity refers to SOP’s and measures designed to prevent the loss, theft, misuse, diversion or intentional release of infectious material and toxins. A Biosecurity plan must be in place and is based on an overarching biosecurity risk assessment for the institution and the work performed. The Biosecurity plan includes, access control, inventory, personnel suitability and reliability, incident and emergency response and information security.

**4.2.1 Access Control**

Controlling the access to and limiting the access of personnel to areas deemed to have a higher biosecurity risk is one method of mitigating the risk of a biosecurity breach. Only those personnel authorized and who have been properly trained shall have access to biohazardous material. The university’s access control policy with respect to designated authority for authorizing access to spaces clearly spells out the role and responsibility of the principle investigator in assigning keys or issuing keypad codes to laboratory spaces. Building Access is the responsibility of Parking and Access Control and the Science Facilities Manager in conjunction with the principle investigator or departmental/program chair. Additional requirements for security screening (such as background checks and security clearances) for work with specific pathogens may be implemented should the biosecurity risk assessment indicate that there is significant increase in the potential harm which might be done with a specific agent. Security screening, if necessary, will be detailed on the Biosafety Permit as a condition of use of the material.

**4.2.2 Inventories**

Keeping an inventory of Biohazardous materials is an important tool in Biosecurity. The detail required for an inventory will vary depending on the Risk Group of the material. An inventory for RG 1 material is not required by the regulations, but best practice would suggest that keeping track of RG 1 material is good lab practice. For RG 2 agents used in a CL 2 lab, the inventory should be a perpetual inventory that is constantly updated as material is added to or taken from the stored supply. It should include the Balance Forward, the amount removed or added, the date of the addition or removal, the person using it and the final balance (This is very similar to the ethanol inventory). It is not necessary to know the inventory down to the # cells, but certainly knowing the number of vials / aliquots or total volume is useful. Only the material in storage must be on the inventory, material actively being used or cultured is not required on its own inventory until it is put into storage (addition to stock) or is removed from storage (used ).

There should be a separate inventory for each type of material.

An example of an inventory for one specific pathogen is below

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Balance Forward | Date | Material added | Material Used | Final Balance | User Signature |
| 10 vials (or volume) | Sept 1, 2012 | 1 vial |  | 11 vials | KK |
| 11 vials | Sept 13, 2012 |  | 2 vials | 9 vials | CW |
| 9 vials | Oct 1, 2012 | 3 vials |  | 12 vials | KK |
|  |  |  |  |  |  |

The use of an inventory allows for an accounting of the material so that the supervisor and the users can track its usage and determine if hazardous material is missing.

Inventories should be posted on the storage device (ultra low freezer for example) so that they can be easily updated and if necessary checked. A verification of the inventory should be performed regularly by lab personnel to ensure the accuracy of the inventory and to help determine if material is missing. The BSO will also inspect the inventory as part of the annual Biosafety Lab Inspection

**4.2.3 Personnel suitability and reliability**

The University needs to ensure that access to infectious materials is restricted to properly trained, competent and responsible personnel. The determination of the suitability and reliability of personnel who have access to biohazardous materials will be dealt with on a case by case basis based on the potential risk of working with high risk organisms otherwise known as security sensitive biological agents (SSBA), weaponization potential of the organism and the personality of the individuals involved. Depending on the circumstance this may require security checks and clearances of personnel as described above in 4.2.1.

**4.2.4 Incident and Emergency Response**

Principle investigators are responsible for ensuring that they have in place written emergency procedures should an unplanned incident occur in their labs. The Biosafety Manual contains general information and procedures which can easily be adapted for the type of material and work which occurs in most labs. Specialized emergency procedures must be identified, recorded and personnel trained in these procedures. Supervisors and principle investigators should record who has received the training. All incidents (safety or security related) must be reported to the BSO and Security.

**4.2.4.a Emergency Spill Response**

Spill response will vary depending on the nature of the spill, the size, the location and the material. Spills should be cleaned up immediately to ensure proper decontamination. If you must leave the scene, the door must be posted informing others of the spill, the hazards and not to enter the room. All spills must be reported to Security and the BSO. Spill cleanup should follow these general guidelines with the specifics being added to the SOP in the Lab.

Spills within Biological Safety Cabinets

* Leave the BSC on
* Cover the spill with absorbent material and flood the spill (from outside the perimeter of the spill to the inside in circular pattern) with the appropriate disinfectant at the appropriate concentration.
* Leave for 20 minutes
* Put on the appropriate PPE.
* Pick up absorbent material
* All items within the cabinet must be disinfected (wiped down or autoclaved)
* All waste must be autoclaved or decontaminated prior to disposal.
* BSC should run for 10-15 mins.
* Decontaminate the surfaces within the BSC.

Spill outside of BSCs, in a lab (large spills)

* Evacuate the area for 30 minus to allow aerosols to settle
* Assemble cleaning supplies and PPE
* Cover the spill with absorbent material and using the appropriate disinfectant at the appropriate concentration, pour disinfectant from outside the perimeter of the spill towards the middle of the spill in a circular pattern.
* Allow disinfectant to act for a minimum of 20 min. If spill is of blood or other heavy organic material, wipe up most of the material, reapply the chemical disinfectant and then allow disinfectant to act for an additional 20 mins.
* All adjacent areas should also be disinfected or wiped down
* All waste must be autoclaved or chemically decontaminated.

Spills occurring in Centrifuges

* Leave lid closed and allow aerosols to settle for at least one (1) hour
* Thoroughly wipe down inside of centrifuge, including the lid with paper towels soaked in disinfectant
* Disinfect the entire rotor, especially the bucket where spill occurred within the centrifuge
* Remove rotor from centrifuge and repeat disinfection.
* Wipe down both rotor and inside of centrifuge with water if bleach was used
* All waste should be autoclaved.

Spills occurring during transport (in public areas)

* Clean-up must be initiated immediately (as hallways are not negatively pressured)
* Follow directives for spills outside of biological safety cabinet.

**4.2.4.b Incident Reporting:**

Incidents or near miss incidents need to be reported to the lab supervisor and principle investigator and to the BSO and Security. An incident report should include the names of those involved, details on the incident or near miss, equipment and procedures involved in the incident or near miss, damage to equipment or facilities if it occurred, nature of injuries and treatment if injury occurred. The BSO or Risk management may choose (or depending on the severity of the incident) may require an investigation. In some cases, the Ministry of Labour may require an investigation as well. Once the incident is reported all parties involved should meet to discuss the incident, its causes and any changes which can be made to the program to mitigate any future reoccurrence of the incident.

A supervisor’s incident report form is included in Appendix 4.

Responding to spills and or accidents in a lab requires that everyone working in the lab know, understand and have access to the emergency response procedures appropriate to the lab and its operations. Principle investigators and lab supervisors should ensure that they have written procedures to deal with the lab specific emergency procedures for the biohazardous material they are working with.

**5.0 Procedures for Working with Biohazardous Material**

**5.1 Procedures for Receiving Biohazardous Material**

Personnel receiving Biohazardous Material need to be trained in Transportation of Dangerous Goods (TDG). The definition of receiving is “accepting and un-packaging the material”. Usually the easiest way to accomplish this is to ensure that at least one person in the lab is the “receiver” and has the training. That one person would open all packages of biohazardous material.

Receiving Personnel should take care when opening packages which contain biohazardous material. The appropriate personal protective equipment (PPE) should always be worn and if the material warrants it, the package should be opened in a biosafety cabinet. The outside of the packaging should be examined for any damage. Damage to the packing material would be indicative of rough handling and should be cause for concern. Personnel opening packages with biohazardous or infectious material should always be aware of the possibility that the container may not be intact.

Signs to watch out for:

1. Damaged outer packaging
2. Wet spots on the outer or inner packaging that would be inconsistent with the situation (if biohazard is shipped as a liquid)
3. Residue on inner packaging (if biohazard is shipped as a solid)

If any of these signs are detected, you should use extra caution when opening the package. The use of a biosafety cabinet when opening a package is highly recommended. If the primary container of the biohazardous material is damaged, then emergency spill clean- up procedures should be invoked, your supervisor and the BSO should be notified immediately.

**5.2 Procedures for Disposal of Biohazardous Material**

Biohazardous material cannot be disposed of without first decontaminating or sterilizing the material.

The following procedures are to be used for decontaminating/sterilizing biohazardous material and then disposing the material.

**5.2.1 Transporting Waste to the Autoclaves** (For labs that do not have an autoclave within the containment zone)

All of the autoclaves outside of individual labs are maintained by Science Facilities. They are tested regularly to ensure that they are operating properly and are trustworthy for the decontamination of material.

Autoclaves are available in ESB A 208, DNA A 108 , LHS C 261 and LHS D 205. The IPCL 3 has its own dedicated autoclave as does the Animal Care Facility.

Biohazardous waste should only be transported in a closed autoclave bag which is then placed in leak-proof, impact resistant, sealable container (like a large tote container). The container should be labelled with a Biohazard symbol and an indication that there may be biohazardous material contained within. The container (with the Autoclave bag and waste within) can then be transported to the Autoclave.

**5.2.2 Preparation for Autoclaving**:

Prior to transporting the biohazardous waste, reserve the use of the autoclave ahead of time in the autoclave log book so it is available for your use. The bag should be placed in the autoclave tray and the waste autoclaved immediately. Ensure that the bag is loosely packed with material and is no more than 2/3 full. This will allow steam to penetrate the contens and effectively sterilize the waste. Autoclave tape should be attached to the outside of the bag. If your permit requires it, insert a bioindicator into the center of the waste (carefully avoiding contact with yourself). Do not put the transport container into the autoclave as it will likely melt under the pressure and heat. Ensure that the volume of material being autoclaved does not exceed the capacity of the autoclave tray in the event the bag and contents fall over during autoclaving.

Only authorized personnel who have been trained in the use of the specified autoclave may operate that autoclave.

**5.2.3 Use of the Autoclave for decontamination**

**The procedures below are extracts from the Canadian Biosafety Standards and Guidelines, 1st ed., Chapter 16, p 270.**

Before loading the autoclave:

* Check inside the autoclave for any items left by the previous users that could pose a hazard.
* Clean the drain strainer
* Confirm that any plastic materials including bags, containers and trays are compatible with autoclaving. Some bags can impede steam penetration while others may melt during the cycle. Only use approved autoclave bags.
* Autoclave bags should be loosely open to allow steam penetration.
* Loosen the caps of liquid containers to prevent bottles from shattering during pressurization. This should be done immediately prior to loading in order to minimize the risk of exposure/contamination if the container is tipped. Vented caps may be a suitable alternative.

Loading the autoclave:

* Load autoclave as per manufacturer recommendations
* Avoid overloading containers and bags (never more than 2/3 full).
* Arrange containers, bags and trays in a manner that allows steam to circulate freely around all items. Avoid stacking or crowding containers, bags and/or trays.
* Place containers in autoclave tray with a solid bottom and walls, to contain spills.
* Never place individual containers on the floor of the autoclave.
* Make sure the door of the autoclave is fully closed (i.e., latched) and that the correct cycle has been selected.
* Start the autoclave.
* Decontaminate the transport waste container with the appropriate chemical decontaminant.

Unloading the autoclave and disposing the waste:

* Don PPE, including eye protection, heat resistant long-cuff gloves, and appropriate protective outer wear.
* Visually check the pressure gauge to ensure that the pressure has decreased inside the chamber.
* Verify the autoclave cycle log to ensure decontamination parameters have been achieved.
* If you used a bio-indicator, follow the appropriate procedure for verification of sterilization
* Once you have verified that the material has been sterilized, date and initial the bag and place in the regular waste container which should be near the autoclave.

Important notes on using an autoclave for decontamination purposes.

1. While information on pathogenic organisms may indicate minimum time to decontaminate, the users must realize the physics involved in an autoclave. In order to bring the interior of a bag of waste to the specified temperature and hold that temperature for the specified amount of time, it is necessary to remember that the heat and steam need to penetrate and the material needs to absorb the heat to come to temperature. So while a small amount of material may be able to reach the appropriate temperature quickly, larger amounts will take longer. It is unlikely that cycles of less than 30 minutes at the temperature and pressure specified will be sufficiently long to decontaminate and sterilize effectively except when using very small quantities of materials. The use of bio-indicators can take some of the guess work out of your methodology. Bio-indicators are used as objective indicators that a load has been properly processed. A variety of makes and types of bio-indicators are available and you should follow the manufacturers instruction on how to use them.
2. Always check the run log to ensure that the autoclave reached the appropriate temperature and pressure and held it for the minimum length of time necessary to ensure sterilization of the material.
3. Autoclaves are very hot. Burn and scald time for the temperatures usually found in autoclaves is less than a second. Wear your PPE and make sure you have the appropriate gloves for handling the material once it comes out of the autoclave.
4. Personnel who use the autoclaves are responsible for ensuring that whatever they autoclave will not cause damage to the autoclave. Autoclave users who autoclave material which cause damage to the autoclave will be held responsible for the cost of the repairs.
5. The transport container should always be chemically decontaminated following exposure to the waste (using the appropriate disinfectant.

**5.3 Chemical Decontamination of Materials Which Can’t be Autoclaved**

Chemical disinfectants are generally used for the decontamination:

* of surfaces and equipment that cannot be autoclaved,
* specimen/sample containers to be removed from the containment zone,
* spills of infectious materials,
* rooms and animal holding spaces.

Every biocontainment lab should have written, in-lab procedures for chemically disinfecting the materials and situations listed above. In particular all biocontainment labs must have a written Spills Response Procedure for each pathogen used in their lab.

The selection and use of chemical disinfectants appropriate for the specified pathogen is dependent on a variety of factors including the resistance of the infectious material, the nature of the object to be disinfected (i.e., hard surface, porous materials), organic load, temperature, relative humidity and pH.

There are several factors that affect the efficacy of chemical disinfectants.

* Organic Load: Tissue, blood, bedding, feces etc… these types of organic loadings protect micro-organisms and toxins from contact with disinfectants and can negate or reduce the effectiveness of disinfectants. Pre-cleaning of materials to be decontaminated, to remove organic material may need to be carried out to achieve proper disinfection. However, the pre-cleaning must be done in a manner to avoid exposure and ensure that all cleaning material are decontaminated before disposal. In some cases, pre-cleaning is neither practicable nor safe. Therefore, in longer contact times (see below) and higher concentrations of the disinfectant may be required for effective decontamination.
* Concentration: Generally speaking the higher the concentration, the faster the time to disinfection, for most disinfectants. High concentrations of certain chemicals may cause damage to the material being disinfected, but lowering the concentration may not result in effective disinfection. Careful consideration must be made as to the concentration of the disinfectant.

**Chemical Decontamination Methods for Non Halogen Chemical Germicides**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Type** | **Effective concentration; contact times** | **Advantages** | **Disadvantages** | **Examples of uses** |
| Alcohols  - | 70-85% ethanol  60-95%  isopropanol 3-30 minutes | Low toxicity Low residue Non corrosive  ---- | Rapid evaporation, reduces contact time Flammable, skin desiccant , Non- sporicidal, ineffective with unconventional agents | Skin disinfectant Surface decontamination Bench top, cabinet wipe down  - ~ |
| Phenolic compounds | 0.04-5%  1 0-30 minutes | Leaves an active residue Biodegradable | Pungent odour, corrosive , toxic Non sporicidal, limited activity against viruses | Disinfection of floors and other surfaces Antiseptic soaps ~ |
| Quaternary Ammonium compounds | 0.05 -1.5%  10-30minutes | Has combined detergent and germicidal activity Stable Working dilutions have low toxicity | Not sporicidal, limited against viruses, myeobaeteria Not readily biodegradable | Surface decontamination Equipment wipe down antiseptic |
| Hydrogen peroxide | 3-30% 1 -60 minutes New “formulated” 6% has additives | Rapid action no residue Low toxicity Environmentally safe 30% is sporicidal | Limited sporicidal activity Corrosive to some metals, irritant Concentration dependant | Surface decontamination Instruments and equipment |
| Peracetic acid | 0.001-0.3% 10-60 minutes gas phase 2-4% 5-120 minutes | Broad spectrum sporicidal at low temperatures Can tolerate organic load Rapid action | Pungent odour Corrosive to some metals Shelf life of dilution is less than 1 week irritant to skin and eyes | Instruments and equipment Gas phase sterilization of chambers for germ free animals |
| Aldehydes Gluteraldehyde | 0.5-2.5% aqueous 2-30 minutes (up to 12 hours to kill all spores) | Broad spectrum Does not corrode metal Can tolerate organic load | Expensive Requires good ventilation pH, temperature dependant Pungent odour Toxic Less than 2 week shelf life | Cold sterilant and fixative Surface decontamination Instruments, equipment, glassware |
| Aldehydes Formalin (37% formaldehyde) | 3-27% formalin in 70-90% water 10-30 minutes | Broad spectrum Inexpensive Does not corrode metal Can tolerate organic load | Pungent odour Irritant Potential carcinogen May require 24 hours to kill spores | Cold sterilant and fixative Surface decontamination Instruments and equipment  ~ |
| Aldehydes Formaldehyde gas (paraformaldehyde) | 1-3 hours | As for formalin  Effective penetration | As for formalin Toxic Flammable Poor penetration of covered surfaces | On site decontamination of biological safety cabinets, HEPA filters Enclosed area |
| Ethylene Oxide Gas | 50-200 mg/L  1-12 hours | Broad spectrum  No heat or moisture  Released  Penetrates packaging materials | Flammable, reactive  Toxic; potential carcinogen and mutagen. Some sterilized items may require 24 hours for out gassing  Requires specialized equipment and training | Heat or moisture sensitive supplies, equipment and instruments |

For a more comprehensive list check out the CBSG ,ed 1. chapter 16, pp. 265-269.

* Contact Time: The contact time is the period of time during which the treated surface remains saturated with the disinfectant. An effective contact time will depend on the disinfectant and the microorganisms present. Faster acting disinfectants are better as some effective contact times are difficult to achieve. For example, alcohols may have bactericidal activity after an extended contact time (e.g., 10 minutes) but they are unlikely to remain on surfaces this long because they evaporate and thus require multiple applications of the disinfectant.
* Temperature: Elevated temperatures generally enhance germicidal action, but may also accelerate evaporation hence reducing contact time. Conversely lower temperatures while increasing contact time, may lower the efficacy of the disinfectant.
* Relative Humidity: RH can influence the activity of some disinfectants, particularly formaldehyde. The nominal RH for formaldehyde fumigation is 70%.
* pH: The activity of some disinfectants may be affected by pH.
* Stability: Some concentrations of disinfectants may not be stable over long periods (NaOCl, alkaline glutaraldehyde) especially in the presence of heat or light. Be familiar with the disinfectant and its effective lifetime.

THE CBSG 1st ed. section 16.2.2 p 265-p269 Table 2 and 3, provides information on the characteristics of several chemical disinfectants including their effectiveness and the contact time required to achieve disinfection and some of the disadvantages of using them.

It is important that decontamination and disinfection techniques be verifiable in many cases. In other words, the selection of a particular method of decontamination must be supported verifiable data and/or references. In some cases it may be necessary to prove, through the use of bioindicators, that the decontamination methodology and disinfectant of choice are effective.

**5.4 Other Methods of Disposal of Biohazardous Waste:**

Disinfection through chemical decontamination or through the use of an autoclave may not always be practical or even possible. Material which cannot be decontaminated in any other way may be disposed of through a 3rd party who will arrange for its incineration. If you have material which cannot be chemically disinfected or by using an autoclave contact the BSO to arrange for this disposal.

**5.5 Sharps Containers and Disposal:**

The disposal of sharps such as scapels, syringes and needles, and other items that may cause cuts or lacerations should only be through the use of the yellow sharps containers. They are available from Science Facilities. They are a sealable, yellow container which may or may not contain a liquid decontaminant. Sharps are placed in the container when they are to be disposed. Once the container is full, the cap is applied, the container should be labelled “Full, for disposal” and the container placed near the regular lab waste container. Custodial staff will dispose of the container as per university procedures.

**6.0 Biosafety Cabinets (BSC)**.

Biological Safety Cabinets (BSCs) are the single most important safety device in the microbiology laboratory and second in overall importance only to safe work practices. There are three classes of BSCs. Class I cabinets have un-recirculated air flow away from the operator that is discharged through a HEPA filter. Class I cabinets provide good operator protections but do not protect the material within the cabinet (the product) from contamination. Class II cabinets have inward air flow for personnel protection, downward HEPA-filtered air for product protection and HEPA filtered exhaust air for environmental protection. They are divided into two types (A and B) based on construction, air flow velocities and patterns, and exhaust systems. Class III cabinets are totally enclosed and gas-tight with HEPA filtered supply and exhaust air. Work is performed with attached long-sleeved gloves. Class III cabinets also protect the worker and the product.

* NOTE: Horizontal Clean Benches or Laminar Flow Hoods which direct air towards the operator are not biological safety cabinets and must not be used for handling infectious, toxic or sensitizing agents.

All three classes of cabinets serve to minimize contact between the operator and the infectious agent by the use of directional airflows. All BSCs contain HEPA filters to ensure the exhaust from the cabinets is free of infectious material. BSCs should be located away from doors, drafts, convection currents, diffusers and high traffic areas. Selection of the proper class of BSC requires careful evaluation of the work involved. Class II (i.e., Type A and Type B) cabinets are designed for work involving microorganisms in CL 2, 3 and 4 facilities. Cabinet air from Type A cabinets may be recirculated back into the laboratory in level 2 and 3 facilities. Ducting a Type A cabinet out of the building is possible, providing the method of ducting uses “thimble” connections (i.e., small opening around the cabinet exhaust filter housing) and the balance of the cabinet exhaust system is not disturbed. The thimble must be removable or be designed to allow for proper certification of the cabinet (i.e., bubble tight damper to seal off the cabinet for decontamination, access port to allow scan testing of the HEPA filter).

Class II, Type B cabinets can be used when manipulating small quantities of chemicals as an adjunct to work with microorganisms in CL2, 3 and 4 labs. Type B1 cabinets recirculate 30% of the air within the cabinet and are suitable for work with minute amounts of chemicals. These cabinets must be “hard ducted” (i.e., direct connection), preferably to their own dedicated exhaust system. The exhaust canopy must allow for proper BSC certification. Type B2 cabinets are total-exhaust cabinets with no air recirculation within them and are suitable for work with small amounts of volatile chemicals. These cabinets are also hard-ducted.

Class III cabinets are designed for work with CL 4 level pathogens.

Only cabinets which meet the National Sanitation Foundation (NSF) Standard No. 49 Class *II Biohazard Cabinetry* (1992) and bear an NSF/ANSI 49 seal are to be purchased and installed in university labs.

BSCs must be tested and verified before they are used upon installation and after repairs or relocation. Testing must be performed by qualified individuals and it is recommended that only NSF accredited field certifiers be used and that it be done annually.

* 1. **Procedures for working in a BSC**
     1. **Before using the cabinet**

1. Don the appropriate PPE
2. Turn off the UV lamp; turn on the fluorescent lamp
3. Disinfect work surfaces with appropriate disinfectant
4. Place essential items inside cabinet
5. Allow the blower to run for 5 – 10 minutes before work.

**6.1.2 Working in the cabinet**

1. Keep all material in the cabinet at least 6” inside of the front grill. Work towards the back of the cabinet as much as is possible
2. Work in a smooth and fluid fashion
3. Organize your work in the cabinet in a “Clean to Dirty” fashion
4. Never cover the front or rear grills with material or impede air flows in any fashion.
5. Material should be discarded in a waste container located towards the rear of the cabinet.
6. Keep a spray bottle of the appropriate disinfectant in the BSC while working to avoid having to move your hands outside of cabinet.
7. Decontaminate the surface of all objects in the BSC should a spill occur.
8. Sustained open flames are prohibited from BSC’s as they can damage the HEPA filter. On demand flames are discouraged but allowed where other alternatives do not exist. Micro or ceramic furnaces or sterile disposable materials should be used where practicable.
9. Working in a BSC **does not** eliminate the need for wearing PPE appropriate for the work being done.

**6.1.3 After Completion of work**

1. Leave blower on at least 5 minutes to purge cabinet
2. Remove and decontaminate equipment and materials with the appropriate disinfectant
3. Disinfect the cabinet surfaces
4. Turn off blower and fluorescent lamp.

**6.1.4 Maintenance**

* Twice daily: Work surfaces wiped down
* Weekly: UV lamp lens, if used, should be wiped clean (dust decrease the intensity of the lamp)
* Monthly: All vertical surfaces wiped down with appropriate disinfectant.
* Annually: Recertification and full cabinet decontamination if necessary for recertification purposes

A word about UV Lights.

UV light at the appropriate wavelength and the proper intensity can be an effective surface sterilizer. However, UV lights are notorious for losing their energy (intensity) in short periods of time and dirt and dust which can collect on the lens can also cause a reduction in the energy received by the surface. Because tubes are not changed regularly, we cannot rely upon UV light in a BSC to be an effective decontaminant for the BSC surfaces. In addition, UV light, has its own hazards. While it is not prohibited, the use of UV lights as an effective decontaminant is not recommended and can not be substituted for other more effective methods of decontamination.

**7.0 Procedures for the Safe Use of Centrifuges**

* Check centrifuge tubes for stress lines prior to use
* Ensure you know how to operate the centrifuge (Read the instructions)
* Avoid overfilling
* Ensure Caps or stoppers are properly in place
* Use sealed buckets or rotors which can be loaded and unloaded in BSCs.
* Ensure all buckets are properly balanced
* Ensure centrifuge achieves run conditions before leaving
* Ensure centrifuge completely stopped before opening lid
* Ensure equipment interlocks working properly
* Check immediately for spills or leaks prior to removing samples
* Clean all spills promptly and completely

**8.0 Procedures for working with Needles and Syringes**

* Avoid the use of needles and syringes whenever possible
* Perform all operations involving biohazardous materials and needles within a BSC
* Fill syringes carefully, avoid frothing or introduction of bubbles
* Shield needles with disinfectant soaked cotton when withdrawing from stoppers
* Do not bend, shear or recap needles. Dispose of needles and syringes in biohazard sharps containers (see section 5.4)

**9.0 Procedures for Handwashing**

* One of the best defenses to prevent exposure
* In CL 1 lab, a non antiseptic soap can be used
* In CL 2 and 3 labs, antiseptic hand washing solutions are required. Most of the common antiseptic solutions contain chlorhexine gluconate or trichlosan. An alternative is to use alcoholic hand rubs. They contain an emollient to counteract the drying action of alcohol.
* Liquid dispensers should be used rather than bars.
* When to wash?
* Before starting any manipulations
* Before leaving the lab
* When hands are obviously soiled
* Before and after completing any task in a BSC
* Every time gloves are removed.
* Before contact with one’s face and hands
* At the end of the day

How to Handwash Properly

1. Turn on faucets and wet hands with tepid water
2. Dispense soap into a cupped hand
3. Spread soap or compound around both hands and between fingers.
4. Wash (lather hands for at least 10 sec. Vigorously rub both sided of hands starting a few inches above the wrist, extending downwards between the fingers and around and under the fingernails. Beware of the creases in your hands
5. Rinse thoroughly under the tepid running water, Rinsing should start above the wrist area and proceed to the tips of the fingers. Note if faucets are not knee or foot-operated do not turn of water yet.
6. Dry hands thorough with paper towels. If hand operated, turn faucets off once hands dry, using the paper towel to protect hands.

**10. Principles of Good Microbiologial Practices:**

The following is an extract from a document originally published by the American Biological Safety Association with some edits for Canadian application.

* Never mouth pipette. Avoid hand to mouth or hand to eye contact in the laboratory. Never eat, drink, apply cosmetics or lip balm, handle contact lenses or take medication in the laboratory.
* Use aseptic techniques. Hand washing is essential after removing gloves and other personnel protective equipment, after handling potentially infectious agents or materials and prior to exiting the laboratory.
* CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) and the CBS recommends that laboratory workers protect their street clothing from contamination by wearing appropriate garments (eg, gloves and shoe covers or lab shoes) when working in *Biosafety Level-2* (*CL-2*) laboratories. In *CL-3* laboratories the use of street clothing and street shoes is prohibited; a change of clothes and shoe covers or shoes dedicated for use in the lab is preferred.
* Needles and syringes or other sharp instruments should be restricted in laboratories where infectious agents are handled. If you must utilize sharps, consider using safety sharp devices or plastic rather than glassware. Never recap a used needle. Dispose of syringe-needle assemblies in properly labeled, puncture resistant, sharps containers.
* Handle infectious materials as determined by a risk assessment. Airborne transmissible infectious agents should be handled in a certified Biosafety Cabinet (BSC) appropriate to the biosafety level (BSL) and risks for that specific agent.
* Ensure engineering controls (e.g., BSC's, eyewash units, sinks, and safety showers) are functional and properly maintained and inspected.
* Never leave materials or contaminated labware open to the environment outside the BSC. Store all biohazardous materials securely in clearly labeled, sealed containers. Storage units, incubators, freezers or refrigerators should be labeled with the *Universal Biohazard* sign when they house infectious material.
* Doors of all laboratories handling infectious agents and materials must be posted with the Universal Biohazard symbol, a list of the infectious agent(s) in use, entry requirements (e.g., PPE) and emergency contact information.
* Avoid the use of aerosol-generating procedures when working with infectious materials. Needle clipping, pipetting mixing, sonication, and centrifugation can produce substantial aerosols. If you must perform an aerosol generating procedure, utilize proper containment devices and good work practice controls to mitigate potential exposures; Tightly cap tubes prior to centrifuging or vortexing; Allow aerosols to settle prior to opening tubes, equipment; Open tubes or equipment inside a containment device whenever feasible; Shield instruments or activities that can emit splash or splatter. Aerosol generating procedures shall be performed in a BSC.
* Use disinfectant traps and in-line filters on vacuum lines to protect vacuum lines from potential contamination.
* Follow your laboratory biosafety plan for the infectious materials you are working with and use the most suitable decontamination methods for decontaminating the infectious agents you use. Know the laboratory plan for managing an accidental spill of pathogenic materials. Always keep an appropriate spill kit available in the lab.
* Clean laboratory work surfaces with an approved disinfectant after working with infectious materials. The containment laboratory must not be cluttered in order to permit proper floor and work area disinfection.
* Never allow contaminated, infectious waste materials to leave the laboratory or to be put in the sanitary sewer without being decontaminated or sterilized. When autoclaving use adequate temperature (121 C), pressure (15 psi), and time, based on the size of the load. Also use a sterile indicator strip to verify sterilization. Arrange all materials being sterilized, so as not to restrict steam penetration.
* Report all accidents, occurrences and unexplained illnesses to your work supervisor and the Biosafety Officer. Understand the pathogenesis of the infectious agents you work with.

Think safety at all times during laboratory operations. Remember, if you do not understand the proper handling and safety procedures or how to use safety equipment properly, do not work with the infectious agents or materials until you get instruction. Seek the advice of the appropriate individuals. *Remember, following these principles of good microbiological practices will help protect you, your fellow worker and the public from the infectious agents you use.*

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| 11. OPERATIONAL PRACTICES in any Containment Lab. **11.1 GENERAL REQUIREMENTS**  The following general practices are required when working in any containment laboratory or animal facility:   * entry must be restricted to laboratory staff, animal handlers, maintenance staff and other persons on official business * only persons meeting specific entry requirements (e.g. immunization, serum screening) may enter containment laboratories unless the facility has been appropriately decontaminated * a health and medical surveillance program must be provided as recommended by Health Canada * personnel must receive training on the potential hazards associated with the work involved and the necessary precautions to prevent exposures to zoonotic agents and release of non-indigenous agents; personnel must show evidence that they understood the training provided; training must be documented and signed by both the employee and supervisor * a documented procedural manual must be written and followed * all persons (including visitors, maintenance staff, etc.) entering the containment area must be trained and know and follow the operational protocols for the project in process; trainees must be accompanied by a trained staff member * persons entering a containment facility must be well prepared and bring all materials they will need with them; if something has been forgotten, traffic patterns must still be adhered to (i.e. do not go back to get it; either phone for someone to bring it or exit via proper protocols) * employees working in the containment area must have general knowledge of the physical operation and design of the facility (e.g. air pressure gradients between zones, directional air flow patterns, alarm signals for air pressure failure, containment perimeter) * traffic flow patterns from clean to dirty areas must be established and adhered to (i.e. move from least to most contaminated areas) * smoke testing (i.e. with a smoke pencil) should be done periodically by lab staff to verify correct airflow * entry/exit protocols for persons, animals, equipment, samples, waste, etc. must be written, posted and followed; general protocols must be supplemented with protocols specific for each project in progress * emergency procedures for entry/exit, spill clean-up, air handling/biosafety cabinet failure, fire, animal escape and other emergencies must be written, posted and followed * in the event of life-threatening emergencies, personal health and safety are a priority; exit protocols must be established whereby routine procedures are bypassed; a reporting area must be identified where further steps must be taken (e.g. disinfecting footwear, changing, showering) prior to leaving * all spills, accidents, overt or potential exposures to infectious materials, and losses of containment (e.g. lab positive pressurization) must be reported immediately to the laboratory supervisor; written records of such incidents must be maintained * an effective rodent and insect control program must be maintained |

**11.2 Minimum Operational Practices Required for Containment Level 2 Laboratories**:

* laboratory personnel must be trained in and follow the safe use of laboratory equipment, biological safety cabinets, procedures to minimize the production of aerosols, decontamination and emergency response
* open wounds, cuts, scratches and grazes should be covered with waterproof dressings
* eating, chewing gum, drinking, smoking, storing food, and applying cosmetics are prohibited
* personal items such as purses and outdoor clothing should be kept separate from work areas
* the work area containing hazardous materials should be kept free from materials not pertinent to the work and that cannot be easily decontaminated (e.g. journals, books, correspondence); paperwork and report writing should be kept separate from such work areas
* laboratory reference material should be kept in the laboratory zone
* hands should be washed frequently (after handling infectious materials, after removing gloves, and before leaving the laboratory)
* open-toed and high-heeled shoes must not be worn in the laboratory
* long hair should be tied back so that it cannot come into contact with hands, specimens, containers, or equipment
* gloves (e.g. intact vinyl or latex) must be worn when handling infectious materials; metal mesh gloves can be worn underneath the latex or vinyl glove to provide protection from sharps and needles
* laboratory coats, gowns or coveralls must be worn when working in the laboratory; this clothing must not be worn in non-laboratory areas (e.g. offices, staff rooms, canteens, libraries)
* protective lab clothing should not be stored in the same locker as street clothing
* contaminated clothing must be decontaminated prior to laundering (unless laundering facilities are within the laboratory zone and have been proven to be effective in decontamination)
* eye and face protection must be worn when it is necessary to guard against splashing hazardous materials, flying particles, and harmful light or other rays
* laboratory doors must be kept closed as required by the facility design
* biological safety cabinets must be used for procedures with potential for producing infectious aerosols (e.g. with zoonotic agents) and with high concentrations or large volumes of zoonotic materials
* contaminated work surfaces must be decontaminated
* all contaminated materials must be decontaminated before disposal or cleaning for reuse
* contaminated equipment leaving the laboratory for servicing or disposal must be appropriately decontaminated
* efficacy monitoring of autoclaves using biological indicators must be done at least weekly, depending on the frequency of use of the autoclave, and records of the results kept on file; cycle log records (i.e. time, temperature and pressure) must also be kept on file

**11.3 Minimum Operational Practices for a Containment Level 3 Laboratories:**

In addition to the general operational practices listed for level 2, the following describes the minimum operational practices required at **containment level 3**:

* a protocol specific to the operation of the lab must be developed and read by personnel; employees must certify in writing that they have understood the material in the protocol
* the laboratory zone must be kept locked
* infectious agents should be stored inside the laboratory zone; agents stored outside the zone must be kept locked, in leak proof containers
* personnel must have demonstrated proficiency in microbiological practices and techniques (e.g. experience in handling infectious organisms or cell cultures)
* personal items such as purses and outdoor clothing must not be brought into the laboratory zone
* a containment check must be performed prior to entering the laboratory zone (i.e. verify negative lab pressurization as designed)
* water seals must be maintained in drainage traps (i.e. through regular sink/shower usage and/or by filling traps in areas that are not being used)
* laboratory samples and supplies may be carried into the laboratory zone or passed through a ventilated pass-box; where the barrier autoclave is used to pass materials into the laboratory, the autoclave must have been cycled prior to opening the outer "clean side" door
* personnel entering the laboratory zone must remove street clothing and jewellery, and change into dedicated laboratory clothing and shoes
* where full body protective clothing is not worn a shower is required on exit from the laboratory; where a known or suspected aerosol exposure has occurred (e.g. dropping infectious materials) a shower is required on exit from the laboratory zone
* a shower (including washing hair, beards) is required on exit from a laboratory zone handling non-indigenous animal pathogens; eye glasses must be disinfected at the containment barrier
* a second layer of protective clothing (i.e. solid-front gowns with tight-fitting wrists, gloves) should be worn over laboratory clothing when directly handling infectious materials (e.g. dedicated for use at the biological safety cabinet)
* contaminated clothing must be decontaminated prior to laundering (unless laundering facilities are within the laboratory zone and have been proven to be effective in decontamination of the microorganisms likely to be encountered)
* all activities with infectious materials are conducted in a biological safety cabinet; where this is not possible, other physical containment devices in combination with personal protective clothing and equipment must be used; no work with open vessels containing infectious materials is conducted on the open bench
* centrifugation of infectious materials must carried out in sealed safety cups or rotors that are loaded and unloaded in a biological safety cabinet
* all contaminated waste materials leaving the laboratory zone must be decontaminated through a double-door autoclave at the barrier before disposal; both doors of the autoclave must not be opened simultaneously
* heat sensitive materials that cannot be autoclaved out of the laboratory zone must be decontaminated at the containment barrier (e.g. fumigated with formaldehyde or vaporized hydrogen peroxide, disinfected using liquid chemicals, or other technology proven to be effective)

The IPCL 3 has a complete set of Standard Operating Procedures which must be reviewed by all lab personnel prior to working in the lab. Personnel who will work in the IPCL 3 must complete the specific Level 3 training sessions.

**11.4 ANIMAL FACILITIES**

Work with animals poses a variety of special hazards including exposure to zoometric agents (naturally occurring or experimentally infected), animal bites and scratches, kicks and crushing injuries, physical hazards (e.g. noise, temperature) and chemical hazards (e.g. cleaning agents, disinfectants). Allergic conditions can result from contact with animal fur or hair, bedding, and animal wastes. At least one-fifth of people who work with laboratory rodents, guinea pigs and rabbits develop allergies. Protection from allergens must be provided through engineering controls, ventilation, use of isolators and cages with filter tops and appropriate use of respiratory protection.

Animal handlers must have knowledge of the species' general characteristics such as behaviour, instincts and physical attributes. Consideration should also be given to their natural ecto- and endoparasites and the zoonotic diseases to which they are susceptible including their route of excretion and dissemination.

The following describes the minimum operational practices required in level 2 **small animal facilities (SA facilities):**

* coveralls and footwear must be worn when working in CL 2 SA facilities
* personnel entering CL 3 SA facilities must change into dedicated facility clothing and shoes
* gloves must be worn when handling infected animals
* hands must be washed after handling animals, after removing gloves and before leaving the facility
* HEPA-filtered respirators are required for handling animals in CL 2 SA facilities where infectious aerosols of zoonotic agents may be generated and cannot be contained within a primary containment device
* eye glasses must be disinfected at the containment barrier
* where a clothing change is not performed on exit from each animal room, disinfectant footbaths are required (the disinfectant must be effective against the microorganisms of concern and changed regularly in accordance with the active life of the disinfectant)
* contaminated clothing must be decontaminated prior to laundering (unless laundering facilities are within the laboratory zone and have been proven to be effective against the microorganisms likely to be encountered)
* each animal room must be labeled with unique hazards and entry requirements (e.g. respiratory protection)
* animal room doors must be kept closed as required by facility design
* water seals must be maintained in drainage traps (i.e. through regular sink/shower/floor drain usage and/or by filling traps in areas that are not being used)
* cages housing infected animals must be appropriately labeled
* careful handling procedures must be employed so as to minimize the creation of aerosols and dissemination of dust from cages, refuse and animals
* proper methods of restraint must be used to minimize scratches, bites and accidental self-inoculations
* all contaminated materials must be decontaminated before disposal or cleaning for reuse
* autoclaving is the preferred method of decontaminating cages prior to washing; temperature of the final rinse water in mechanical cage washers should be at least 82oC
* animal carcasses and tissues must be incinerated or processed through new technology proven to be effective (e.g. tissue autoclave); carcasses must be transported from the animal room for disposal in leak proof containers that are appropriately labeled.

**11.5 POST-MORTEM ROOMS**

Hazards in the post-mortem (PM) room are not limited to splashes and aerosols of infectious materials. Accidents can be caused by cutting instruments, sharp ends of cracked bones, slippery floors, electrical equipment, chemical fixatives and disinfectants.

General precautions:

* only authorized staff are allowed to use the necropsy facilities
* staff must be trained in the use of all equipment and tools (e.g. electric hoist/monorail, tools, PM table, incinerator)
* staff must be trained in proper disinfection and cleaning procedures
* the area must be kept neat and tidy; equipment, paper, reports, etc. should be stored securely and not be accumulated in the PM room to facilitate cleaning and decontamination; floors should be clear of obstructions
* specific protocols for each project must be developed and followed; these include entry/exit protocols (for people, animals, equipment and samples), protective clothing and equipment, disinfection and cleaning protocols, use of the incinerator and autoclaves, and emergency procedures

Preparation for necropsy:

* protective clothing appropriate to the AP containment level and potential hazards must be worn in the PM room; this should include the removal of street clothing and donning of protective clothing and footwear; HEPA-filtered respirators are required when the potential for infectious aerosols exist; waterproof aprons, gloves and eye/face protection (face shield, goggles) should also be worn; a safety helmet is required when operating an electrical hoist/monorail
* specific protocols must be developed for the movement of animals and carcasses into the PM room (e.g. hoist for large animals, cart for small livestock, secure containers for poultry and laboratory animals)

Necropsy Procedures:

* necropsy safety procedures specific to the species involved must be followed (i.e. use of cutting instruments to avoid injury)
* the animals (especially birds and small lab animals) should be wetted with water and/or disinfectant prior to necropsy
* skill-full technique is required to prevent excessive spread of contamination and the formation of aerosols originating from fluids and tissues (this is particularly important for work with zoonotic agents); every effort should be made to confine the spread of contamination; this is especially true when there is a likelihood of material being dropped from an elevated position

Cleaning and disposal procedures:

* upon completion of the post mortem, all necropsy tools and instruments must be decontaminated by autoclaving or disinfection (the disinfectant must be effective against the microorganisms of concern); as some disinfectants are inactivated in the presence of organic materials, gross contamination should be removed prior to disinfection
* disposable sharps, needles, blades, glass slides, etc. must be discarded into an appropriate sharps container for decontamination
* the necropsy table, floor and other contaminated work areas must be cleaned and disinfected at the end of an experiment using an appropriate procedure; preliminary washing using a general purpose disinfectant/detergent should be done; special care must be exercised when using a hose to wash the area (i.e. prevent the spread of contamination and formation of aerosols); decontamination of the PM room can then be achieved by spraying or fumigating with a disinfectant effective against the microorganisms of concern
* specimens (fresh, frozen or fixed) for further study should be placed in leak proof containers, appropriately labeled; the outside of the container must be cleaned and disinfected at end of necropsy or upon exit from the PM room; samples may only be opened in a laboratory zone of the same AP containment level
* all animal waste must be incinerated or processed through new technology proven to be effective (e.g. tissue autoclave); the incinerator or tissue autoclave should be located adjacent to the PM room
* where large specimens must be divided into smaller pieces and transported to an incinerator, pieces should be placed carefully into leak proof containers to avoid splashes and aerosols; the outsides of containers must be cleaned down and disinfected prior to transport out of the PM room; the containers must be labeled with the contents and the name and phone number of a contact person

Exit procedures:

* the requirement for showering out of the PM room is dependent on the microorganism of concern; a full shower out of the facility (including washing hair, beards and glasses) is mandatory when working with zoonotic level 3 and 4 agents, and non-indigenous agents
* contaminated protective clothing must be decontaminated prior to disposal or re-use; contaminated laundry is autoclaved prior to processing (unless using a pass-through laundry machines proven to be effective against the microorganism of concern)

**12.0 Personnel Protection**

The effective use of vaccines is often an overlooked form of protection against exposures. If vaccines exist for the pathogen being used, get immunized against it.

Although the preferred method of protection is by reducing exposure at the source, to protect against the failure of the primary containment, PPE can become an important line of defense.

In considering the use of PPE two criteria should be used: 1. degree of protection offered and 2. the ease of use. Once the PPE has been identified it is the responsibility of both the supervisor and the user to ensure that PPE is used and used properly.

Each type of containment level (1, 2, 3, and 4) requires specific protective laboratory clothing.

**12.1 Personal Protective Equipment**

12.1.1 Laboratory Coats, Gowns and Head Coverings

The lab coat has two uses: to protect street clothing from biological or chemicals spills, and to offer some additional body protection

* Level 2 lab: lab coat, gown or smock or uniform. It is recommended that they not be 100% cotton due to the lack of flame retardancy and poor resistance to a number of chemicals.
* Level 3: solid front or wrap around gowns. A 2 piece scrub suit is mandatory under the solid front gown.

Head coverings

Generally not required in most biological areas, unless a complete change of clothing is required for access, and where product protection is required.

* + 1. Shoes and shoe coverings

Any area where there is a significant risk of dropping heavy objects should require the use of industrial safety shoes. For general biological use, comfortable shoes such as tennis shoes or nurses shoes are recommended. Sandals are not allowed in laboratories using biohazards due to the potential exposure to infectious agents. A change from street shoes is strongly recommended for those working in level 3 facilities and for those working with infected animals in animal rooms. Alternately, shoe coverings may be used in level 2 and level 3 labs when a complete change of shoes and a dedicated pair of shoes are not required.

* + 1. Gloves

Gloves are the most widely used form of PPE. They are used for a wide variety of hazards including protection from heat, cold, solvents, caustics, toxins, infectious microorganisms, radioisotopes, cuts and animal bites and acids. Unfortunately, there is no ideal glove that will protect against all hazards. Gloves are made from a variety of materials including, rubber (latex), neoprene, neoprene-latex, nitrile, polyurethane, PVC etc... . Selection should depend on the hazard encountered. If biological work will include the use of chemical solvents such as toluene, benzene or carbon tetrachloride; rubber, neoprene, neoprene-latex, and PVC will be de-graded. Choose the glove appropriate to the task. If you are unsure, contact the vendor as to the correct choice of gloves for the application you intend.

In microbiological labs, surgical gloves of latex, rubber or vinyl are generally the preferred choice. (Latex gloves are slowly being removed from service due to the increase in allergic responses to latex). They offer a high level of dexterity and a higher level of sensitivity; unfortunately they offer very little if any protection against needle sticks, sharps and animal bites. Remember that gloves are the weakest component of the PPE. Gloves should overwrap the cuff and lower sleeve of the laboratory clothing. When handling infected tissues during necropsies, often stainless steel mesh gloves are worn to protect against accidental cuts.

Removing gloves: With your non dominant hand, grasp the glove material on the inside of the wrist below the edge of the glove on your dominant hand. Be careful not to touch your skin. Pull glove off (so that it is inside out). Using the just removed glove (the inside is clean), grasp the glove of the non - dominant hand as you did the first glove, just above the inside edge and pull off glove (inside out again). Ball both gloves together and dispose.

* + 1. Respiratory Protection

Two types of respiratory protection exist. Those which remove the hazardous particulates or those which supply clean air. A respirator such as a full face, half face cartridge respirator or N 95 type masks require fit testing to ensure a proper seal around the nose, mouth and face. These type of respirators would be worn in atmospheres that pose an infectious or toxic hazard such as an animal room where infectious agents could be excreted in urine.

The second type of respirator which supplies clean air includes hoods, helmets and full suits or self contained breathing apparatus. These are more bulky and ease of movement is often restricted.

Single use paper dust masks are not classified as true respirators. They do not offer adequate respiratory protection in infected animal rooms or other areas where infectious aerosols may be present, because one cannot be ensured of an adequate fit. They are acceptable for use during surgical procedures in order to maintain a sterile surgical field.

* + 1. Eye / Face Protection

Eye or facial protection is important because biological work may often use concentrated alkalis, and acids, concentrated disinfectants, including phenolics and quaternary ammonium compounds which can cause severe eye damage and blindness if splashed into the eye. Infection can also occur through the conjunctiva if certain pathogenic microorganisms are splattered into the eye. Full Face respirators or half face respirators plus splash goggles are often recommended when respirable aerosols or droplets may be produced.

Safety glasses are intended to provide impact protections, but should not be used to protect against splashes. For these hazards, safety goggles or face shields should be used. Ordinary glasses offer better splash protection than nothing at all, however, they do not replace the need for approved safety eyewear. The use of Contact Lenses in laboratories is discouraged at Trent as in the event of an accident where the lens needs to be removed (for flushing) the time required to remove the lens prior to flushing may result in additional injury. In addition some chemical vapours may be absorbed through the lens and trapped behind it.

**13. Working with Potential Biohazards outside of the Lab.**

Working in the natural environment can also create situations where there is exposure to potentially pathogenic agents. Avian influenza, West Nile disease, Hanta virus, Lyme’s disease and Rabies are just some of the more commonly reported diseases which result in infections just from being in the natural environment.

When assessing the risk of exposure to a potential pathogen in the field some questions to think about are:

* 1. What are the possible pathogens I may be exposed to.
  2. Is this animal/situation known to contain or harbour pathogens which may affect human health?
  3. Is the pathogen vector based (like West Nile and mosquitoes)?
  4. Could exposure result in severe illness?
  5. What is the vector or mode of transmission of the pathogen? (water-borne, blood-borne, zoonotic).
  6. Is a vaccine warranted or available?
  7. What is the level of interaction you will have (measuring, taking samples, dissections?)
  8. Are you at risk of getting bitten or scratched?

Ensuring that you have the proper procedures and personal protective equipment to mitigate the risk of exposure in the situations outlined above is important. Precautions similar to those used by primary care givers or first responders are probably sufficient for most situations (excluding bites and scratch risks). However, you should have the same vaccinations that they have as well (hepatitis A and B, tetanus**). It is strongly recommended that all personnel working with animals have an up-to-date tetanus vaccination.**

**14. Inspections and Audits**

Inspections or audits of the Biosafety Program in your lab are useful tools to measure the effectiveness of the controls, procedures and PPE used in your lab. An annual inspection will be done by the BSO for containment level 2 labs and above. Other inspections should be done periodically as a way a verifying that things are going the way they should and according to your Lab SOP’s. Visual inspections of the lab and furniture also allow you to identify repairs which might be needed to services, furniture or flooring. Audits are typically a much more involved process where the entire program is reviewed for its effectiveness. This would normally be done on a much less frequent basis and often only where lab incidents would suggest a breakdown in the safety program.

Appendix 1. Resources

* 1. Canadian Biosafety Guidelines 1st ed. 2013. Public Health Agency of Canada. Ottawa. www.canadianbiosafetystandards.collaboration.gc.ca
  2. Containment Standards for Facilities Handling Aquatic Animal Pathogens, 1st ed., Biohazard Containment and Safety Science Branch, Canadian Food Inspection Agency. 2010. Ottawa. <http://www.inspection.gc.ca/animals/aquatic-animals/imports/pathogens/facilities/eng/1377962925061/1377963021283>
  3. Containment Standards for Facilities Handling Plant Pests.1st ed. Biohazard Containment and Safety Science Branch, Canadian Food Inspection Agency. 2007. Ottawa. [www.inspection.gc.ca/english/sci/bio/plaveg/placone.shtml](http://www.inspection.gc.ca/english/sci/bio/plaveg/placone.shtml)
  4. Laboratory Safety and Biosecurity. [www.phac-aspc.gc.ca/lab-bio/index-eng.php](http://www.phac-aspc.gc.ca/lab-bio/index-eng.php)
  5. Human Pathogens and Toxins Act. [www.phac-aspc.gc.ca/lab-bio/regul/hpta-lapht-eng.php](http://www.phac-aspc.gc.ca/lab-bio/regul/hpta-lapht-eng.php)
  6. Human Pathogens and Toxins Regulations. [http://gazette.gc.ca/rp-pr/p2/2015/2015-03-11/html/sor-dors44-eng.php](.%20http:/gazette.gc.ca/rp-pr/p2/2015/2015-03-11/html/sor-dors44-eng.php)
  7. World Health Organization, Biosafety. [www.who.int/topics/biosafety/en/](http://www.who.int/topics/biosafety/en/)
  8. Canadian Biosafety Standards 2nd ed. 2015. Public Health Agency of Canada. Ottawa.

<http://canadianbiosafetystandards.collaboration.gc.ca/cbs-ncb/index-eng.php>

**Appendix 2. Biosafety Work Permit Application:**

The format of this application on the ROMEO research database will look different but the information required will generally be the same. However, the application on ROMEO will be the required submission method and information requested may change from time to time. Please log in to ROMEO to complete an application for a Biosafety Work Permit for work with Human, Animal and Plant or Soil Pathogens or material which may contain such pathogens.

Bio-Safety Permit Application Page 1 of 8



Office of the Dean Science

Office of the V.P. Research

**Bio-Safety Project Permit Application and Local Risk Assessment**

This protocol must be completed by each Principal Investigator holding a grant administered by Trent University or supervising a research project where the use of biohazardous infectious materials are described and used. This form must also be completed if animal work is proposed involving the use of biohazardous agents or animals carrying zoonotic agents infectious to humans or wildlife. Completed forms are to be sent to the Office of the V.P. Research for distribution to the Bio-Safety Committee. For questions regarding the completion of this form please contact the Bio- Safety Officer at ext 7061. Any changes to this form or to the projects described within must be completed and forwarded on to the Bio-Safety Committee for reassessment. Information on Bio-Safety at Trent can be accessed on the Web at [www.trentu.ca/sciencedean.](http://www.trentu.ca/sciencedean)

Project Administrative Details

|  |  |  |  |
| --- | --- | --- | --- |
|  | | | Project # (For internal use only) |
| **Project Title and Brief Description** | | | |
| **Principal Investigator** |  | | |
| **Department** |  | | |
| **Office Number** |  | | |
| **Phone Number** |  | | |
| **Email Address** |  | | |
| **Location of Experimental Work to**  **be carried out** | **Building** | | **Room** |
| For work being performed at affiliated institutions or away from Symons Campus please indicate full address. If the affiliated institution has a safety officer, their signature of approval will be required prior to review by the Bio-Safety Committee review. | | **Name and Address of Institution** | |

Bio-Safety Project Approval and Materials Registration – Page 2 of 6

|  |  |
| --- | --- |
| **Title of Grant(s) Associated with this Project**  Please attach a brief description of your work, such as the Research Grant Summary that explains the biohazards used as well as the hazard risk assessment. | |
| **Funding Agency/Agencies** | |
| **Names of all personnel working under the Principal Investigator in the location listed above:** | |
| **1.** | **4.** |
| **2.** | **5.** |
| **3.** | **6.** |

Biohazardous Materials

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | **1.0 Microorganisms** | | | | | | |
|  | | | **Does your work involve the use of microorganisms?** Yes No If not proceed to 2. | | | | | | |
| Name of Microorganism(s) | | Is the micro-organism a human pathogen. | | Is the micro-organism known to be a terrestrial animal pathogen? | Is the micro-organism an Aquatic Pathogen | Is the microorganism known to be a zoonotic agent? | Is the microorganism known to be a plant pest? |
| 1 |  | Yes No | | Yes No | Yes No | Yes No | Yes No |
| 2 |  | Yes No | | Yes No | Yes No | Yes No | Yes No |
| 3 |  | Yes No | | Yes No | Yes No | Yes No | Yes No |
| 4 |  | Yes No | | Yes No | Yes No | Yes No | Yes No |
| 5 |  | Yes No | | Yes No | Yes No | Yes No | Yes No |
| **For each microorganism listed above please indicate the original source of the material.**  1: 2: 3: 4: 5: | | | | | | | | |
| **For each microorganism please specify the Risk Group:**  1: 2: 3: 4: 5: | | | | | | | | |
| **For each microorganism describe the routes of transmission: (Inhalation, Ingestion, Absorption and/or Direct Contact)**  1: 2: 3: 4: 5: | | | | | | | | |
| **For each microorganisms describe the appropriate chemical decontaminant to be used in the event of a spill. (type and concentration)**  1: 2: 3: 4: 5: | | | | | | | | |

|  |  |  |
| --- | --- | --- |
| **2.0 Cell Culture** | | |
| **Does your work involve the use of cell cultures?** Yes No If no proceed to 3. | | |
| **Cell Type** | **Is this cell type used in work?** | **Established or Primary\*** (\*derived from fresh tissue) |
| Human | Yes No | Established Primary |
| Rodent | Yes No | Established Primary |
| Other | Yes No | Established Primary |
| **Supplier of primary cell culture tissue?** | | |
| **List specific cell lines.** | | |
| Please describe the work you will be doing with the cultured material(s). | | |

|  |  |  |
| --- | --- | --- |
| **3.0 Use of Human Source Materials** | | |
| **Does your work involve the use of human source materials?** Yes No If no proceed to 4. | | |
| **Indicate if the following will be used in the lab.** | | |
| Human blood (whole) or other bodily fluids? | Yes No | If Yes, please specify. |
| Human blood (fraction) or other bodily fluids? | Yes No | If Yes, please specify. |
| Human Organs (unpreserved)? | Yes No | If Yes, please specify. |
| Human tissues (unpreserved)? | Yes No | If Yes, please specify. |

|  |  |  |
| --- | --- | --- |
| **4.0 Genetically Modified Organisms and Cell Lines** | | |
| **Will genetic modifications be made to the organism or cell line?** Yes No If no please proceed to 5.0 | | |
| **Will genetic sequences from the following be involved?** | | |
| Genes from any CDC class 1 pathogens | Yes No | If Yes, please specify. |
| Other human or animal pathogen and/or their toxins | Yes No | If Yes, please specify. |
| Will intact genetic sequences be used from SV 40 Large T antigen? | Yes No | If Yes, please specify. |
| Will intact genetic sequences be used from known oncogenes? | Yes No | If Yes, please specify. |
| Will a live vector(s) (viral or bacterial) be used for gene transduction? | Yes No | If Yes, name vector. |
| Will virus be replication defective? | Yes No | |
| Will virus be infectious to humans or animals? | Yes No | |
| Will this be expected to increase the containment level required? | Yes No | |

|  |  |  |
| --- | --- | --- |
| **5.0 Animal Experiments** | | |
| Will any of the agents listed be used in live animals? | Yes | No |
| Name of animal species to be used: |  | ACC Protocol Number |
| If using murine cell lines, have they been tested for murine pathogens? | Yes | No |

Bio-Safety Project Approval and Materials Registration – Page 5 of 6

|  |  |
| --- | --- |
| **6.0 Use of Animal Species with Zoonotic Hazards** | |
| Will any animals or their organs, tissues, lavagges or other bodily fluids including blood be used? | Yes No |

|  |  |  |
| --- | --- | --- |
| **7.0 Biological Toxins** | | |
| **Will toxins of microbiological origin be used?**  If no please proceed to 8.0 | Yes | No |
| If yes, please name the toxin: |  | |
| What is the LD 50 of the toxin? |  | |

|  |  |  |
| --- | --- | --- |
| **8.0 Import Requirements** | | |
| **Will the agent be imported?** Yes No If no please proceed to 9.0 | | |
| Has an Import permit been obtained from CFIA for Aquatic Pathogens or Plant Pests? | Yes | No |
|  |  |  |
| Has a copy of the permit been sent to the BSO? | Yes | No |
| **9.0 Description of Experimental Procedure** | | |
| Please describe your experimental procedures which are directly related to your use of pathogens or material which may contain pathogens. Specifically outline those procedures which may produce aerosols or may be have a risk of spreading contamination. | | |

|  |  |
| --- | --- |
| **10.0 Training Requirements for Personnel named on Form** | |
| All personnel named on the above form who will be using any of the above named agents are required to complete/attend the following training courses on the Science Safety Program or by the BSO.   * Science Safety Program Core Principles * Laboratory Safety Orientation * Bio-Safety * WHMIS   As the principal investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1-8 have been trained. | |
| Signature | Date |

Bio-Safety Project Approval and Materials Registration

Local Risk Assessment to be completed by PI

|  |
| --- |
| 11. Local Risk Assessment  **Hazardous Characteristics of Laboratory Procedures (Check any that will be used with biohazardous agents)** |
| Working with Animals (potential for bites/scratches) |
| Sharps Use, Needles |
| Glass |
| Pipetting |
| Mixing |
| Pouring infectious materials |
| Lyophilizing |
| Cell sorting |
| Blenders |
| Centrifuge |
| Sonicator |
| Vortex |
| Grinding |
| Vigorous Shaking |
| Homogenizing |
| Flaming inoculating loops |
| Large volume of biohazardous material in use, greater than 1 L |
| Toxin production |
| Cryogenic techniques |
| Collection of Environmental Samples |
| Culturing Environmental Samples |
| Collection of Human tissues, bodily fluids |
| Manipulation of Human tissues, bodily fluids |
| Opening containers of infectious materials whose internal pressures may be different from ambient (e.g. heated samples) |
| Biohazardous materials in powdery form |
| Transport biohazardous materials outside of the lab |
| Ship/Receive/Transport biohazardous materials outside of the lab building |

**Hazards Associated with Work Practices, Safety Equipment and Facility Safeguards**

PPE in Use

Gloves When?

Labcoats When?

Safety Glasses When?

Face Shields When?

Spill Kit Available, contents?

Biosafety cabinet available?

Centrifuge Safety Cups

Sealed Centrifuge Rotors

Medical Surveillance suggested

**Emergency Procedures**

In Writing

Available in Lab

|  |  |  |  |
| --- | --- | --- | --- |
| **Principal Investigator Commitment** | | | |
| I, agree to conduct my research in accordance with all of the regulations and guidelines (Federal, Provincial and Institutional) which this project may fall under. I agree that all of the information above is accurate to the best of my knowledge and I agree not to change the project, with the exception of improving safety, without notifying and getting the approval of the Bio-Safety Committee first. I agree to work within the confines of the Work Permit and the conditions outlined in the Work Permit. | | |
| Signature of Principal Researcher |  | Date |

**Appendix 3. Local Risk Assessment Summary (to be completed by BSO based on the information provided in Biosafet Work Permit Application)**

Name Of PI:

Room or Facility work is to be done within:

ROMEO Project #:



Section 1 - Risk Assessment:

1. Risk group of agent(s): 1  2  3
2. Is a vaccine available? Y  N  Not Required  Not Applicable
3. Is a standard treatment available? Y  N
4. Is there a splash potential? Y  N
5. Does the procedure generate aerosols? Y  N
6. Does the procedure involve high concentration/volume? Y  N
7. What is the route of transmission? Aerosol(inhalation)  droplets/ingestion  Direct contact
8. Number of organisms for infection: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_
9. Known laboratory acquired infections? Y  N
10. Animals subjected to biohazards? Y  N
11. Non-standard manipulations Y  N



Section 2 – Controls in Place:

1. Facility meets or exceeds CL applied for: Y  N
2. BSC available for aerosol generating procedures? Y  N
3. Procedures listed on BRAf are appropriate to control exposure? Y  N
4. PPE in use is adequate to prevent transmission of agent? Y  N
5. Facility users are trained and up to date? Y  N
6. Self-monitoring for LAI sufficient? Y  N
7. Medical surveillance recommended or required? Y  N



Section 3 – Recommendations/Restrictions:

BSO/Committee has recommendations/restrictions (to be listed on permit): Y  N  (list)

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_



Date BSC Review: ­­­­­­­­­­­­­­­­­­­­­­­­­­­\_\_\_\_\_\_\_\_\_\_\_\_\_ BSO’s Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Chair’s Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

yy/mm/dd

**Appendix 4. Biohazardous Material Work Permit:**

Biosafety Permit

|  |  |  |
| --- | --- | --- |
| Biosafety Permit # | Research Project # | Name of Principle Investigator |
|  |  |  |

Contact Information

|  |  |  |  |
| --- | --- | --- | --- |
| Office # | Phone # | Email address | Location of work to be performed |
|  |  |  |  |

Project Information

|  |
| --- |
| Title of Project |
|  |

|  |
| --- |
| Brief Description of Project |
|  |

|  |  |  |  |
| --- | --- | --- | --- |
| Pathogen or Hazard | Risk Group of Pathogen or Hazard | Human or Animal | Source (Purchased or Sampled) |
|  |  |  |  |

Conditions

|  |
| --- |
|  |
|  |

Approvals

|  |  |  |
| --- | --- | --- |
| Biosafety Officer | Biosafety Committee | External (if required) |
|  |  |  |

Note: The Principle Investigator agrees that this project can only proceed provided that the conditions listed above are followed. Any deviation from the Conditions will render this permit Void.

**Appendix 5. Supervisors Incident Report Form**

# Trent University

**Supervisor’s Incident Investigation Report Form**

(The Regulations for Industrial Establishments under the Occupational Health and Safety Act of Ontario,

Section5, Notice of Accidents, requires that additional information must be reported LQ DGGLWLRQ to that provided on the WSIB form. Supervisors are required to conduct a thorough investigation of incidents resulting in injuries or illness.

This form is to be completed by the supervisor in addition to the WSIB Form 7 (should one be required). This form must be sent to the Environmental Health and Safety 2IILFHU within 2 days of the incident.)

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| --- | --- | --- | --- | --- | --- |
| **Name:** | | | | | **Date of Incident:** |
| **Job Title:** | | | | | **Location of Incident:** |
| **Department:** | | | | | **Supervisor:** |
| **Phone:** | | | | | **Phone:** |
| **WSIB Form 7 Completed:** |  | **Y** |  | **N** |  |

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| --- | --- | --- | --- | --- |
| **Briefly describe the events leading to the incident or injury, what was being done at the time, describe the injury and what actually happened, and include a description of any equipment or machinery involved. Attach an additional page if necessary.** | | | | |
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| **Check one or more factors that may have contributed to the incident/injury:** | | | | |
| **Task Related:** |  | Hazardous procedure used |  | Inadequate Personal Protective Equipment |
|  | | | | |
|  |  | Improper position or posture |  | Incorrect, defective or unavailable tools |
|  | | | | |
| **Material/Equip:** |  | Inadequate guarding |  | Inadequate labeling |
|  | | | | |
|  |  | Unsafe design or construction |  | Inadequate lockout/tagout |
|  | | | | |
| **Environment:** |  | Poor weather conditions |  | Inadequate lighting/ventilation |
|  | | | | |
|  |  | Poor housekeeping |  | Poor workstation layout |
|  | | | | |
| **Personal:** |  | Inexperience of person |  | Lack of training |
|  | | | | |
|  |  | Unusual stress |  | Operating without authority |
|  | | | | |
| **Organization:** |  | Inadequate maintenance |  | Lack of safety procedures |
|  | | | | |
|  |  | Lack of safety inspection |  | Inadequate supervision |
|  | | | | |
| **Other:** (explain) | | | | |
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| **Briefly describe the action taken to investigate the incident and the steps taken to prevent a recurrence.** | | | |
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| **Check one or more actions taken or planned to prevent a recurrence:** | | | |
|  | To repair or replace tool or equipment |  | To improve personal protective equipment |
|  | To install guard or safety device |  | To provide, update, or develop training |
|  | To revise or develop a procedure |  | To provide closer supervision |
|  | To improve preventative maintenance program |  | To request an ergonomic assessment |
|  | To improve or increase inspections |  | To improve housekeeping |
|  | To contact supervisor for assistance |  | To contact EHS for assistance |
|  | To contact supplier/manufacturer for assistance |  | To contact Physical Resources for assistance |
|  |
|  | To contact HR for assistance (discipline) | Other (explain) |
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**Probable Recurrence Rate**

Frequent Occasional Rare

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| --- |
| **Investigated by Supervisor Reviewed by Department Head (name and signature) (name and signature)** |
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|  |
| **Date: Date:** |

Send the form within 48 hours of the incident to: Environmental Health and Safety Officer

Risk Management Office Trent University, Blackburn Hall rm108 Fax: 705-748-1009

Jan. 2011

1. Wedum, Arnold G. 1997. History and Epidemiology of Laboratory-Acquired Infections (In Relation to the Cancer Research Program). JABSA, 2(1) pp. 12-29. ABSA. [↑](#footnote-ref-1)