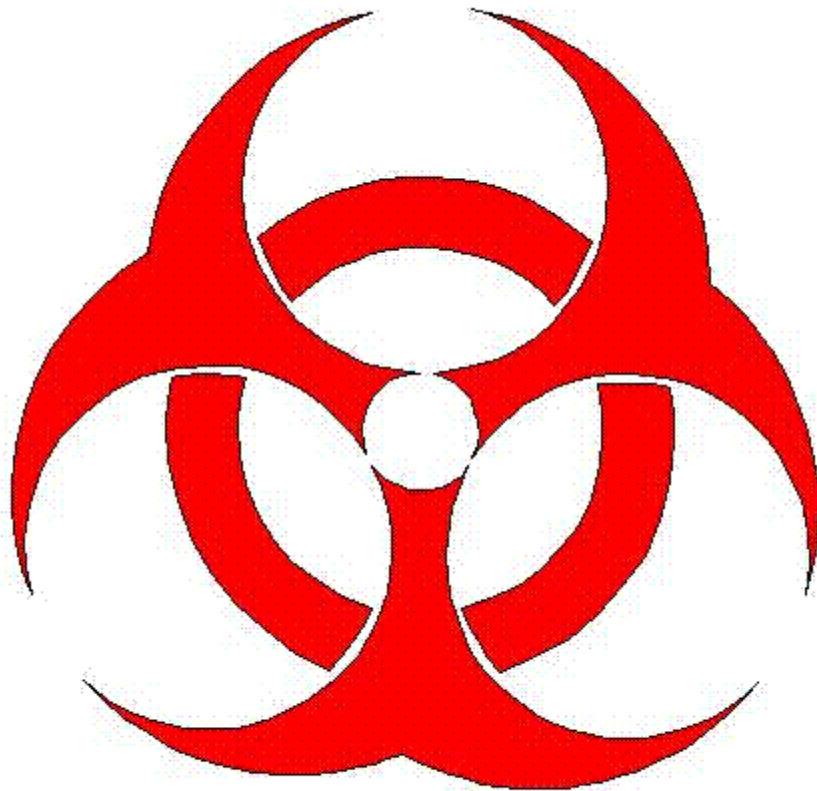


# **Biological Safety Manual**



**Kwantlen Polytechnic University**

**May 2009**

## Emergency Numbers

Any disaster, unusual occurrence, utility malfunction, or equipment failure that presents imminent danger to life and limb or property is an emergency and is to be reported immediately by telephone. For further information about Kwantlen emergency response plan, please refer to Kwantlen Polytechnic University Policy No. F4 Emergency Response Plan (See Appendix E).

### **General Contact Numbers**

Campus telephones ----- Local 8-1-1  
Fire/Policy/Ambulance----- 9-1-1  
Emergency telephone—Richmond ----- 604-599-2676  
Facilities Supervisor----- 604-599-2240 or 2400  
First Aid and Security ----- Dial 0 or call 604-599-2676

### **Emergency Contact Information for Biosafety Committee**

Manpreet Cheema----- 604-518-9439  
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### **Kwantlen Polytechnic University Emergency Response Group**

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Karen Hearn, Director, Facilities ----- 604-599-2442  
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### **Biological Safety Cabinets Maintenance**

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BIOLOGICAL SAFETY MANUAL**

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## 1. INTRODUCTION

### 1.1 KWANTLEN POLYTECHNIC UNIVERSITY HEALTH AND SAFETY POLICY

Kwantlen Polytechnic University has a responsibility to provide a policy and procedure to ensure that work is being conducted safely and in conformity with the British Columbia – Occupational Health and Safety Act, the Environmental Protection Act, Health Canada Biosafety Guidelines and Kwantlen Polytechnic University Policy No G.22 Safety & Health Program (refer to Appendix E).

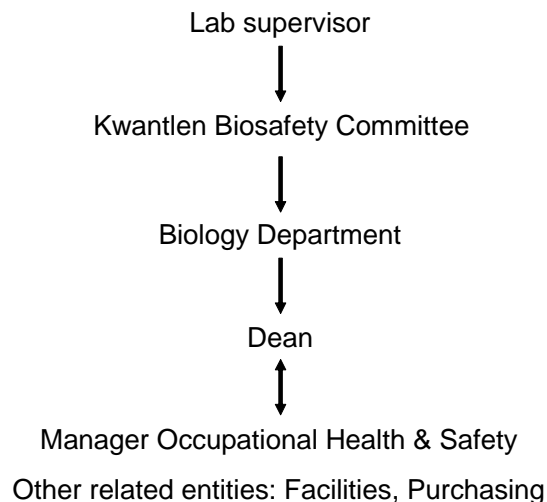
According to the Safety and Health Program G.22, the University has setup this Biological Safety Manual to identify and reduce the risks associated with work conducted using potentially hazardous biological substances.

### 1.2 THE BIOSAFETY COMMITTEE RESPONSIBILITIES

The Biosafety Committee affirms a responsibility to set and enforce appropriate standards of safety for work with potentially hazardous biological substances within Kwantlen Polytechnic University.

Terms of Reference

#### Organizational Chart:



Kwantlen Polytechnic University Biosafety Committee is responsible for ensuring that all activities within the microbiology laboratory of Kwantlen Polytechnic University involving infectious biological substances are conducted safely and in compliance with the Health Canada Laboratory Biosafety Guidelines (3<sup>rd</sup> edition, 2004). Infectious biological substances include viruses, bacteria, fungi, parasites, and other microorganisms that, by virtue of their replicative properties, are potentially harmful to humans and other living organisms.

The Biosafety Committee assumes the following responsibilities:

- To make recommendations to the Kwantlen Polytechnic University regarding actions and policies related to biosafety at Kwantlen Polytechnic University.
- To monitor and promote compliance with the established policies and procedures as set out in the most current version of the Health Canada Laboratory Biosafety Guidelines, and other government guidelines related to biosafety.
- To develop and recommend policies and procedures to meet or exceed the requirements for containment level 2 (CL2) as stated in the most current version of the Health Canada Laboratory Biosafety Guidelines.
- To approve protocols involving the use of potentially biohazardous agents and to confirm the appropriate containment level for the work.
- To verify that the appropriate facilities and procedures are in use, and to ensure that appropriate procedures for the use, storage and disposal of named biological agents are followed.
- To review, recommend and act as an expert resource for biosafety education and training program at Kwantlen Polytechnic University.
- To recommend to Kwantlen Polytechnic University that appropriate institutional occupational health programs be put in place as necessary to achieve the outcomes stated above.



### **1.3 STUDENT AND EMPLOYEE RESPONSIBILITIES**

#### **Before starting to work:**

- Employees should ensure that they have received and understood the biosafety training, any relevant Standard Operating Procedures (SOPs), the Biosafety Manual, and any other relevant safety information provided by management.
- Students are responsible to read and understand the safety procedures outlined in their laboratory manual and should be trained by their laboratory supervisor during the initial lab section.

#### **When working in the laboratory, both employees and students should:**

- Be aware of their duties and responsibilities, wear personal protective equipment as suitable, and perform work in accordance to standards set forth in the Health Canada Biosafety Manual.
- Collaborate with their laboratory supervisor to carry out their responsibilities.
- Inform their supervisors in the event of any accident/incident, spill, exposure or unsafe act.

### **1.4 TRAINING**

#### **Staff**

It is mandatory for the Biosafety committee, laboratory supervisors, laboratory workers and students who work with microorganisms to have appropriate training before they can start working in the microbiology laboratory.

The Biosafety committee and laboratory supervisors must attend an approved Biosafety Course and obtain a certificate of completion. The trained Biosafety committee will provide biosafety training to other laboratory workers and students.

Biosafety training should be documented and recorded in the form of an issued training certificate for the workers and a training binder to archive copies of all training. The training binders should be archived by the Facilities Department and a copy of the biosafety training binder(s) should also be kept in the laboratory at a specified location.

The Biosafety committee should provide an annual biosafety training course refresher to all laboratory workers.

In addition, the Biosafety committee, laboratory supervisors and laboratory workers must read and understand this Biosafety manual before starting any work in the microbiology laboratory.

### **Students**

Students should read and understand the safety procedures outlined in their laboratory manual and should be trained by their laboratory supervisor in the appropriate lab section. The student Biosafety training should encompass (but not necessarily be limited to) the following points:

- The concept of containment level and risk groups as they apply to the microbiology laboratory.
- Good microbiology practices.
- Biological safety cabinet operations.
- Accidental exposure and spills response.

### **1.5 IMPORTATION PERMITS FOR HUMAN PATHOGENS**

Importation of human pathogens in Canada is regulated by Importation of Human Pathogens Regulations (1994) to ensure proper laboratory handling and containment of these pathogens. Importation permits are issued by the Office of Laboratory Security, Biosafety Division after appropriate evaluation and approval of laboratory facilities. The application form can be obtained from <http://www.phac-aspc.gc.ca/ols-bsl/pathogen/index.html>.



A microbiology laboratory working with containment level 2 (CL-2) microorganisms must have a valid permit before importation. The Biosafety Committee should perform a self-inspection to ensure that the microbiology facility meets the Laboratory Biosafety Guidelines' requirement (please refer to Section 5.3.2). The self-inspection is subject to verification by Health Canada inspectors at any time.

After completion, the original application form should be sent to:

Office of Laboratory Security  
Centre for Emergency Preparedness and Response  
Public Health Agency of Canada  
100 Colonnade Road, Loc.: 6201A  
Ottawa, Ontario K1A 0K9  
Tel: (613) 957-1779  
Fax: (613) 941-0596

A permit will be issued and faxed to the applicant upon receipt of the application and its satisfactory assessment (usually within 5 working days). The original permit will follow by regular mail.

## **1.6 IMPORTATION PERMITS FOR ANIMAL PATHOGENS**

Importation of animal pathogen is regulated by the Canadian Food Inspection Agency (CFIA) under the Health of Animal Act, 1990, and the Health of Animal Regulations. Permits are required for the importation of all animal pathogens into Canada. The application form can be downloaded from <http://www.inspection.gc.ca/english/for/pdf/c5083perimpe.pdf>.

Completed applications should be sent either by fax at (613) 228-6129 or via regular mail to the following address:

Canadian Food Inspection Agency  
Biohazard Containment and Safety Unit  
159 Cleopatra Drive, Ottawa, Ontario, K1A 0Y9 room 1040

The CFIA has a list of animal pathogens that are currently under review. To determine the pathogenicity of a particular microorganism species, contact CFIA at:  
<http://www.inspection.gc.ca/english/sci/bio/anima/animaqueste.shtml>

After evaluation and approval, an import permit will be issued which must accompany the pathogen into Canada. A single or multiple-entry permit will be issued according to the particular situation. The import permit will specify the conditions under which the pathogen is to be maintained and work is to be carried out.

### **1.7 IMPORTATION PERMITS FOR BOTH HUMAN AND ANIMAL PATHOGENS**

For importation of pathogens that are common to both animals and humans, an import permit is required from the Canadian Food Inspection Agency as well as the Public Health Agency of Canada.

## **2. SAFETY PRACTICES AND PROCEDURES**

### **2.1 ROUTES OF EXPOSURE**

Laboratory workers who deal with infectious substances are at risk of exposure to the substances they handle. The most common routes of exposure to infectious substances are:

- percutaneous inoculation (from a needle)
- inhalation of aerosols generated by non-careful work practice or accident
- contact of mucous membranes and contaminated material
- ingestion

Among these, aerosols exposure is considered to be the greatest biohazard facing laboratory workers. Laboratory workers can minimize the risks associated with work involving these infectious substances through the application of appropriate laboratory safety practices outlined below.

The reader is encouraged to consult the references in Appendix C for further information on routes of exposure and information about Biosafety.

### **2.2 GENERAL LABORATORY SAFETY PRACTICES**

The following are general laboratory safety practices applicable for CL1 and CL2 facilities.

- A Biosafety Manual must be available for all laboratory personnel and it must be understood and followed.
- The laboratory and the prep room must be kept neat, tidy and clean, and storage of materials not pertinent to the work must be minimized.
- Eating, drinking, smoking, storing food or utensils, applying cosmetics, and inserting or removing contact lenses are activities not permitted in the laboratory. Contact lenses are not protective devices, they are only allowed when other forms of corrective eyewear are not suitable.



- Doors to the microbiology laboratory should NOT be left open.
- Access to the microbiology laboratory is limited to authorized personnel.
- All laboratory personnel entering the laboratory must understand the hazards with which they will come in contact with during their work. They must be trained in appropriate safety precautions and procedures. The laboratory supervisor shall provide training in laboratory safety, and competence in safe techniques must be demonstrated before work is allowed with hazardous or toxic agents.
- Hazard warning signs, indicating the containment level or the risk group of the agent used, must be posted outside the microbiology laboratory operating at Containment Level 2 (for more information about containment level and risk group, please refer to Section 5).
- Open wounds, cuts, scratches and grazes should be covered with waterproof dressings.
- Pregnant women and people who have an immuno-compromised condition and who work in, or enter the laboratory, must identify themselves to laboratory staff so they may be notified of the associated risks.
- Students and/or laboratory workers should be protected by appropriate immunization where possible.
- Protective laboratory clothing (uniforms, coats, gowns) must be readily available and used as required. Laboratory clothing is hung on hooks near the microbiology laboratory exit (refer to Floor Plan at the back of the manual). Protective laboratory clothing should be worn properly by all personnel including students, visitors, trainees, and others entering or working in the laboratory. Protective laboratory clothing must not be worn in non-laboratory areas (for more information about personal protective equipment, please refer to section 2.3).

- Suitable footwear with closed toes and heels and preferably with non-slip soles must be worn in the laboratory area.
- Gloves must be available near the biological safety cabinet. They must be worn for all procedures that may involve direct skin contact with toxins and infectious materials. Wearing jewelry (especially rings and hand jewelry) in the laboratory is discouraged. Gloves must be removed carefully and decontaminated with other laboratory waste before disposal.
- Face and eye protection (e.g., glasses, goggles) must be worn at all times to protect the face and eyes from splashes, impacting objects, harmful substances, or UV light.
- Oral pipetting is prohibited in the laboratory.
- Long hair must be tied back.
- Work benches must be cleaned and wiped down with the appropriate disinfectant at the end of the day and after any spill of potentially hazardous material. Loose or cracked work surfaces must be repaired or replaced.
- All technical procedures must be performed in a manner that minimizes the creation of aerosols (refer to Section 2.4.2).
- All contaminated or infectious liquid must be autoclaved before disposal. The outside of the container must be disinfected with 70% EtOH when necessary before sending to autoclave.
- The use of needles and syringes and other sharp objects must be strictly limited and the disposal of such items should be into suitably designed and labeled containers.
- Hands must be washed after gloves are removed, before leaving the laboratory, and after handling materials that are known or suspected to be contaminated, even when gloves have been worn.



- All spills, incidents (accidents and near misses) and overt or potential exposures must be reported to the laboratory supervisor or acting alternate, as soon as possible after the event. Appropriate medical evaluation, surveillance, and treatment must be sought and provided as required. See Flowchart in Section 4.0 for emergency response procedure.
- Disinfectants effective against the agents in use must be available for immediate use at all times.

## **2.3 PERSONAL PROTECTIVE EQUIPMENT (PPE)**

### **Laboratory Coats or gowns**

- The laboratory coat has 2 uses: to protect street clothing from biological or chemical spills, and to offer some additional body protection.
- Containment level 2 (CL-2) requirements: lab coat, gown, smock or uniform is recommended to be made of 100% cotton due to resistance to a number of chemicals. Note that lab coats that are fastened in the front are permissible. These coats should NOT be worn outside the containment laboratory.
- All non-disposable laboratory coats and dirty lab coats in particular should be laundered following guidelines from the Director Materials Management.

### **Head coverings**

- Generally not required in most CL2 biological areas, unless a complete change of clothing is required for access, and where product protection is required.

### **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 nurse's shoes are recommended. Open-toe sandals are not allowed in laboratories working with biohazardous agents, due to potential exposure to infectious agents.

## **Gloves**

- Gloves should be readily available and 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.
- Gloves are made from a variety of materials including, rubber (latex), neoprene, nitrile, polyurethane, etc. Selection should depend on the anticipated hazards to be encountered. If biological work will include the use of chemical solvents such as toluene, benzene or carbon tetrachloride, then rubber, neoprene and PVC based gloves will not be suitable as these agents can degrade them. A comprehensive list of chemical compatibility of different glove materials can be obtained from most glove suppliers.
- In microbiological laboratories, surgical gloves of rubber (latex) or vinyl are generally the preferred choice. They offer a high level of dexterity and a higher level of sensitivity; unfortunately they offer very little if any protection against needle sticks or sharps. Gloves are typically the weakest component of PPE.
- Some studies have shown however, that double layers of gloves or the addition of a pair of neoprene glove will increase the layer of protection from the normal 0.008-0.01 inches to 0.03 inches, resulting in a lower exposure dose in the event of needle stick injury.
- Gloves should over wrap the cuff and lower sleeve of the laboratory clothing.

## **Respiratory protection**

- Two types of respiratory protection exist: (1) Those that supply clean air, or (2) those that remove the hazardous particulates.



- (1) A full face or half face cartridge respirator that supplies clean air. These types of respirators must be properly fitted prior to use. These types of respirators are worn in atmospheres that pose an infectious or toxic hazard such as in an animal room where infectious agents could be excreted in urine.
- (2) Single-use paper dust masks for removal of hazardous particles. These devices are not classified as true respirators but are suitable and adequate for the types of risks that may be found in a CL2 facility. They are effective for removal of potentially hazardous particles.

### **Eye or Face Protection**

- Eye or face protection is important because biological work may often involve the use of concentrated alkalis, acids, concentrated disinfectants, including phenolics and quaternary ammonium compounds which can cause severe eye damage and blindness if splashed. 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 protection, but should not be used to protect against splashes. For these hazards, safety goggles or face shields should be used. Ordinary prescription glasses offer better splash protection than no protection at all, however, they do not replace the need for approved safety eyewear.
- In a microbiological laboratory, the use of contact lenses is allowed only when other forms of corrective eyewear are not suitable. However, in the event of an accident where the lens needs to be removed, it is probable that hands would not be completely decontaminated prior to removal of the lens, and hence there is an increased risk for infection.

## **2.4 SAMPLE HANDLING**

### **2.4.1 WORKING WITH PATHOGENS**

In general, the risks to laboratory workers or students working with a virus that only infects and causes disease in rodents is lower than the risks associated with working with human cell culture. Some microorganisms (viruses, bacteria, fungi, etc.) are species specific, selectively infecting and causing disease in a limited number of host species. Unrelated and distantly related species may not be similarly affected by the same infectious microorganism, due to differences in physiology, metabolism, or biochemistry.

However, disease causing micro-organisms can be spread or transmitted from one host to the next, directly or indirectly, by a number of methods, including aerosol generation and inhalation, skin and mucous membrane contact with contaminated surfaces, contact contamination of an open wound or lesion, and autoinoculation via a cut, laceration or puncture with a contaminated instrument.

As a result, all laboratory workers and students who perform work in the microbiology laboratory involving infectious substances should adopt good microbiology practice in addition to the general laboratory safety practices to minimize the risks associated with infectious pathogens.

### **2.4.2 GOOD MICROBIOLOGY PRACTICE.**

Working with microorganisms requires good microbiology practice. In general, the use of good microbiology practice aims to prevent both contamination of the laboratory workers with the organism(s) and contamination of the organism(s) or test subjects with organisms from the surrounding environment.

The principles of good microbiology practice should be applied to work with all types of microorganisms irrespective of containment level.

## **Aseptic technique:**

Aseptic technique refers to procedures that are performed under sterile or near-sterile conditions. In the context of microbiology, it refers to the creation and maintenance of a sterile microenvironment to grow and maintain a microorganism strain of interest and prevents all contact with contaminants. The physical microenvironment is defined by the borders of an appropriate holding vessel such as a flask, bottle, tube, or petri dish and the microorganisms can either be grown on a solid agar medium or be suspended in a broth, diluent or other fluid medium.

## **Aseptic technique principles:**

1. All systems used in the procedure including the inside of the vessel, the growth medium, and any objects used in the manipulative processes, must be sterile prior to use;
2. While performing experiments, one should always:
  - Wear appropriate personal protective equipment (PPE) including lab coats, gloves, face shields, etc, as appropriate;
  - Work with a Bunsen burner and be within the sphere of a Bunsen burner flame. The opening of the tubes/flasks should be passed quickly through the Bunsen burner flame during manipulations in which liquid media and/or culture is added or removed. The upwards current of hot air created by the Bunsen prevents contaminated air or particles dropping into the culture when the lid is open;
  - Keep the vessel closed except for the minimum time required to introduce or remove materials;
  - Avoid placing tops and lids down on the benches when removing from vessel openings;
  - Ensure that loops and pipette tips are sterile before use, and are not contaminated by casual contact with the bench, fingers or the outside of the bottle during handling. Loops and pipette tips should be decontaminated or disposed of in a safe manner immediately after use.

### **Aerosols Minimization:**

- Mix liquid volumes by gentle rolling and swirling. Avoid mixing by vigorous shaking;
- When pipetting, avoid generating bubbles and splash by putting the pipette tip into a liquid or onto a surface, and gently ejecting contents;
- Vessels should be in close proximity to each other when transferring liquids between them to prevent falling drops and splashing;
- Use loops only after they have cooled down from flaming;
- To avoid leakage in a centrifuge, never over-fill centrifuge pots, tubes, containers;
- Always place tubes, bottles, flasks etc., in racks or other suitable containers to avoid accidental dropping and or tip-overs.

### **Disinfection and decontamination:**

- Appropriate disinfectants/decontaminants such as 70% ethanol, domestic bleach (10%) should be used to decontaminate bench, work surfaces and the inside of biological safety cabinets before and after work or as required;
- All surfaces, which have been exposed to the spill or otherwise contaminated with a biological agent, should be decontaminated with a suitable disinfectant. Refer to Table 1.

**Table 1** Classes of Microorganisms and Suitable Decontaminants

Decontaminants	Vegetative bacteria	Mycobacteria and fungi	Spore forming bacteria	Lipo virus	Non-lipo virus
10% domestic bleach	+	+	+	+	+
70% Ethanol	+	+	-	+	Variable
6% formulated Hydrogen peroxide	+	+	+	+	-
0.1 –2% Quaternary ammonia (up to 2g/L)	+	-	-	+	-
1-5% Phenolic compounds (up to 5ml/L)	+	+	-	+	Variable
2% Glutaraldehyde (2g/L)	+	+	+	+	+
5.0% Formaldehyde	+	+	+	+	+

\* WHO Laboratory Biosafety Manual, Third Edition & Princeton University Environmental Health & Safety

For detail information regarding each of the above specific decontaminants, please see Table 2.

In addition to above techniques, good microbiology practice encompasses a wide range of other working methods including:

### Safe use of Centrifuges

- Routinely check centrifuge tubes and rotors for cracks and signs of stress;
- Do not overfill;
- Ensure caps or stoppers are properly in place;
- Ensure all buckets are properly balanced;
- Ensure centrifuge achieves run conditions before leaving unattended;
- Ensure centrifuge has completely stopped before opening lid;
- Check centrifuge cavity for spills or leaks prior to removing samples;
- Clean all spills promptly and completely.



## Safe use of Needles and Syringes

- Avoid using needles and syringes whenever possible;
- If possible, perform all operations utilizing syringes and needles, within a biological safety cabinet;
- Fill syringes carefully, avoid frothing or introduction of bubbles;
- Shield needles with disinfectant-soaked cotton when withdrawing from stoppers;
- If possible, do not bend, shear or recap needles;
- If needles must be recapped, use a one-handed scoop method or holder
- All used needles and syringes are to be disposed in a suitable, properly and clearly labeled puncture resistant container.

## Maintenance of Equipment

- Microscopes: use 70% ethanol to disinfect the stage, eyepieces, knobs and any other contaminated parts at the end of the day.
- Water baths: Clean water bath using disinfectant such as phenolic detergent every week.

## Hand washing

- Containment Level 2 (CL2) requires antiseptic hand washing solutions. Common antiseptic solutions contain chlorhexine gluconate or trichlosan.
- Liquid dispensers should be used rather than soap bars
- Hands should be washed:
  - Before starting any manipulations;
  - Before leaving the lab;
  - When hands are obviously soiled;
  - Before and after completing any task in a biosafety cabinet;
  - Every time gloves are removed;
  - Before contact with one's face or mouth;

## 2.5 DECONTAMINANT AGENTS

There are a variety of chemical decontaminant agents commonly available for use in a biological laboratory. Their uses along with advantages and disadvantages are listed in



**Table 2** Chemical decontaminant agents

Decontaminants	Advantages	Disadvantages	Uses
10% domestic bleach	widely available, broad spectrum, inexpensive, active against all microorganisms	highly toxic, corrosive to skin and metals; lose strength gradually, inactivated by organic matter, deteriorates under light and heat.	general disinfectant, soak contaminated metal-free materials
70% Ethanol	low toxicity, no residue, easy to use, non corrosive	rapid evaporation, reduces contact time; flammable, skin desiccant, non sporicidal, easily inactivated by organic matter, no cleansing properties, ineffective with unconventional agents	surface decontamination, bench top, cabinet wipe down.
6% formulated Hydrogen peroxide	rapid action, no residue, low toxicity, environmentally safe	limited sporicidal activity, store away from heat and light, corrosive to some metals	surface decontamination
0.1-2% Quaternary ammonia	has combined detergent and germicidal activity, non-staining, inexpensive, stable, low toxicity	not sporicidal, inactivated by organic matter, water hardness and anionic detergents, limited against viruses, mycobacteria, low biodegradability	surface decontamination, equipment wipe down
1-5% Phenolic compounds	leaves an active residue, biodegradable	unpleasant odor, corrosive, toxic, irritant, can penetrate the skin, non sporicidal, no activity against bacterial spores	disinfection of floors and other surfaces
2% Glutaraldehyde	broad spectrum, non-corrosive, can tolerate organic material	expensive, limited shelf-life, irritant, pH and temperature dependant, pungent odor	cold sterilant and fixative, surface decontamination
5.0% Formaldehyde	broad spectrum at >20°C, does not corrode metal, less susceptible to inactivation by organic material	pungent odor, irritant, suspected carcinogen, low acting (requires at least 30min and up to 24hours) and needs a relative humidity level of about 70%, used in fume-hood or well-ventilated area, loss of decontaminant activity when refrigerated	cold sterilant and fixative, surface decontamination

\* WHO Laboratory Biosafety Manual, Third Edition & Princeton University Environmental Health & Safety



## **2.6 WASTE MANAGEMENT**

For information regarding general waste management at Kwantlen Polytechnic University, please refer to Kwantlen Polytechnic University Policy No. F13 Waste Management/Environment (see Appendix E).

The microbiology laboratory is responsible for the separating, packaging and treating of its laboratory waste prior to its removal and disposal. The following procedures apply to waste contaminated with or containing **biological agents** only.

Biological waste includes:

1. Liquids such as cell culture or bacterial media, agar, and supernatants.
2. Non-sharps and solid laboratory waste (e.g. empty plastic cell culture flasks and petri dishes, empty plastic tubes, gloves, wrappers, absorbent tissues, etc.) which may be, or are known to be contaminated with viable biological agents.
3. Laboratory sharp waste that is known or suspected to be contaminated with hazardous biological agents.

### **2.6.1 PACKAGING LIQUID AND SOLID BIOLOGICAL WASTE**

#### **1. *Biohazard Liquid waste***

Liquids should be collected in leak-proof containers such as flasks or bottles.

These containers should withstand autoclaving temperatures since they will undergo steam sterilization. In addition, the containers should be covered with a lid or aluminum foil bearing heat sensitive autoclave tape; however, they should not be sealed shut in order to prevent pressure buildup.

#### **2. *Biohazard Solid waste***

Gloves, empty plastic cell culture flasks and petri dishes, empty plastic tubes, wrappers, absorbent tissues, and any other non-sharp, solid biohazard waste, which may be, or have been, contaminated with viable biological agents should be collected in biohazard autoclavable bags. All bagged biohazard waste must be closed by twisting but not sealed tight to allow pressure equalization, and taped using a heat sensitive tape.

## 2.6.2 TREATING LIQUID AND SOLID BIOLOGICAL WASTE

### ***Sterilization and Disinfection***

Biohazardous agents should be disinfected using steam sterilization/autoclave procedures. As a very general rule of thumb, a 20 min cycle at 121°C and 15 psi is the minimum autoclave cycle to use for steam sterilization.

Depending on the volume of waste to be sterilized, it may be necessary to extend the duration of exposure to high temperature steam under pressure. Steam sterilization is generally not recommended for laboratory waste contaminated with a combination of viable biological agents and significant amounts of hazardous chemical materials.

Liquid waste containers and the biohazard autoclavable bags containing the non-sharp solid waste must be placed into an autoclavable tray of sufficient size to contain all liquid in case the vessel fails or breaks inside the autoclave chamber.

Indicator strips and heat sensitive tape must be used to ensure proper autoclaving temperature has been reached.

### **Disposal**

After steam sterilization, the liquid wastes and non-sharp solid wastes should be allowed to cool down before being removed from the autoclave.

Innocuous liquids may be disposed of via the laboratory drainage system and flushed away with large amounts of tap water.

Melted agar must NOT be poured into sinks or floor drains. It should be allowed to cool and solidify and disposed of as a solid waste.

Autoclaved waste should have a new label placed over the biohazard label that reads "Autoclaved/Decontaminated".

Sterilized non-sharp solid waste should be placed in the appropriate location for pickup and disposal by janitorial personnel.



## **Autoclave Maintenance**

The laboratory technician should confirm cycle effectiveness on a monthly basis by running empty chamber and/or full load cycles and using biological indicators strips. A formal protocol should be developed, followed, and data recorded.

The autoclave should be placed on an annual inspection/certification schedule. The laboratory technician staff is responsible for establishing the maintenance schedule and ensuring that it is followed.

### **2.6.3 SHARPS WASTE MANAGEMENT**

Sharps waste at Kwantlen Polytechnic University is categorized as needle/blade waste, and glassware waste.

#### **Needle/blade waste**

Needle/blade waste is defined as hypodermic, suture, or IV needles, syringes with needles, lancets, scalpels, blades and similar metallic sharp or pointed items for disposal that are capable of causing punctures, cuts, or tears in skin or membranes.

- All needle/blade waste that is contaminated with biological agents must be collected in the designated red needle/blade waste container bearing the biohazard symbol.
- When this red needle/blade waste container is  $\frac{3}{4}$  full, it should be snapped closed and sent for autoclaving prior to incineration.
- Needles should not be recapped, purposely bent or broken by hand, removed from disposable syringes, or otherwise manipulated by hand.
- All non-contaminated needle/blade waste should be collected in a separate puncture-proof container. When it is  $\frac{3}{4}$  full, it should be snapped closed and sealed for a laboratory technician to dispose of.

#### **Glass waste**

Glassware waste includes any disposable intact or broken laboratory containers such as flasks, beakers, bottles, etc; small glass containers, ampoules and tubes; or glass pipettes.

- Glassware waste must not be placed into regular garbage containers or plastic bags of solid waste.



- All glassware containers must be empty and be autoclaved if they are contaminated with viable biological agents handled in the laboratory.
- After sterilization, intact glassware is to be cleaned for reuse while broken glassware should be disposed of into the designated broken glass container.

### **Disposal of glass waste**

- After sterilization of glass waste, it is to be removed from the autoclave and placed in a hard plastic container labeled “Sterilized glassware”. When the container is  $\frac{3}{4}$  full, Facilities should be notified to remove the glass waste.

## **2.7 BIOLOGICAL SAFETY CABINET (BSC)**

### **2.7.1 INTRODUCTION**

Biological safety cabinets (BSC's) are the single most important safety device in the microbiology laboratory. All BSC's contain HEPA filters to ensure the exhaust from the cabinets is free of infectious material. BSC's should be located away from doors, drafts, convection currents, diffusers and high traffic areas. There are 3 classes of cabinets: Class I, II, and III. The Biological safety cabinets at Kwantlen are Nuair Class II Type A2 (formerly referred to as Type A/B3).

### **Class II Cabinets**

Class II cabinets are designed for personnel, product and environmental protection. They are designed for work involving microorganisms in containment levels 2, 3 and 4 laboratories. Thus, Class II cabinets are commonly used in biomedical research laboratories. There are two general subtypes of class II: A or B. They differ in airflow velocities: 1) the amount of air recirculated over the work surface and 2) the amount of air exhausted. The BSC's at Kwantlen Polytechnic University are Class II, Type A2 cabinets (refer to Appendix A for a schematic diagram of this type of cabinet). Class II, Type A2 BSC's have the following characteristics,

- They are open fronted.
- They are suitable for work with minute quantities of volatile toxic chemicals and trace amounts of radionuclides.
- They provide good protection to the worker, as well as to the work being performed.

- The cabinet air may be re-circulated back into the laboratory or ducted out of the building by means of a "thimble" connection (i.e., a small opening around the cabinet exhaust filter housing) whereby the balance of the cabinet is not disturbed by fluctuations in the building exhaust system.
- The thimble is designed to allow for proper certification of the cabinet (i.e., provide access to permit scan testing of the HEPA filter).
- They maintain a minimum average face velocity of 0.5 m/s (100 ft/min).
- They have ducts and plenums under negative pressure.

### 2.7.2 OPERATION OF BSC

#### Starting up the BSC:

- Turn off UV lights (if necessary) and turn on fluorescent light and cabinet blower, allow the blower to run for at least 5 minutes before work is performed.
- Wipe the interior surfaces with 70% ethanol.
- Check the air intake and exhaust grilles for obstructions.
- Switch on the alarm if the cabinet is equipped with an alarm.

#### Working in the BSC:

- Wear protective clothing and gloves.
- Perform operations as far to the rear of the work area as possible.
- All materials required for the procedure should be sprayed with 70% ethanol before loading into the cabinet. Make sure the air grilles are not obstructed.
- Gloved hands should be sprayed with 70% ethanol before working in the BSC.
- Have available, a biohazard bag and a leak-proof container at the rear of the cabinet to keep contaminated solid biohazard waste and liquid biohazard waste, as necessary.
- Unless necessary, do not work with open flames inside the cabinet.

#### After working in the BSC:

- Allow the blower to run for 5 minutes with no activity.
- Close or cover open containers (e.g. tip boxes, culture media etc) and spray 70% ethanol to disinfect them before removing them from the cabinet.



- Spray 70% ethanol to disinfect other objects that may be contaminated before removing them from the cabinet.
- Ensure that contaminated gloves and all other contaminated materials are placed into biohazard bags or the leak-proof container within the cabinet.
- Close all biohazard bags and tape them before removing them from the cabinet.
- Cover all biohazard liquid waste with a lid or aluminum foil and tape them with an autoclave tap before removing them from the cabinet.
- Use 70% ethanol to disinfect interior surfaces of cabinet; periodically remove the work surface and disinfect the area beneath it (including the catch pan) and wipe the surface of the UV light with disinfectant.
- Turn off the fluorescent light and cabinet blower.
- Turn on the UV light if appropriate (do not turn on when people are working close by).
- Remove gloves and dispose of them as appropriate; wash hands.

### **2.7.3 MAINTENANCE OF BSC'S**

- Decontaminate work surfaces after work is done or twice a day.
- If installed, wipe clean the UV lamp every week.
- Wipe down all vertical surfaces every month.
- If UV lamps are used, verify UV lamp intensity annually.
- Inspect, test and certify all BSC's every year using trained service personnel to ensure that the cabinet is functioning as intended by the manufacturer. For BSC failure or inspection, please contact the Biology Laboratory Technician who will report deficiencies through the Dean to Director of Facilities.

### 3. INVENTORY MANAGEMENT

Inventory management is an important aspect of the microbiological laboratory. The following points should be followed:

- It is the responsibility of laboratory technicians to oversee and maintain laboratory inventory at levels sufficient to meet needs and demands and allowing for an overage in case of emergencies.
- All newly arrived laboratory supplies should be identified and recorded in a logbook system that includes the following information: vendor company name, batch size and arrival date, etc as appropriate.
- First in First out (FIFO) principle should be applied to all laboratory supplies. Stock should be rotated with the oldest stock always being used first.
- The laboratory technician should closely monitor the stock level, how fast each stock is used up and then determine a reasonable re-order point for them.
- In the laboratory, all media/cultures should be clearly labeled with their name, lot #, expiry date, the date they are made, ingredients, and the person who prepared them.
- All drawer and cupboards should be clearly labeled. Inventory lists should be reviewed and updated regularly. Depending on the level of use, it is recommended that the inventory list be updated every other week.
- All containers of hazardous substances should be Workplace Hazardous Material Information System (WHMIS) compliance labeled and be classified under Material Safety Data Sheets (MSDS). Please refer to Kwantlen Policy No. G13 Workplace Hazardous Material Information System (WHMIS) for more information. The microbiology laboratory should have its own MSDS for hazardous substances used in the laboratory. These MSDS should be easily accessible in the laboratory and should be updated annually. The Facilities department should also receive a copy of the current MSDS. Refer to the facility floor plan on the backside of the manual cover for the storage location of MSDS.
- Hazardous substances in compatible groups should be stored in secure areas.
- Inorganic acids should be kept separate from organic acids; flammable substances should be kept separate from acids; oxidizing and reactive substances should be kept away from organic chemicals etc.
- Personal protective clothing should be hung on hooks near the laboratory exit; different sizes of gloves should be available and near the biological safety cabinets.



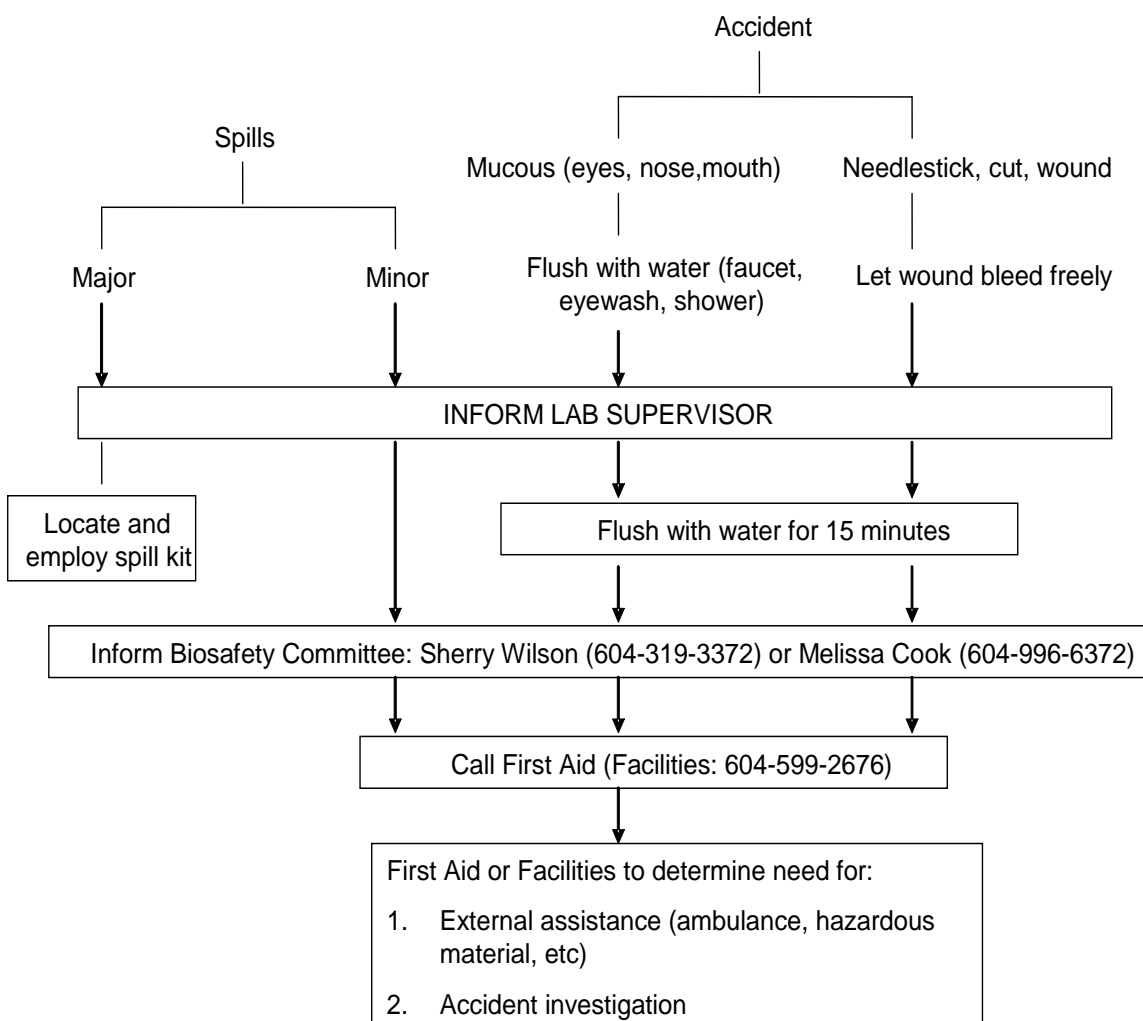
- Infrequently used items should be stored in cupboards and drawers; the laboratory technician(s) should monitor these items over time for instability.
- Laboratory technician(s) should, on a regular basis (suggest monthly), sort through items stored in fridges and freezers, on shelves and benches, etc. and properly dispose of unwanted items to prevent clutter.

## 4. EMERGENCY RESPONSES

All spill and exposure incidents should be reported to laboratory supervisor.  
Please refer to flowchart below for emergency response procedure.

### EMERGENCY RESPONSE PROCEDURE

For extreme emergencies, call 911 directly



Affected laboratory students or workers should seek medical attention. For extreme emergencies, call 9-1-1 directly. For non-urgent accidents, contact Facilities. Facilities personnel will attend and determine the procedures to be followed. Note that a fully functional first aid room is available on campus; there is always a fully trained designated attendant on site who has access to a portable first aid bag and oxygen tank. For general information about Kwantlen emergency response plan, please refer to Kwantlen Polytechnic University Policy No. F4 Emergency Response Plan and Policy No. F4a Emergency Response/Communication Plan (See Appendix E).

The emergency response flowchart, including procedures for dealing with spills or other laboratory exposures at Kwantlen, should be kept in the laboratory at all times and be easily located in an emergency.

## **4.1 SPILLS**

### **Contents of Spill kit**

- One box of 10 Eliminator Spill Control pillows 13 cm x 30 cm (with extras of these pillows located above the flammable cupboard in Linda's prep room)
- 100 Kimberly Clark, White Kleen Guard disposable aprons 28 x 40 inches
- 1 econo- mercury spill kit
- 1 Spill-X-A Acid neutralizer 1.13 kg
- 2 Spill-X-C Caustic neutralizer 0.90 kg
- 2 Spill-X-S Solvent Absorbent 0.45 kg
- 1 information pamphlet including information, instructions, and amounts to use to clean up a spill.
- 1 Gas Mask Willson types TIGW & TLGW Gas Mask including instruction and 2 canisters for acids, ammonia, carbon monoxide and organic vapors

For spill kit location, refer to floor plan on the backside of this Biosafety manual cover.

Not all spills in the laboratory will require use of the spill kit. The general guiding principle will be the size of the spill. Larger spills of potentially toxic liquids will generally require use of the spill kit whereas smaller spills may be addressed without the spill kit.

#### 4.1.1 CLEANING UP SPILLS WITH A SPILL KIT

- Use the spill control pillow or absorbent material to absorb the spilled liquid;
- Put the used pillow or absorbent material into an autoclavable bag or covered container and autoclave it;

##### **For large spills ( $\geq 1.0$ L) in a laboratory**

- If appropriate, evacuate the area for 30 minutes to allow aerosols to settle.
- Assemble cleaning supplies, and wear personal protective equipment (PPE).
- Pour concentrated disinfectant such as 10% bleach from outside to inside.
- Cover with absorbent, and allow disinfectant to act for 20 minutes
- All adjacent areas should also be disinfected or wiped down.
- Autoclave all waste.
- Decontaminate all surfaces exposed to the spill with a suitable disinfectant (please refer to Table 1 in Section 2.4.2 for a suitable disinfectant).

#### 4.1.2 CLEANING UP SPILLS WITHOUT A SPILL KIT

##### **For spills within a biological safety cabinet**

- Do not turn off ventilation.
- Wear personal protective equipment (PPE).
- Flood the spill with 10% bleach and cover with absorbent paper.
- Leave it on for 20-30 minutes.
- Pick up with absorbent material and send to autoclave.
- All other items within the cabinet should also be wiped down or autoclaved.

##### **For spills in a centrifuge**

- Leave lid closed and allow aerosols to settle for at least 1 hour.
- Wear personal protective equipment (PPE).
- Thoroughly wipe down inside of centrifuge, including the lid with paper towels soaked with disinfectant.
- Disinfect the entire rotor, especially the bucket where spill occurred within the centrifuge.
- Remove rotor from centrifuge and repeat disinfection.



- Rinse both rotor and inside of centrifuge with water if bleach was used.
- Autoclave all waste.

### **For spills in laboratory or prep area**

- Evacuate the area right away for 30 minutes to allow the aerosols to settle.
- Wear personal protective equipment (PPE).
- Pour concentrated disinfectant such as 10% bleach around the spill, then use paper towel to slowly walk the disinfectant from outside to inside.
- Allow the disinfectant to mix with the spill material for 20 minutes.
- Then absorb the liquid with absorbent paper and place in autoclavable bag.
- Autoclave all the waste.
- Decontaminate all the surfaces exposed to the spill.

## **4.2 EXPOSURE TO INFECTIOUS AGENTS**

The following emergency response procedures shall be followed when a student or worker has been exposed to infectious agents via inhalation, a needle stick, cut or puncture wound, via ingestion or mucous membrane contact, or via non-intact skin contact. Also refer to emergency response procedures flowchart in Section 4 for details.

### **Student or Worker**

- Immediately flush the exposed site with water.
  - a. For needle stick, cut or puncture wound, wash with soap and water for at least 15 minutes after allowing the wound to bleed freely.
  - b. For mucous (eyes, nose, mouth) membrane, flush with water at the nearest faucet or eye wash station for at least 15 minutes. See Floor plan for location.
- **Inform the laboratory supervisor.**
- Seek prompt medical attention as directed.

### **Kwantlen employee**

- Refer the affected students/workers to Facilities and/or the nearest hospital emergency department or emergency clinic.

## 5. CLASSIFICATION OF RISK GROUPS AND CONTAINMENT LEVELS

### 5.1 RISK GROUPS

Infectious organisms have been categorized into risk groups to identify the relative hazards they may pose to individuals and the community. Refer to the current edition of Health Canada Biosafety guidelines for further information about risk groups.

Please refer to Appendix B for a list of microorganisms categorized according to Risk Group.

#### 5.1.1 RISK GROUP 1 (low individual and community risk)

Risk Group Level 1 is defined as any biological agent that is unlikely to cause disease in healthy workers or animals. It includes those microorganisms, bacteria, fungi, viruses and parasites that are unlikely to cause disease in healthy workers or animals. A good example would be level 1 strains of *Escherichia coli* (*E. coli*).

#### 5.1.2 RISK GROUP 2 (moderate individual risk, low community risk)

Risk Group Level 2 organisms are defined as any pathogen that can cause human disease but under normal circumstances is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures rarely cause infection leading to serious disease. Effective treatment and preventative measures are available and the risk of spread is limited. Please refer to Appendix B for a list of microorganisms categorized according to Risk Group.

#### 5.1.3 RISK GROUP 3 (high individual risk, low community risk)

Risk Group Level 3 is defined as any pathogen that usually causes serious human disease or can result in serious economic consequences, but does not ordinarily spread by casual contact from one individual to another, or that causes disease untreatable by antimicrobial or anti-parasitic agents.

#### 5.1.4 RISK GROUP 4 (high individual risk, high community risk)

Risk Group Level 4 includes pathogens that usually produce very serious human or animal diseases, often untreatable, and may be readily transmitted from one individual to another or from an animal to human or vice-versa directly or indirectly, or by casual contact.



**Note that microorganisms to be used in the CL2 microbiology laboratory at Kwantlen Polytechnic University must fall under the Risk group 1 and/or 2 category.**

## **5.2 CONTAINMENT**

The principles of containment are applied to the basic design of laboratory facilities and the working practices of all the people in the laboratory. The purpose of containment is not only to prevent the escape of microorganisms out of the laboratory but also to ensure the safety of workers in the laboratory.

The activity of working with and manipulating micro-organisms should always be carried out using principles of good laboratory practice and be done in properly designed containment laboratories. There are 4 different levels of containment and the containment level that a particular microorganism should be handled in is indicated by the corresponding risk group of the microorganism. Table 3 shows the recommended containment level for different risk group agents.

**Table 3** Risk Groups with Recommended Containment Level and Class of BSC's

Risk group	Risk Assessment	Examples	Containment Level	BSC
1	low individual, low community risk	<i>Bacillus circulans</i> , <i>Bacillus subtilis</i> , <i>Micrococcus luteus</i> , <i>E. coli</i> K12	1	N/A
2	moderate individual risk, low community risk	<i>Bacillus cereus</i> , <i>Mycobacterium smegmatis</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella cholerae-suis</i>	2	Class I or Class II
3	high individual risk, low community risk	<i>Coxiella burnetti</i> , <i>Mycobacterium bovis: non-BCG strains</i> , <i>Yersinia pestis</i> , <i>Bacillus anthracis</i> <i>Histoplasma capsulatum</i> ,	3	Class I or Class II
4	high individual risk, high community risk	<i>Marburg virus</i> , <i>Ebola virus</i> , <i>Crimean-Congo hemorrhagic fever virus</i> , <i>Herpesvirus simiae</i>	4	Class III

### 5.2.1 CONTAINMENT LEVEL 1 (CL1)

This is required for a basic laboratory that handles agents requiring containment level 1. CL1 requires no special design features beyond those suitable for a well-designed and functional laboratory. Biological safety cabinets (BSC's) are not required. Work may be done on an open bench top, and containment is achieved through the use of practices normally employed in a basic microbiology laboratory.

### 5.2.2 CONTAINMENT LEVEL 2 (CL2)

This is required for a laboratory that handles agents requiring containment level 2. The primary exposure hazards associated with organisms requiring CL2 are through the ingestion, inoculation and mucous membrane route. Agents requiring CL2 facilities are not generally transmitted by airborne routes, but care must be taken to avoid the generation of aerosols (aerosols can settle on bench tops and become an ingestion hazard through contamination of the hands or splashes).

Primary containment devices such as BSC's and centrifuges with sealed rotors or safety cups are to be used as well as appropriate personal protective equipment (i.e., gloves, laboratory coats, protective eyewear). As well, environmental contamination must be minimized by the use of hand washing sinks and decontamination facilities (autoclaves).

### **5.2.3 CONTAINMENT LEVEL 3 (CL3)**

This is required for a laboratory that handles agents requiring containment level 3. These agents may be transmitted by the airborne route, often have a low infectious dose to produce effects, and can cause serious or life-threatening disease. CL3 emphasizes additional primary and secondary barriers to minimize the release of infectious organisms into the immediate laboratory and the environment. Additional features to prevent transmission of CL3 organisms include appropriate respiratory protection, HEPA filtration of exhausted laboratory air and strictly controlled laboratory access.

### **5.2.4 CONTAINMENT LEVEL 4 (CL4)**

This is the maximum containment available and is required for facilities manipulating containment level 4 agents. These agents have the potential for aerosol transmission, often have a low infectious dose and produce very serious and often fatal disease; there is generally no treatment or vaccine available. This level of containment is characterized by a functionally isolated unit, and when necessary, is structurally independent of other areas. CL4 emphasizes maximum containment of the infectious agent by complete sealing of the facility perimeter with confirmation by pressure decay testing; isolation of the researcher from the pathogen by his or her containment in a positive pressure suit or containment of the pathogen in a Class III BSC line; and decontamination of air and other effluents produced in the facility.

## **5.3 LABORATORY CONTAINMENT REQUIREMENTS**

### **5.3.1 CL1**

#### **PHYSICAL REQUIREMENTS**

- A room separated from public areas by a door is required. There are no particular restrictions on locating the facility near public or heavily traveled corridors, however doors should remain closed.



- Coatings on walls, ceilings, furniture, and floors should be cleanable. Windows that can be opened should not be near working areas or containment equipment and should be equipped with fly screens.
- There are no special air handling requirements beyond those concerned with proper functioning of the biological safety cabinets, if used, and those required by building codes.
- Hand washing facilities must be provided, preferably near the point of exit to public areas.
- Separate areas should be provided for hanging street clothing and laboratory coats.
- Eye wash stations may be required by local statute.

## **OPERATIONAL REQUIREMENTS**

- The general laboratory safety practices described in Section 2 must be followed. In addition, where chemical disinfection procedures are employed, effective concentrations and contact times must be used. Chemical disinfectants used to decontaminate materials to be removed from the laboratory must be replaced regularly.

### 5.3.2 CL2

In addition to the requirements of Containment Level 1, the following are required.

#### PHYSICAL REQUIREMENTS

- The laboratory should be located away from public areas, general offices, and patient care areas.
- A biohazard sign with appropriate information must be posted on the entrance to the laboratory.
- Laboratory furnishings and work surfaces should be impervious and readily cleanable.
- Coat hooks must be provided for laboratory coats near the exit.
- An autoclave must be available in or near the laboratory.
- Laboratory doors should be self-closing.

#### OPERATIONAL REQUIREMENTS

- Class I or II biological safety cabinets are required for all manipulations of agents that may create an aerosol. The biological safety cabinet must have been tested and certified within the previous 12 months according to accepted standards.
- Inspection and retesting is mandatory if the cabinet is relocated. Minor relocations may be exempt if the move is supervised by the testing technologist, to ensure that the equipment has not been subjected to undue stress. At the time certification is carried out, the testing technologist should ascertain that the users are familiar with the containment capability of the equipment under various operating conditions. The testing technologies should also familiarize users with precautions to be taken in its use.
- Air from these cabinets may be recirculated to the room only after passage through a high efficiency particulate air (HEPA) filter.
- Centrifugation must be conducted with closed containers or aerosol proof safety heads or cups. These should be opened only in the biological safety cabinet.
- Animals or insects that have been experimentally infected must remain in the laboratory or appropriate animal containment facility.

- An emergency plan for handling spills of infectious materials must be developed and be ready for use whenever needed. Laboratory workers must be educated about the emergency plans.
- Vacuum lines used for work involving the agent must be protected from contamination by HEPA filters or equivalent equipment.
- A laboratory coat, to be worn only in the laboratory area, is required. Coats that fasten on the front are permissible. These coats shall not be worn outside the containment laboratory.
- Special care should be taken to avoid contamination of the skin with infectious materials. Gloves must be worn when handling infected animals or when skin may be exposed to infectious materials.
- Contaminated glassware must not leave the facility. Decontamination must be carried out using procedures demonstrated to be effective. If there is no autoclave or incinerator in the laboratory, contaminated materials must be disinfected chemically or be double bagged and transported to the autoclave or incinerator in durable, leak proof containers which are closed and wiped on the outside with disinfectant before leaving the laboratory.
- Service personnel and cleaning staff that enter the facility must be informed of the hazards that might be encountered. Cleaning staff should clean only the floors. The laboratory personnel have the responsibility for rendering the facility safe for routine cleaning. Periodic intensive cleaning must be done at regular intervals. Cleaning and maintenance staff should receive appropriate immunization and medical surveillance.

**The Biosafety Committee should ensure a self-inspection for the CL2 laboratory is performed before any students or workers start working in the CL2 microbiology laboratory. The CL2 checklist set out by the Public Health Agency of Canada can be obtained at <http://www.phac-aspc.gc.ca/ols-bsl/containment/index.html>. Note that the self-inspection report may be subject to verification by Public Health Agency of Canada inspectors at any time.**

## 5.4 RISK ASSESSMENT

The goal of the risk assessment is to confirm that the containment level of the existing facility is adequate for the type of microorganism and the type of work being conducted. It is mandatory to conduct a risk assessment for each hazard that will be used in the microbiology laboratory at Kwantlen Polytechnic University. Risk assessment should be carried out by a number of individuals with different responsibilities and varieties of expertise. These may include but are not limited to a laboratory supervisor, senior microbiologist, the Biosafety committee, and/or a principal investigator. All students, laboratory workers and supervisors must be aware of the potential hazards of working with a particular organism or microbe.

Part 1 of a risk assessment is to identify the hazard by determining the following:

### Step 1: Identify the risk

- What is the identity (name) of the biohazard? (e.g. *Aspergillus niger*)
- Is it infectious to humans/animals, or both? Is the animal a reservoir of the agent?

### Step 2: Quantify the risks which may include but not be limited to:

- What is the recommended Risk Group of the organism (1 to 4 according to Health Canada biosafety guidelines)
- What are the quantity and/or concentration of the infectious material being used?
- How stable is the infectious agent (inherent biological decay rate)?
- What is the infectious dose of the particular agent?
- What is the mode of infection?
- What is the route of administration?
- What is the potential for aerosol generation?

### Step 3: Consider other factors which may include but not be limited to:

- What is the condition of the host?
- Is a vaccine available, how effective is it?
- Is there any drug therapy available at the moment (ie. antibiotics)?
- What type of work is proposed (*in vitro*, *in vivo*, aerosol challenges?)
- Will there be use of recombinant organisms?



- What is the skill level of the handlers?

Information about the microorganisms used in the microbiology laboratory may be available on ATCC website <http://www.atcc.org/Home.cfm>. Material Safety Data Sheets for infectious microorganisms (biological agents) are also available from the Public Health Agency of Canada website <http://www.phac-aspc.gc.ca/msds-ftss/index.html>.

Part 2 of risk assessment is to quantify the risks identified in Part 1 and assign risk values to them in order to be able to rank different kinds of risks and probabilities of adverse events.

A weighting system is proposed to methodically assess the risk associated with the handling and manipulation of different microbiological strains. The weighting system is based on the *likelihood* of personnel being infected by an agent. The weighting system is outlined in Table 4 and should be used for every microbial strain that is introduced into the facility. A series of risk parameters from Step 1 are listed along with assigned score rankings in the second column. The higher the likelihood of infection, the higher the assigned score rankings. For each risk parameter, a score is assigned and scores are totaled to produce a final score value.

The final score value is compared to three categories in Table 5 and provides a rational assessment of the risk to human health in the CL-2 facility.

The list of outlined risk parameters in Table 4 is not necessarily complete and the assigned score rankings are not necessarily fixed. Table 4 is meant to serve as a starting point to conduct routine risk assessment for each microbiological strain that is used in the CL-2 laboratory. The score categories shown in Table 5 are also not necessarily fixed. ***When conducting an actual risk assessment for a new microorganism that is intended to be brought into Kwantlen, the adequacy of the content in Table 4 and Table 5 should be carefully reviewed. Consideration of all aspects of the activities, facilities, equipment, personnel and biological agents should be included.***

It should be noted that all weighting systems are subjective to a large extent, and professional judgment plays an important part in allocating ranks or scores to specific weighting criteria.

**Table 4** Risk Assessment and Weighting System

Microorganism (Latin name and/or ATCC identifier): _____		
Risk Parameter	Score Ranking	Score
Is the strain infectious to humans?, animals, or both?	Animals = 2 Humans = 5 Both = 7	
What is the agent's risk group/level as defined by ATCC or Health Canada? If it is higher than level 2, not permitted to be used in the CL-2 facility.	L1 = 2 L2 = 5 L3 = N/A L4 = N/A	
What is the amount/volume of material being used?	Low = 3 Med = 5 High = 7	
What is the infectious dose of the strain? This requires a literature search to determine.	No infectious dose known = 1 Usage below infectious dose = 3 Usage above infectious dose = 10	
What is the portal of entry of the strain and for each portal, what is the likelihood of the portal of entry being available?	Oral consumption = 5  Inhalation = 5  Exposure through an open wound = 10  Contact with mucous membranes = 10	
What is the potential for aerosol generation? This would depend on the projected manipulations that will be taking place in the facility, ie. pipeting, centrifugation	Low = 3 Medium = 5 High = 7	
What is the agent's stability?	Low = 3 Med = 5 High = 7	
Is there a vaccine available for treatment?	Yes = 3 No = 10	
Are there other treatments available ie. Antibiotics?	Yes = 3 No = 10	
What are the skill level of handlers (ie. Students and staff)?	More experience = 3 Little experience = 8	
	<b>Total score</b>	

**Table 5** Total score ranking

<b>Total Score</b>	<b>Risk assessment conclusion</b>
<49	CL-2 containment for this microorganism is adequate and poses minimal risk to staff and personnel. Facilities and procedures are sufficient
50 – 65	CL-2 containment for this microorganism poses moderate risk to staff and personnel. Facilities and procedures are sufficient to handle any accidents or biohazard exposure, which may occur.
66+	CL-2 containment for this microorganism is not adequate for sufficiently reducing the risk of accidental infection to personnel and staff. Risk to staff and personnel are unacceptable.

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Appendix A: Class II Type A2 Biological Safety Cabinet

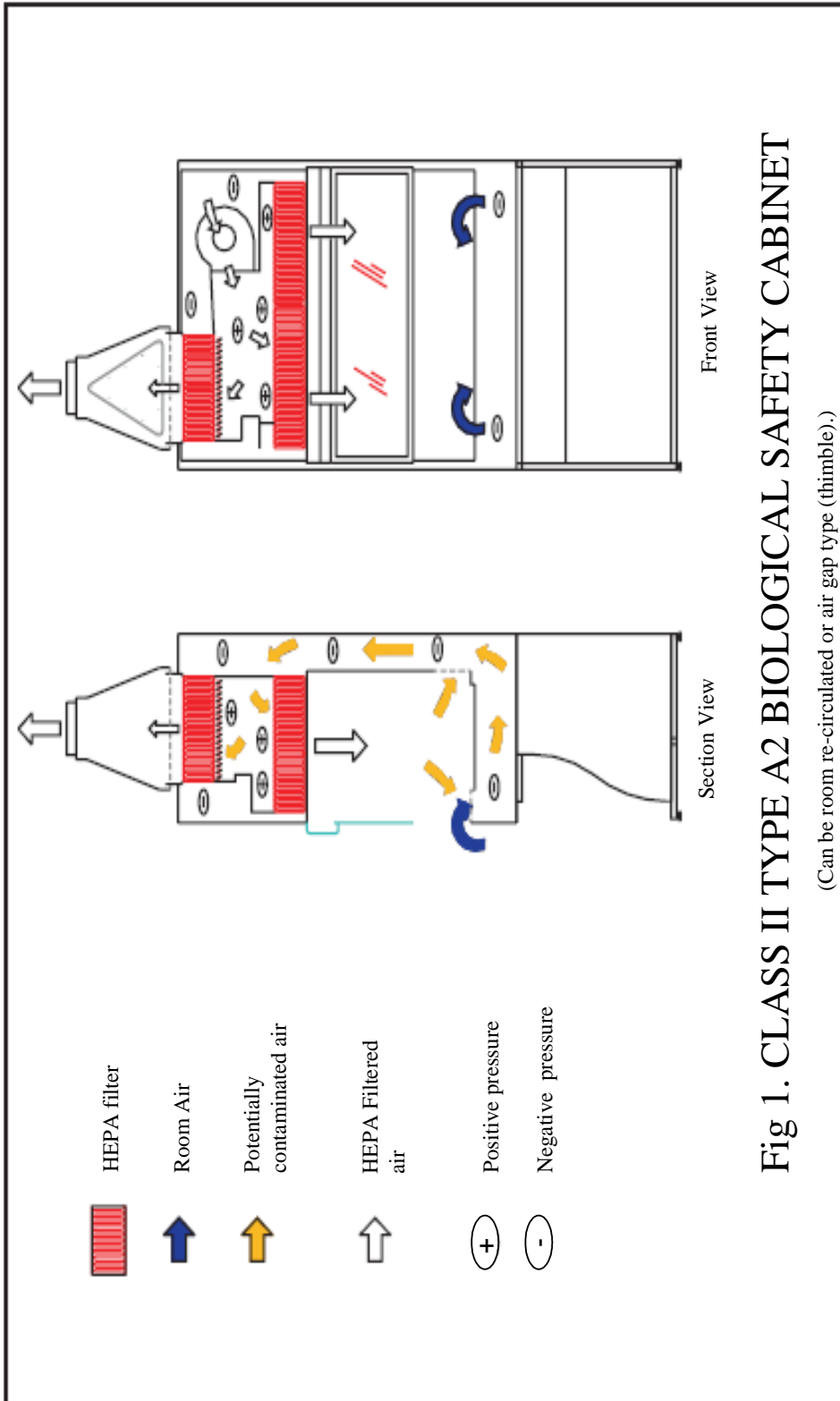


Fig 1. CLASS II TYPE A2 BIOLOGICAL SAFETY CABINET

(Can be room re-circulated or air gap type (thimble).)

## Appendix B: Risk Group Agent list

### RISK GROUP 1 AGENTS REQUIRING CONTAINMENT LEVEL 1

(low individual and community risk)

This group includes those microorganisms, bacteria, fungi, viruses and parasites that are unlikely to cause disease in healthy workers or animals.

Many agents are referred to in the literature by a variety of names and, before assuming that an unlisted agent is assigned to Risk Group 1, its characteristics and pathogenicity must be verified in consultation with Kwantlen Polytechnic University Biosafety Committee.

### RISK GROUP 2 AGENTS REQUIRING CONTAINMENT LEVEL 2

(moderate individual risk, limited community risk)

A pathogen that can cause human or animal disease but, under normal circumstances, is unlikely to be a serious hazard to healthy laboratory workers, the community, livestock or the environment. Laboratory exposures rarely cause infection leading to serious disease. Effective treatment and preventive measures are available and the risk of spread is limited.

#### **BACTERIA, CHLAMYDIA, MYCOPLASMA**

Actinobacillus: all species

Actinomyces pyogenes (C. pyogenes)

Bacillus cereus

Bartonella bacilliformis, B. henselae, B. quintana, B. elizabethae

Bordetella pertussis, B. parapertussis and B. bronchiseptica

Borrelia recurrentis, B. burgdorferi

Campylobacter spp: C. coli, C. fetus, C. jejuni

Chlamydia pneumoniae, C. psittaci (non-avian strains), C. trachomatis

Clostridium botulinum, Cl. chauvoei, Cl. difficile, Cl. haemolyticum,

Cl. histolyticum, Cl. novyi, Cl. perfringens, Cl. septicum, Cl. sordellii, Cl. tetani

Corynebacterium diphtheriae, C. haemolyticum, C. pseudotuberculosis,

C. pyogenes (A. pyogenes)

Edwardsiella tarda  
The logo for PE PharmEng Technology Inc. features the letters 'PE' in a large, bold, blue font. To the right of 'PE', the words 'PharmEng' are written in a smaller, blue, sans-serif font, with 'Pharm' on the top line and 'Eng' on the bottom line. Below 'PharmEng', the words 'Technology Inc.' are written in a very small, blue, sans-serif font.

Erysipelothrix rhusiopathae (insidiosa)  
Escherichia coli: enterotoxigenic / invasive / hemorrhagic strains  
Francisella tularensis Type B, (biovar palaeartica), F. novocida  
Fusobacterium necrophorum  
Haemophilus influenzae, H. ducreyi  
Helicobacter pylori  
Legionella spp.  
Leptospira interrogans: all serovars  
Listeria monocytogenes  
Mycobacteria: all species except M. tuberculosis and M. bovis (non-BCG strain), which are in Risk Group 3  
Mycoplasma pneumoniae, M. hominis  
Neisseria gonorrhoeae, N. meningitidis  
Nocardia asteroides, N. brasiliensis  
Pasteurella: all species except P. multocida type B, which is in Risk Group 3  
Pseudomonas aeruginosa  
Salmonella enterica (S. choleraesuis)  
Salmonella enterica serovar arizonae (Arizona hinshawii)  
Salmonella enterica serovar gallinarum-pullorum (S. gallinarum-pullorum)  
Salmonella enterica serovar meleagridis (S. meleagridis)  
Salmonella enterica serovar paratyphi B (S. paratyphi B) (Schottmulleri)  
Salmonella enterica serovar typhi (S. typhi)  
Salmonella enterica serovar typhimurium (S. typhimurium)  
Shigella boydii, S. dysenteriae, S. flexneri, S. sonnei  
Staphylococcus aureus  
Streptobacillus moniliformis  
Streptococcus spp: Lancefield Groups A, B, C, D, G  
Treponema carateum, T. pallidum (including T. pertenue), T. vincentii  
Ureaplasma urealyticum  
Vibrio cholerae (including El Tor), V. parahaemolyticus, V. vulnificus  
Yersinia enterocolitica, Y. pseudotuberculosis

## **FUNGI**

Cryptococcaceae



Candida albicans  
Cryptococcus neoformans  
Moniliaceae  
Aspergillus flavus  
Aspergillus fumigatus  
Epidermophyton floccosum  
Microsporum spp.  
Sporothrix schenckii  
Trichophyton spp.

## **VIRUSES**

Arthropod-borne viruses are identified with an asterisk (\*). Only those viruses, which may be associated with human or animal disease, have been included in this list. Agents listed in this group may be present in blood, CSF, central nervous system and other tissues, and infected arthropods, depending on the agent and the stage of infection.

### Adenoviridae

Adenoviruses: all serotypes

### Arenaviridae

Lymphocytic choriomeningitis virus: laboratory adapted strains

Tacaribe virus complex: Tamiami, Tacaribe, Pichinde

### Bornaviridae

Borna disease virus

### Bunyaviridae\*

Genus Bunyavirus

Bunyamwera and related viruses

California encephalitis group, including LaCrosse, Lumbo and

Snowshoe hare virus


Genus Phlebovirus: all species except Rift Valley fever virus

Caliciviridae: all isolates, including Hepatitis E and Norwalk virus

### Coronaviridae

Human coronavirus: all strains

Genus Torovirus

 Transmissible gastroenteritis virus of swine  
Technology Inc.

Hemagglutinating encephalomyelitis virus of swine

Mouse hepatitis virus

Bovine coronavirus

Feline infectious peritonitis virus

Avian infectious bronchitis virus

Canine, Rat and Rabbit coronaviruses

#### Flaviviridae\*

Yellow fever virus: 17D vaccine strain

Dengue virus: serotypes 1, 2, 3, 4

Kunjin virus

Hepatitis C virus

#### Hepadnaviridae

Hepatitis B virus, including Delta agent

#### Herpesviridae

Alphaherpesvirinae

Genus Simplexvirus: all isolates including HHV 1 and HHV 2, except Herpes B virus which is in Risk Group 4

Genus Varicellavirus: all isolates including varicella / zoster virus (HHV 3) and pseudorabies virus

Beta herpesvirinae

Genus Cytomegalovirus: all isolates including CMV (HHV 5)

Genus Muromegalovirus: all isolates

Gamma herpesvirinae

Genus Lymphocryptovirus: Epstein Barr Virus (HHV 4) and EB-like isolates

Genus Rhadinovirus: all isolates except H. ateles and H. saimiri in Risk Group 3

Genus Thetalympocryptovirus: all isolates

Unassigned Herpes viruses: includes HHV 6 (human B-lymphotrophic virus), HHV 7, HHV 8, etc.

#### Orthomyxoviridae

Genus Influenzavirus:

Influenza virus type A: all isolates

Influenza virus type B: all isolates

Influenza virus type C: all isolates

#### Papovaviridae



Genus Papillomavirus: all isolates

Genus Polyomavirus: all isolates

Paramyxoviridae

Genus Morbillivirus: all isolates except Rinderpest virus

Genus Paramyxovirus: all isolates

Genus Pneumovirus: all isolates

Parvoviridae

Genus Parvovirus: all isolates

Picornaviridae

Genus Aphthovirus:

Genus Cardiovirus: all isolates

Genus Enterovirus: all isolates

Genus Hepatovirus: all isolates (Hepatitis A)

Genus Rhinovirus: all isolates

Poxviridae

Chordopoxvirinae (poxviruses of vertebrates)

Genus Avipoxvirus: all isolates

Genus Capripoxvirus:

Genus Leporipoxvirus: all isolates

Genus Molluscipoxvirus

Genus Orthopoxvirus: all isolates except Variola virus and Monkeypox virus that are  
in Risk Group 4

Genus Parapoxvirus: all isolates

Genus Suipoxvirus: Swinepox virus

Genus Yatapoxvirus

All other ungrouped poxviruses of vertebrates

Reoviridae

Genus Orbivirus: all isolates


Genus Orthoreovirus: types 1, 2 and 3

Genus Rotavirus: all isolates

Retroviridae

Oncovirinae

Genus Oncornavirus C

 Subgenus Oncornavirus C avian: all isolates

Subgenus Oncornavirus C mammalian: all isolates except HTLV-I and HTLV-II

Genus Oncornavirus B: all isolates

Lentivirinae: all isolates except HIV-I and HIV-II

Spumavirinae: all isolates

#### Rhabdoviridae

Genus Vesiculovirus: all laboratory-adapted strains

Genus Lyssavirus: Rabies virus (fixed virus)

#### Togaviridae

Genus Alphavirus\*

Semliki forest virus

Sindbis virus

Chikungunya virus: high-passage strains

O'Nyong-Nyong virus

Ross river virus

Venezuelan equine encephalitis virus: only strain TC-83,  
no animal inoculation

Genus Rubivirus

Rubella virus

Genus Pestivirus

Bovine diarrhoea virus

Border disease virus

Genus Arterivirus

Equine arteritis virus

#### Unclassified viruses

Other Hepatitis viruses

Astro viruses

Chronic infectious neuropathic agents (CHINAs): Scrapie, BSE  
(except Kuru and Creutzfeldt-Jakob Disease agents in Risk Group 3)

## PARASITES

Infective stages of the following parasites have caused laboratory infections by ingestion, skin or mucosal penetration or accidental injection. Preparations of these parasites known to be free of infective stages do not require this level of containment.



## PROTOZOA

Babesia microti  
Babesia divergens  
Balantidium coli  
Cryptosporidium spp.  
Entamoeba histolytica  
Giardia spp. (mammalian)  
Leishmania spp. (mammalian)  
Naegleria fowleri  
Plasmodium spp. (human or simian)  
Pneumocystis carinii  
Toxoplasma gondii  
Trypanosoma brucei, T. cruzi

## HELMINTHS

### Nematodes

Ancylostoma duodenale  
Angiostrongylus spp.  
Ascaris spp.  
Brugia spp.  
Loa loa  
Necator americanus  
Onchocerca volvulus  
Strongyloides spp.  
Toxocara canis  
Trichinella spp.  
Trichuris trichiura  
Wuchereria bancrofti

### Cestodes

Echinococcus (gravid segments)  
Hymenolepis diminuta  
Hymenolepis nana (human origin)



Taenia solium

Trematodes

Clonorchis sinensis

Fasciola hepatica

Opisthorchis spp.

Paragonimus westermani

Schistosoma haematobium

Schistosoma japonicum

Schistosoma mansoni

## **RISK GROUP 3 AGENTS REQUIRING CONTAINMENT LEVEL 3**

(high individual risk, low community risk)

A pathogen that usually causes serious human or animal disease, or which can result in serious economic consequences but does not ordinarily spread by casual contact, from one individual to another, or that can be treated by antimicrobial or antiparasitic agents.

### **BACTERIA, CHLAMYDIA, RICKETTSIA**

Bacillus anthracis

Brucella: all species

Burkholderia (Pseudomonas) mallei, B. pseudomallei

Chlamydia psittaci: avian strains only

Coxiella burnetii

Francisella tularensis type A (biovar tularensis)

Mycobacterium bovis: non-BCG strains

Mycobacterium tuberculosis<sup>1</sup>

Pasteurella multocida, type B

Rickettsia: all species

Yersinia pestis

<sup>1</sup>Note: Preparation of smears and primary culture of M. tuberculosis may be performed at Level 2 physical containment using Level 3 operational procedures and conditions. All other manipulations of M. tuberculosis require Containment Level 3 physical and operational conditions.

### **FUNGI**

Moniliaceae

Ajellomyces capsulatum (Histoplasma capsulatum, including var. duboisii)

Ajellomyces dermatitidis (Blastomyces dermatitidis)

Coccidioides immitis

Paracoccidioides brasiliensis

### **VIRUSES**

Arthropod-borne viruses are identified with an asterisk (\*).

Arenaviridae

 Lymphocytic choriomeningitis virus: neurotropic strains

## Bunyaviridae

Unclassified Bunyavirus

Hantaan, Korean haemorrhagic fever and epidemic nephrosis viruses including

Hantavirus pulmonary syndrome virus

Rift Valley fever virus

## Flaviviridae\*

Yellow fever virus: wild type

St. Louis encephalitis virus

Japanese encephalitis virus

Murray Valley encephalitis virus

Powassan encephalitis virus

## Herpesviridae

Gammaherpesvirinae

Genus Rhadinovirus: Herpesvirus ateles, Herpesvirus saimiri

## Retroviridae

Oncovirinae

Genus Oncornavirus C

Human T-cell leukemia / lymphoma virus2

Genus Oncornavirus D

Mason-Pfizer monkey virus

Viruses from non-human primates

Lentivirinae

Human immunodeficiency viruses (HIV): all isolates2

## Rhabdoviridae

Genus Vesiculovirus: wild type strains

Genus Lyssavirus

Rabies virus (street virus)

## Togaviridae

Genus Alphavirus\*

Eastern equine encephalitis virus

Chikungunya virus

Venezuelan equine encephalitis virus (except Strain TC-83)

Western equine encephalitis virus

## Unclassified Viruses



Chronic infectious neuropathic agents: Kuru, Creutzfeldt-Jakob Disease agents (level of precautions depends on the nature of the manipulations and the amount of sera, biopsy / necropsy materials handled)

Laboratories engaging in primary isolation and identification of HTLV or HIV may perform these activities in Containment Level 2 laboratories (physical conditions) using Containment Level 3 operational procedures and conditions. All research and production activities require Containment Level 3 physical and operational conditions.

## **PARASITES**

None

## **RISK GROUP 4 AGENTS REQUIRING CONTAINMENT LEVEL 4**

(high individual risk, high community risk)

A pathogen that usually produces very serious human or animal disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice-versa, directly or indirectly, or by casual contact.

### **BACTERIA**

None

### **FUNGI**

None

### **VIRUSES**

Arthropod-borne viruses are identified with an asterisk (\*).

#### Arenaviridae

Lassa, Junin, Machupo, Sabia, Guanarito viruses

#### Bunyaviridae\*

Genus Nairovirus

Crimean-Congo hemorrhagic fever virus

#### Filoviridae

Marburg virus

Ebola virus

#### Flaviviridae\*

Tick-borne encephalitis complex including Russian Spring-Summer encephalitis virus

Kyasanur forest virus

Omsk hemorrhagic fever virus

#### Herpesviridae

Alphaherpesvirinae

Genus Simplexvirus: Herpes B virus (Cercopithecine herpesvirus 1)

#### Poxviridae

Genus Orthopoxvirus



Variola virus

Monkeypox virus

## **PARASITES**

None

## Appendix C: Reference

1. Laboratory Biosafety Guidelines, Health Canada, 2004, 3<sup>rd</sup> edition, Ottawa.
2. Laboratory Biosafety Manual, WHO, 1993, 2<sup>nd</sup> edition, Geneva.
3. Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH, 1999, 4<sup>th</sup> edition, Washington.

## Appendix D: Kwantlen Polytechnic University Policies



































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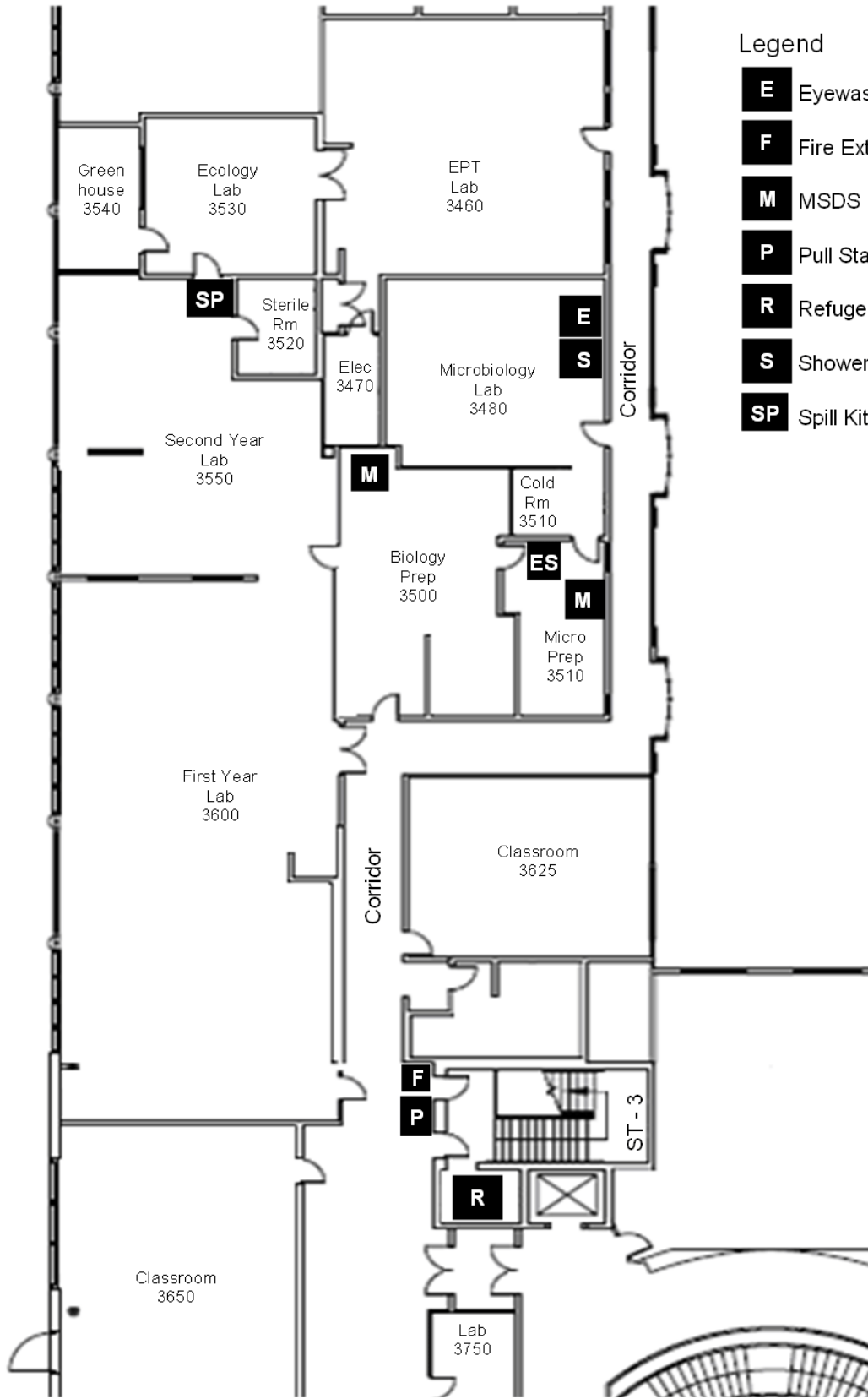
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Legend

- E** Eyewash
- F** Fire Extinguisher
- M** MSDS
- P** Pull Station
- R** Refuge
- S** Shower
- SP** Spill Kit