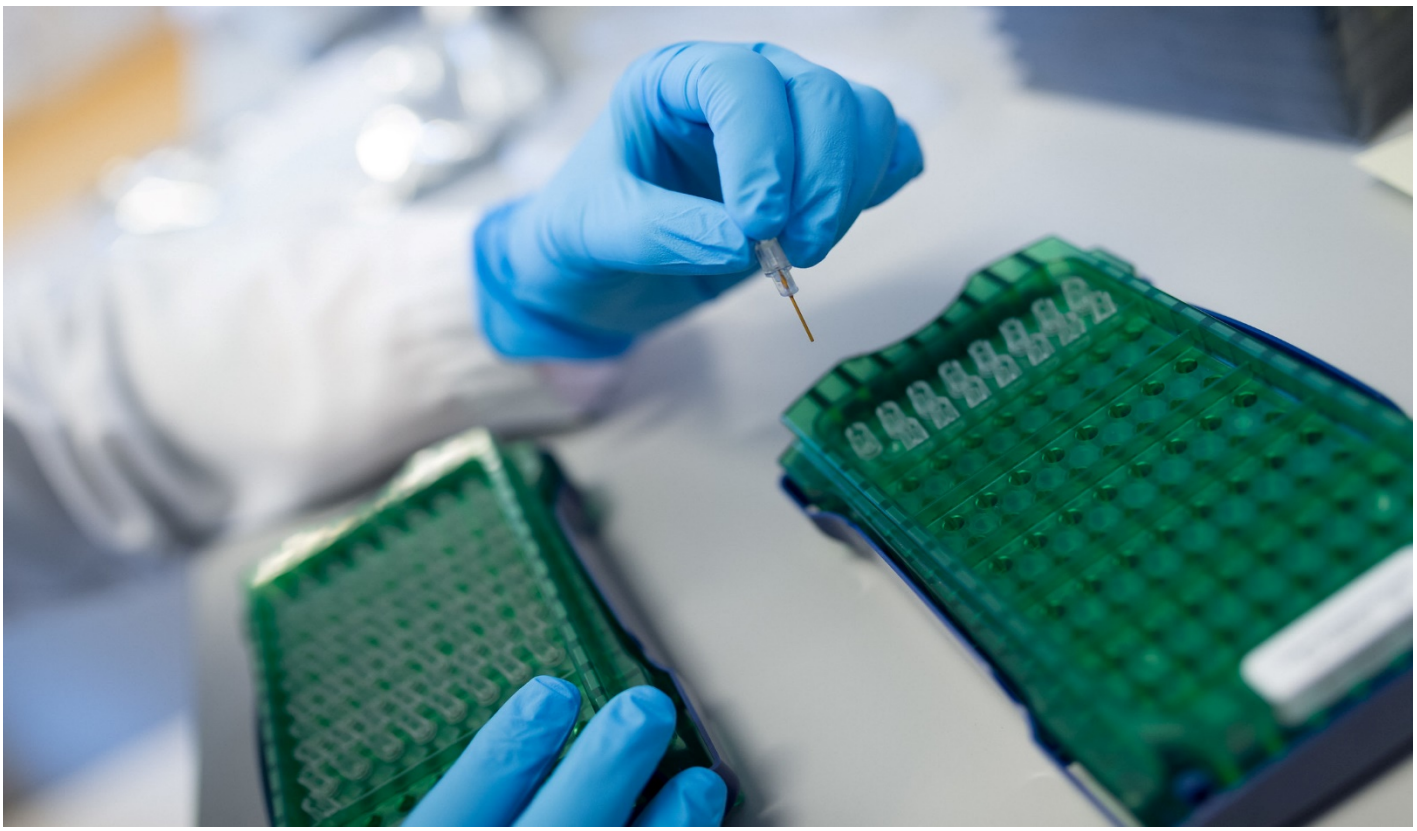
UBC Biosafety User Reference Manual 2020



THE UNIVERSITY OF BRITISH COLUMBIA

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UBC Biosafety Office	822-4353
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Forward

This manual has been developed by the Office of Biosafety, Safety & Risk Services, UBC, with the endorsement of the University's Biosafety Committee. It is intended to provide information to protect workers and the surrounding environment from possible exposure to biohazardous agents. The information also serves to protect experiments and research by controlling the unwanted spread of contamination.

The premise is that no experiment should be considered so important as to jeopardize the wellbeing of the worker or the environment. The planning and implementation of safety practices to prevent laboratory-acquired infections and to eliminate the spread of contamination must be part of every laboratory's routine activities.

The handling of biological agents and recombinant DNA requires the use of precautionary measures dependent on the agents involved and the procedures being performed. It is the purpose of this manual to provide background information and guidelines to be used in conjunction with other resources for the evaluation, containment and control of biohazardous materials in the research laboratory.

Implementation of these procedures is the responsibility of the Principal Investigator and depends largely on the efforts of laboratory supervisors and employees. It is essential to seek additional advice and training when needed to conduct research in a manner which is safe to employees, students and the surrounding community. To assist in this, the services and resources of the Safety & Risk Services are available. The Biosafety Advisor can be reached at 604.822.4353.

University of British Columbia SC-1

Purpose

To articulate the University's objective of providing a safe, healthy and secure environment for all members of faculty and staff, students and visitors, and to delineate responsibility for achieving it.

Policy

The University aims to provide a safe, healthy and secure environment in which to carry on the University's affairs. All possible preventive measures are taken to eliminate accidental injuries, occupational diseases and risks to personal security.

Compliance with the Workers' Compensation Act, WHMIS and related legislation is the minimum standard acceptable. All students and members of faculty and staff are encouraged to strive to exceed these minimum legal standards and to eliminate unnecessary risks.

Definitions

An administrative head of unit is a Director of a service unit, a Head of an academic department, a Director of a center, institute or school, a Principal of a college, a Dean, an Associate Vice President, the Registrar, the University Librarian, a Vice President or the President.

A supervisor is a person, not necessarily an administrative head of unit, who has been delegated supervisory responsibility for others working or studying at UBC.

A worker is a person who is an employee, student or volunteer for the University of British Columbia.

Duties and Responsibilities

The University

It is the responsibility of the University acting through administrative heads of unit to:

- provide a safe, healthy and secure working environment;
- ensure regular inspections are made and take action as required to improve unsafe conditions;
- ensure that health, safety, and personal security considerations form an integral part of the design, construction, purchase and maintenance of all buildings, equipment and work processes;
- provide first aid facilities where appropriate;
- support supervisors and safety committees in the implementation of an effective health, safety and security program;
- ensure compliance with WorkSafeBC, Public Health Agency of Canada, Canadian Food Inspection Agency, and other applicable legislation;
- establish department or building safety committees;
- communicate with the university community or affected groups about events or situations when potentially harmful conditions arise or are discovered;

- ensure adequate resources are available to implement appropriate procedures.

The Supervisor

It is the responsibility of supervisory staff to:

- formulate specific safety rules and safe work procedures for their area of supervision;
- ensure that all employees under their supervision are aware of safety practices and follow safety procedures;
- provide training in the safe operation of equipment;
- inspect regularly their areas for hazardous conditions;
- correct promptly unsafe work practices or hazardous conditions;
- be responsive to concerns expressed about personal security and investigate any accidents, incidents or personal security concerns which have occurred in their area of responsibility;
- report any accidents or incidents involving personal security to the appropriate University authority; participate, if requested, on department or building safety committees.

Individual Students and Members of Staff and Faculty

It is the responsibility of individual students and members of faculty and staff to:

- observe safety rules and procedures established by supervisory staff, administrative heads of unit and the University;
- be safety-conscious in all activities, be they work, study or recreation;
- report as soon as possible any accident, injury, unsafe condition, insecure condition or threats to personal security to a supervisor or administrative head of unit;
- use and care for properly personal protective equipment provided by the University; participate, if elected or appointed, on departmental or building safety committees.

Detailed Procedures

Joint Health and Safety Committees work to achieve these objectives by providing education and reviewing policies and procedures. Local Safety Teams carry out the safety programs within their areas and make recommendations to ensure that the safety objectives of the University can be achieved. The terms of reference for these committees are available through the Department of Safety & Risk Services.

The Department of Safety & Risk Services and UBC Campus Security assist departments to implement and maintain effective health, safety and personal security programs, liaise with the regulatory authorities on behalf of the University and support the activities of the University's Safety Committees.

For more information, please consult with the Department of Safety & Risk Services and/or the UBC Campus Security.

Introduction

The University of British Columbia is committed to providing a safe and healthy workplace for its faculty, staff, and students and to ensure the protection of the community and the environment. To meet this commitment the Department of Safety & Risk Services has developed and implemented numerous health and safety programs.

The University Health and Safety Policy states that "the University shall be administered so as to ensure that health, safety and accident prevention form an integral part of the design, construction, purchase and maintenance of all buildings, equipment and work processes". These practices are not only concerned with the safety and health of faculty, staff and students but also of primary importance is the protection of the community and the environment.

Human error and poor laboratory practice can compromise the best of laboratory safeguards designed specifically to protect the laboratory worker. The primary factor in the prevention of laboratory accidents and laboratory associated infections is a fully trained faculty and staff. To accomplish this it is essential that faculty and staff receive the appropriate training in laboratory safety measures.

The Department's Biosafety Program has been developed to meet this need and to ensure compliance with all federal, provincial and local standards and regulations. Its purpose is to ensure the safe handling of biohazardous materials in all research and teaching facilities under the auspices of the University of British Columbia. The main focus of the program is the protection of faculty, staff and students. Ways to protect research and the environment are also considered.

Definition of Biosafety

Biosafety or Biological Safety encompasses all aspects of containment to prevent any exposure to and accidental release of infectious biological material. This also includes the containment of plants and animals.

Definition of Biosecurity and Writing a Plan

A biosecurity plan is implemented to prevent theft, misuse or intentional release of pathogens. A pathogen is defined as an agent that can cause disease in humans or animals. The type of biosecurity plan that is created and implemented will depend on the nature of the facility, the type of research and diagnostics conducted and the local environment. Personnel from varying levels of administration can be involved in the creation of the biosecurity plan. Key features of a biosecurity plan should include facility security, inventory of pathogens and emergency protocols for security incidents.

Facility security

In this part of the plan, strategies used to prevent the entry of unauthorized personnel and the theft of pathogens must be examined. For instance, access to pathogens is restricted somehow (i.e. kept under lock and key) and the facility has specific security protocols in place to minimize

the entry of unauthorized personnel (i.e. Key card access, identity badges, protocols for locking doors).

Inventory of Pathogens

When working with pathogens, labs must have an inventory of all pathogens that indicates the risk group of each item. Depending on the risk associated with the pathogen varying levels of information is necessary for the inventory. Access to the pathogens must be restricted to specific laboratory personnel and a tracking system must be established to determine if vials are unaccounted for. Personnel who do have access to the pathogens must be documented and kept on file. For security sensitive microbial toxins, an ongoing inventory must be kept for each vial. More information on inventories can be found here.

Emergency Protocols for security incidents

In those cases where there have been unauthorized personnel entering the building or pathogen samples stolen, misused or intentionally released, an emergency protocol must be in place. In this protocol, it must state a clear procedure on who needs to be contacted about the theft or unauthorized entry (i.e. Supervisor, security, law enforcement agencies, SRS etc.)

Definition of a Biohazard

There is a distinction between biological materials and biohazardous materials. Biological materials encompasses all materials containing genetic information and capable of replication. Included in this are viruses and prions. Biohazardous materials are defined as infectious agents or hazardous biological materials that present a risk or potential risk to the health of humans, animals to the environment. It should be noted that the following terms are used interchangeably throughout this document: biohazard, infectious material, infectious agent, and pathogen. The risk can be direct through infection or indirect through damage to the environment. Biohazardous agents can be classified into 10 groups:

Bacteria

This single celled organism lacks a nucleus and is classified in three phenotypes: Gram-positive (cocci or bacilli), Gram-negative (cocci or bacilli), and mycoplasmas (those that lack a cell wall). When a bacterium causes harm and disease in human and/or animal hosts, they are referred to as bacterial pathogens. Some examples include: *Escherichia coli, Mycobacterium tuberculosis, Staphylococcus aureus*, and *Bacillus anthracis.*

Viruses

These smallest of the replicating organisms have no metabolism of their own and are obligate parasites that depend on their host's machinery for replication. Viruses are classified according to their replication strategy, structure and the organization of their genome – double stranded DNA, single-stranded DNA, RNA & DNA reverse transcribing, double stranded RNA, negative-sense single-stranded RNA, positive-sense single-stranded RNA and subvirual agents. Some examples include: Influenza virus, Human Immunodeficiency Virus (HIV), Rabies virus, and Ebola virus.

Fungi

These eukaryotic microorganisms are larger in size and have organelles, including a nucleus, vacuoles, and mitochondria. Two main categories, yeast and mold, are known to have species that cause disease in human and/or animal hosts. Various molds have been known to release spores and some toxins, thus increasing the risk to using certain strains. Some examples include: *Aspergillus niger, Candida albicans,* and *Histoplasma capsulatum.*

Parasites

Protozoa and helminthes that live on or within a larger host organism at the host's expense are considered parasites. There are two categories of parasites, endo and ecto-parasites. Endoparasites live within the tissues or cells, but cause infections that are generally treatable. Ectoparasites live on the external surface or within the skin of their host and cause infestation. Some examples of pathogenic protozoa include*: Plasmodium falciparum, Leishmania donovani*, and *Giardia lamblia*. Some examples of pathogenic helminthes include: *Trichinella spiralis* (nematode), *Enterobius vermicularis* (pinworm), and *Hymenolepis nana* (tapeworm).

Zoonotic Pathogens

Zoonotic pathogens and toxins that are capable of infecting both animals and humans. Zoonoses are a greater risk with any activities involving first generation wild-caught animals that may be infected and carry a pathogen indigenous to the animal's natural environment. Some examples include: Salmonellosis, Plague, Rabies, Toxoplasmosis, and prions.

Prions

Prions are small infectious, proteinaceous particles generally accepted to be responsible for causing a number of neurodegenerative diseases known as Transmissible Spongiform Encephalopathies (TSEs) that affect humans and animals. Some examples of TSE in animals include BSE, Kuru, and Scrapie.

Toxins

Toxins are poisonous substances that are produced by bacteria, animals or plants. They are usually active at very low concentration and vary in size. They range from small molecules to larger molecules such as, peptides or proteins. In addition, they vary greatly in their severity, ranging from mildly poisonous to deadly. Some toxins are also able to cause illness upon contact or absorption with body tissues. There are two main classes of toxins, endotoxins and exotoxins. Exotoxins are often heat labile proteins and polypeptides that are produced and secreted or released by a variety of species. Some examples include: Tetanus toxin, Cholera toxin, and Pertussis toxin. Endotoxins are structural molecules that are embedded in the outer layer of the cell wall of certain Gram negative bacteria. There are also some toxins that can be isolated from higher organisms such as fungi, plants, and animals (venom and tetrodotoxin).

Recombinant DNA

Recombinant DNA (rDNA) is formed when combining genetic material from more than one source, natural or synthetic, to produce novel DNA. A gene in its own natural genome may not pose a risk. However, the risk level can change when the gene is combined with another gene or

modified in some way that affects is expression or function. Consequently, modifications that are done to the DNA must be examined and proper assessments must be done to determine potential effects of the modified DNA. There are three kinds of rDNA: Genetically Modified Organisms (GMOs), Viral Vectors, and Synthetic DNA (sDNA).

- A GMO can be as simple as rDNA cloned into a bacterial or viral host to over-express a specific gene for further study. More complex GMOs include transgenic and knock-out animals where their genome is altered by the insertion or removal of DNA segments respectively.
- Viral Vectors are transfer vehicles used to deliver genetic material into host cells for subsequent gene expression. Genetic modifications are typically made to these vectors to improve gene delivery efficiency and to enhance the safety of the system. Some examples include: recombinant Adenovirus and HIV based lentivirus.
- sDNA refers to artificial segments of DNA that are chemically synthesized in vitro without an initial DNA template.

Cell Lines

While cultured cells on their own do not pose a risk to the worker, the biological agents that could potentially be contained within the cell or the media must be considered. In addition, the cell cultures could also contain other infectious agents that could be released into the environment. One of the primary hazards of manipulating any cell line is the expression of latent viruses. Endogenous viral sequences have been found in a variety of cell lines derived from mammalian species, including humans. Additional care should be taken with primary cultures.

Human blood and tissue

Like cultured cells the risk with human blood and tissues is with what they may contain. There are numerous blood borne pathogens such as HIV, hepatitis B & C, and M.tb. More details can be found in the Blood and Body Fluids: Exposure Control Plan. Note that clinical samples collected from infectious patients maybe subject to more stringent containment practices, for instance samples

Viral Vectors – Assessing Risk

Viral vectors are gene delivery systems that exploit the natural abilities of viruses to deliver genetic material into host cells.

There are many different systems for targeting different cell types for different purposes, but the commonly used systems are based on:

- Lentivirus
- Adenovirus (AdV)
- Adeno-associated virus (AAV)
- Retrovirus

The characteristics that impact the risks associated with vector handling and usage include:

The **class of viral envelope**: an indicator of host range:

Ecotropic – narrow

Amphotropic – moderate

Pantropic – wide

Replication deficiency: the viral vector can infect/enter cells but no new viral particles are produced.

Self-inactivation: retroviral systems may have sections of the viral genome deleted, preventing replication competence.

The **gene inserted**: may be benign or a 'hot' gene expected to cause disease or other toxic effect.

The amount of viral genome remaining: the less of the original virus remaining, the safer the system is considered to be.

containing Zika virus, SARS-CoV-2, West Nile virus or HIV.

Biological Substance Types:

Other biological substances many pose an indirect threat to environmental health and thus fall under the oversight of the Biosafety Program.

Invasive Species

Plants, animals and micro-organisms in an area where they are not indigenous which can adapt, spread quickly and do not have natural predators in the new environment. They have potential to cause serious economic or environmental damage. Aegilops cylindrica (jointed goat grass) is an example of an invasive species regulated by CFIA.

Transgenic or Genetically Engineered Organism

A plant, animal or invertebrate in which one or more genes or genetic constructs or traits have been introduced using recombinant DNA techniques (also known as genetic engineering), which could be considered to include the insertion of genetic material from the same or different species.

Plant Pest

Any living organism injurious to plants, plant products or by-products, including insects, mites, diseases, vermin, animals and weeds. Dendroctonus micans (European Spruce Beetle), Tetranychus truncatus Ehara (Spider mite), and Synchytrium endobioticum (Potato wart) are all classified as pests and regulated by CFIA.

Susceptible Species

A species of aquatic animal in which infection has been demonstrated by the occurrence of natural cases or by experimental exposure to the pathogenic agent that mimics natural transmission pathways. Where the pathogenic agent is considered a reportable and immediately notifiable disease by CFIA, acquisition and holding of the aquatic animal may require quarantine measures and facility certification. Examples include Poecilia reticulata (Guppies) and Danio rerio (zebrafish) that are both susceptible to Spring viraemia of carp.

PV Potentially Infected Materials (PIM) - A Global Public Health Risk

Polio is a highly infectious disease transmitted primarily by fecal-oral route that may initially present as fever, fatigue, headache and vomiting. Assymmetric paralysis, in some cases fatal, may occur 1-2 weeks post infection.

The Global Polio Eradication Initiative (GPEI) launched by the World Health Organization in 1998 is the largest public health effort in the history. As of 2015 wild type poliovirus (PV) type 2 has been eradicated. Eradication efforts continue for types 1 and 3. After eradication is complete, a release of PV -infected material into a PV-free community may have devastating public health consequences.

Of significant concern are human and environmental samples collected in times and places from populations where wildtype PV was present or live vaccine (OPV) was in use. Since the majority of PV infections are asymptomatic or present mild symptoms, this potential for PV infection would have likely gone unrecognized at time of collection.

specimens classified as PV PIM would be destroyed. Learn more here.

Human Pathogens and Toxins Act

The Parliament of Canada has passed Bill C-11, the Human Pathogens and Toxins Act (HPTA), which contains prohibitions and requirements relating to the full range of laboratory activities. A new federal program and regulatory framework will be created based on requirements of the Canadian Biosafety Standard, 2nd Edition, 2015 (CBS). The Human Pathogens and Toxins Act enables the Human Pathogens and Toxins Regulations. Under this Act and Regulation, any institution or group working with Risk Group 2 or higher organisms is required to hold a Human Pathogen and Toxin License. On behalf of all UBC-affiliated researchers, UBC holds licenses for Risk Group 2 and Risk Group 3 pathogens and security sensitive microbial toxins under trigger quantities. These licenses stipulate the buildings in which RG2 and RG3 organisms may be handled and stored.

The BSO must be notified of all pathogens imported into or exported out of Canada. The BSO must also be informed of all transfers of regulated human pathogens within Canada between license holders. If you have any questions regarding this process, please contact the following member of the Biosafety Safety Advisor at 604-822-4353.

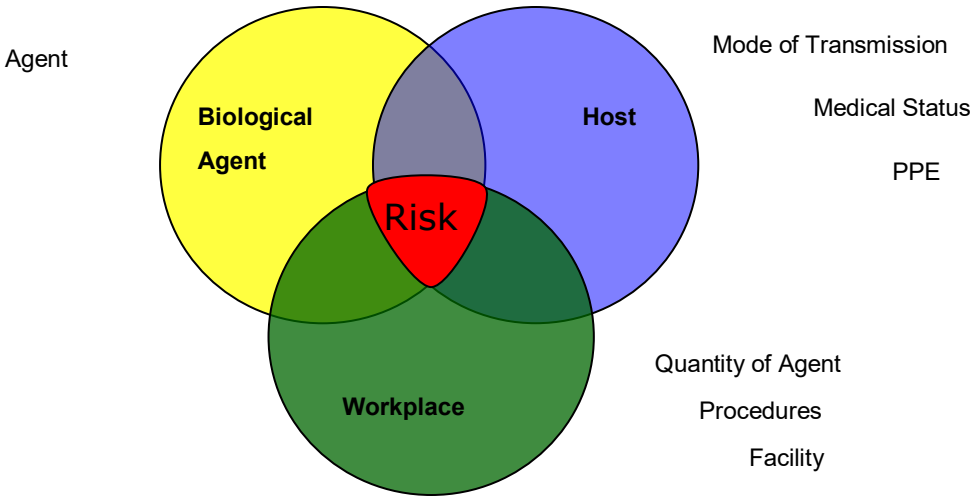
Regulating Bodies

In Canada, there are two Federal regulating bodies for work with biohazardous materials. The Public Health Agency of Canada (PHAC) is the authority of biosafety and biosecurity for human pathogens. The Canadian Food Inspection Agency (CFIA) is the authority of biosafety and biosecurity for animal, plant, and aquatic pathogens. PHAC administers the Human Pathogens & Toxin Regulations and section 160 of the Health of Animals Regulations. The Canadian Biosafety Standard, 2nd Edition, 2015 was developed through collaborations between PHAC and CFIA and is the guiding document for compliance under the act and regulation. The Canadian Biosafety Handbook is a companion document provided to help researchers meet the compliance requirements set out in the Standards. In addition to these publications, there are periodically published Biosafety Advisories to address emerging public health issues. An example of such an advisory concerning SARS-CoV-2, the causative virus for COVID-19, can be found here.

Risk Assessment

Before starting work with any biological agent, a proper risk assessment must be done to determine the appropriate work procedures and containment level in which the work can be safely completed. For this to occur the following must be assessed: Biological agent, Host (or person working with the agent), and the Workplace.

The figure below summarizes how biological agents, the host and the workplace come together to form a risk.



Biological Agent Assessment

Assessment of the biological agent must examine what the agent is and which risk group it belongs to.

Risk Factors

Bacteria, viruses, fungi or other infectious agents are studied because they cause disease. Since many of these agents are pathogenic to humans, animals, or other forms of life, their use in the laboratory poses risks which vary with each agent and the way it is used. PHAC has developed the Canadian Biosafety Handbook which can be used to assist in classifying biological agents into different Risk Groups. Pathogen Safety Data Sheets are available through the PHAC website for well-characterized pathogens that detail the characteristics, classification, and control measures necessary for working safely with the agent. Pathogens are assigned to risk groups by weighing a number of factors associated with the biological agent. The factors used are described below:

Pathogenicity:

Pathogenicity is the ability for biological material (including toxins) to infect a human, animal, or plant host.

Virulence:

Virulence is defined as the severity of the disease that the pathogenic biological agent causes. When examining the severity of the disease, pathogenic biological agents that will cause death are considered to be the most virulent. The duration of the disease is also a factor. Microorganisms causing chronic illness have a greater virulence to those causing acute symptoms.

Infectious Dose:

Infectious dose is defined as the amount of infectious biological material needed to cause disease. Certain biological agents will require a large amount of particles to cause disease, while others only minimal amounts. As such, infectious dose is a crucial risk factor that must be considered when performing risk assessments.

Stability:

Stability is the ability of the biological agent to remain biologically active when outside a host. Certain agents are able to remain infectious for days or weeks when left on the open bench, while other agents degrade and become inactive within minutes.

Route of Infection:

Route of infection is defined as the way the biological agent can infect a host. Typically, biological agents can infect a host through the following routes: inhalation, ingestion, direct inoculation, mucous membrane and skin contact. This risk factor is very important to consider as it helps determine the precautions that must be taken when the agent is being manipulated in the laboratory.

Communicability:

Communicability looks at how easily a microorganism can be passed from one host to another. Agents such as human coronavirus, Mycobacterium pneumoniae and Bordatella pertussis can be passed easily to other hosts through casual contact, while other agents such as HIV, Hepatitis B and Hepatitis C can only be passed through exposure to body fluids. Knowing the communicability of the agent helps in determining the precautions required to prevent its spread to not only other laboratory workers, but to the general public.

Environmental Impact:

Environmental impact examines how the biological agent may affect the general environment if it is released. It is important to not only look at the agents that affect humans, but also those that affect plants and animals. The release of plant or animal pathogens could have both environment and economic detrimental consequences.

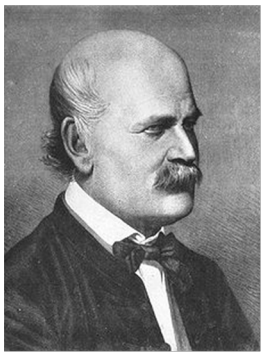
Vaccines:

When working in the laboratory, the risk posed by a biological agent can be alleviated by the existence of vaccines or other preventative measures and therapies. Please consult with the Occupational and Preventive Health Unit prior to performing work with biohazardous materials.

Host Range:

Host range is considering the range of species that are affected by the pathogen. Hosts can be either primary, intermediate or dead end.

Ignaz Semmelweis



In 1847, Ignaz Semmelweis worked in the Vienna Hospital as an obstetrician. He was introduced to the concept of medical students washing their hands with a mild calcium hypochlorite solution after working with cadavers. And although this action brought a decrease in childbed fever, it was still thought that each case was a unique case. Ignaz believed and publicly stated that simple cleanliness was the way to prevent childbed fever. He expanded his washing techniques to include tools as well as hands.

In 1849, Ignaz was not invited to stay at the Vienna Hospital due to his beliefs and in 1851 accepted a small post in Hungary. He virtually eliminated childbed fever in the 6 years he had the post. Regardless of his success, the medical community would not accept his theory and he was ostracized from the community. He died in 1861 at age 47 never knowing the impact he had on the medical field.

20 years later Pasteur offered a theoretical explanation to Ignaz's observations and called it germ theory. Now Ignaz Semmelweis is considered to be a pioneer in antiseptic techniques.

Laboratory Acquired Infections

Laboratory acquired infections are described as those which result from laboratory work, whether it occurred in a laboratory worker, or in another person who happened to be exposed, as a result of work with infectious agents.
It is not always easy to define a laboratory acquired infection or to conclude with certainty that one has occurred. Problems occur when the research being done involves microorganisms that are commonly found in the community. There is always a possibility that the illness was contracted during the hours the individual was not at work. If it can be shown that the illness was the result of a spill or exposure to an unusually large amount of the microorganism, then a case for laboratory acquired infection may be proven. Bacteriological or serological typing of the organism from the individual may also suggest that it is a current laboratory strain.

Exposure Reporting

It is required that all spills, possible exposures and incidents involving biohazardous materials be documented and reported to the Principal Investigator and centrally through www.cairs.ubc.ca. The CAIRS report will ensure that the Department of Safety & Risk Services and the University Occupational Preventive Health Unit are notified. The Biosafety Advisor will use the information you provided in your report to log the required notification to the Public Health Agency of Canada.

Routes of Exposure

Microorganisms can enter the body through accidental inoculation, ingestion, mucous membrane, direct contact or aerosols. In laboratory-acquired infections the route may not be the same as when the disease is acquired naturally. The dose or number of organisms required to initiate infection is often difficult to ascertain and depends on the route of exposure.

It is not unreasonable to expect that any person who works with pathogenic microorganisms will be more likely than members of the community to become infected. There is evidence that some organisms cause more laboratory infections than others and that the incidence of infections varies according to the nature of the work and the health status of the worker. Attention must be given to ways in which laboratory workers may become infected. From 1979 to 1999 there were 223 reported cases of *M. tuberculosis* as compared to 2 reported cases of *Mycoplasma pneumoniae*.

In the absence of adequate exposure control measures workers may contract laboratory acquired infections via accidental puncture/penetration, ingestion, contact with mucous membranes, inhalation or by direct contact with infectious materials.

Puncture or Penetration

Infection may arise as the result of pricking, jabbing or cutting the skin with infected instruments or objects such as hypodermic needles, scalpels, and broken contaminated glassware. For this reason, the use of sharps and glassware is discouraged when working with Risk Group 2 and higher pathogens.

It is critical to analyze sharps-related injuries in the workplace to identify hazards and trends. Some common trends are needle sticks from recapping, cuts from picking up contaminated broken glass, and disposal of "quick-release" scalpel blades. To help minimize the exposure to sharps, individuals are required to dispose of the sharp after every use. Sharps should be disposed of in a closable, puncture resistant, leak proof container and disposed of following the procedures in the Hazardous Waste Management Manual.

Ingestion/Exposure to Mucous Membrane

Infection through ingestion or exposure to mucous membranes most commonly occurs via facial contact with contaminated hands but may also result from a splash or spray to the face.

The most effective protective measure against accidental ingestion is washing hands thoroughly. Laboratory personnel should wash their hands for 90 seconds every time they remove their gloves, have a potential exposure, or are about to leave the laboratory. This has been proven to be the most effective way of protecting oneself against infectious biological materials. The hand washing procedure is found in Appendix A.

Other effective measures include using engineering controls that offer splash protection such as biological safety cabinets and wearing a face shield or eye protection. It is never permissible to pipet by mouth, eat, drink, smoke or apply cosmetics in a laboratory space.

Accidental ingestion of laboratory agents can result from:

Mouth pipetting

Mouth pipetting is a practice that is strictly prohibited in lab settings. The use of mechanical devices is not only safer but also allow for more accurate pipetting.

Eating, drinking and smoking

Ingestion of microorganisms occurs as a result of, eating, drinking or smoking in laboratories. There are documented cases of finger to mouth transmission leading to infection.

Splashes to the face and eyes

Laboratory workers usually know when they are splashed or sprayed in the face or eye by infectious material. The common cause of such accidents is the violent separation under pressure of needles and syringes. The eyes seem to be particularly vulnerable to splash infections and approved splash protection must be worn when handling hazardous materials under these conditions. Where splashes do occur, exposed regions should be washed gently but thoroughly.

Spillage and direct contact

It is good practice to assume that all work surfaces and equipment may be contaminated. When material containing microorganisms is spilled, or when containers break and shed some or all of their contents, the event may pass unnoticed. The result is that the work bench or equipment may remain contaminated. The same result is

found when decontamination procedures are not entirely effective. The fingers may then transfer organisms to the mouth or the eyes. A member of an ophthalmic unit counted the number of times two others touched their faces in the course of half an hour (Fiewett 1980). One doctor touched or rubbed their eyes 27 times and another 15 times. It must also be remembered that microorganisms may enter the blood stream through cuts and abrasions on the hands and fingers. Proper hand washing is essential to protection from exposure to microorganisms by accidental contact.

Infections by Aerosol

When a liquid is forced under pressure through a small hole, or if a fine jet of liquid is allowed to impinge onto a solid surface, the result is a cloud of very small droplets referred to as aerosols. If these droplets contain bacteria or other forms of infectious material, they are referred to as infected air-borne particles. These particles can remain suspended in air and be moved throughout the room by air currents generated by ventilation and the movements of people. The smaller the particle, the greater their potential for traveling long distances.

Infectious air-borne particles do not necessarily have their origins in aerosols. Lyophilized cultures, dried bacterial colonies, dried material on stoppers and caps of culture tubes and bottles, fungal and actinomycete spores can all be released when the containers are opened. These are all considered sources of air-borne particles that may contain viable organisms and lead to possible infection.

Of greatest concern is the release of infected air-borne particles that may contain, but not limited to, organisms which cause diseases such as tuberculosis, psittacosis, Q fever, pulmonary mycoses and, in some special circumstances, brucellosis. If the organisms are contained not in aqueous but in proteinaceous fluids (e.g. sputum, mucus, serum), evaporation will be much slower as these materials tend to retain water. The droplets will settle more rapidly; fewer will remain suspended in air and fewer infected air-borne particles, available for wider dispersion, will be produced.

Formation of aerosols can be controlled by the use of proper techniques or special equipment. For example, both screw-capped safety cups and sealed centrifuge heads permit use of a centrifuge in an open laboratory with minimum risk of aerosols, provided the cup or head is opened in a Biological Safety Cabinet (BSC). Also, special blenders are available which prevent the escape of aerosols produced during use. However, while the use of available safety devices is recommended, their use is not a substitute for good technique.

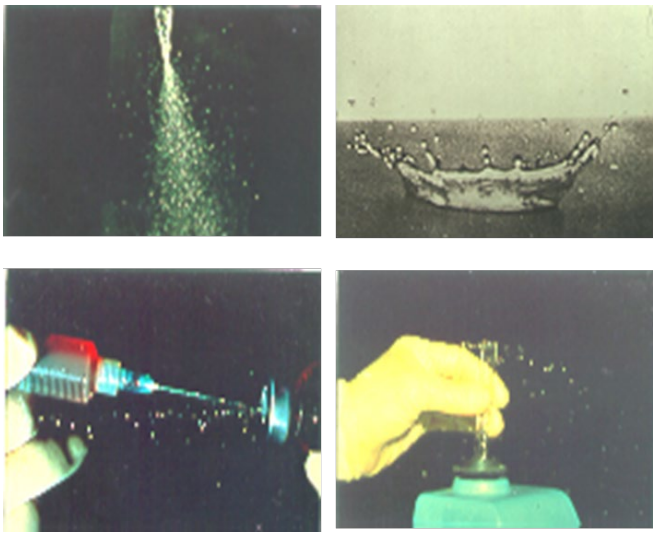
Once formed, aerosols can be captured by high efficiency particulate air (HEPA) filters or removed from the laboratory by room ventilation methods. A "chemical" fume hood or a containment cabinet provides a partial barrier against airborne materials, including aerosols, while a gas-tight biological cabinet forms an absolute barrier. A partial barrier for a centrifuge, fermentor or freeze-drying apparatus can be obtained by positioning an exhaust hood or canopy over the apparatus provided there is sufficient exhaust to sweep away any aerosol resulting from an incident. When working with biohazardous

materials all procedures that may result in the creation of aerosols must be performed within a NSF49 approved certified BSC.

Aerosol production and dispersal in laboratories

It has been shown that many laboratory techniques, using both simple and mechanical equipment, as well as common laboratory accidents, produce aerosols consisting of various sizes of particles. These techniques include:

- bacteriologists' loops
- pipettes
- syringes and needles
- opening tubes and bottles
- centrifuges and blenders
- harvesting of eggs and other virological procedures
- lyophilization and breakage of cultures.



Many laboratories maintain solutions of microorganisms that are routinely diluted for testing and counting. These dilute solutions present the greatest hazard because the droplets formed contain high concentrations of microorganisms. When they dry, the nuclei they leave are very small and light. The size and weight of particles influence the distance they travel on air currents. Even when these aerosols are produced at bench level there are numerous sources of air currents which influence where they will travel. For example, bunsen burners create considerable updrafts that rapidly disperse aerosols throughout the room. The operator may be protected by the up-draft, but other occupants will not be.

Other laboratory equipment generate rapidly moving or convection currents, thereby creating excellent conditions for the dispersal and inhalation of air-borne particles. Larger particles and droplets, which do not evaporate, can also contribute to the hazards of infection by contaminating surfaces. As suggested above, fingers may be contaminated in this way and microorganisms can therefore be transferred to the mouth and eyes, making it essential that lab personnel wear the appropriate personal protective equipment and wash their hands prior to leaving the laboratory.

Risk Groups

The Canadian Biosafety Handbook uses the above risk factors to classify biohazards into four distinct risk groups.

Risk Group 1 Agents

*"*A microorganism, nucleic acid or protein that is either not capable of causing human or animal disease or is capable but unlikely to do so (Canadian Biosafety Handbook 2016)". These are agents that have a **low** individual and **low** community risk. Examples in this group include Baker's yeast, non-pathogenic strains of E. coli, non-infectious animal tissues, most mouse cell lines, and some recombinant DNA work.

Risk Group 2 Agents

"A pathogen or toxin that poses a **moderate** risk to the individual and a **low** risk to community. Effective treatment and preventive measures are available and the risk of spread of diseases caused by these pathogens is low (Canadian Biosafety Handbook 2016)". Examples include Haemophilus influenza, Salmonella, Hepatitis B virus and HIV that is non-cultured.

Risk Group 3 Agents

"A pathogen that poses a **high** risk to the individual and a **low** risk to the community. These pathogens are likely to cause serious disease in individuals. Effective treatment and preventive measures are usually available and the risk of spread of the disease caused by these pathogens is low for the community (Canadian Biosafety Handbook 2016). Examples include Bacillus anthracis, all species of Brucella, Mycobacterium tuberculosis and propagated HIV.

Risk Group 4 Agents

"A pathogen that poses a **high** risk to the health of individuals and a high risk to community. These pathogens are likely to cause serious disease in an individual which can often lead to death. Effective treatment and preventive measures are not usually available and the risk of spread of disease caused by these pathogens is **high** for the community. All of the agents in this group are viruses. Examples include Monkeypox, Lassa virus, Herpes simian B virus and Ebola.

Laboratory Animals

The use of animals at UBC for teaching, testing and research is a privilege and not a right. A series of procedures and policies have been developed or adopted to ensure that the use of animals at UBC remains sensitive to the needs of the animals as well as to the goals of teaching and research.

The Canadian Council on Animal Care (CCAC), the national body overseeing the use of animals in teaching and research in Canadian institutions, is the driving force behind many of the policies. Institutions are required to follow the policies announced by the CCAC to remain in compliance and be eligible for research grants from the major granting agencies. Policies emanating from national or foreign groups with specific interests or concerns in their fields (e.g. Canadian Psychological Association, Society for Neuroscience) may be adopted.

Zoonotic Diseases

The use of experimental animals and insects poses special problems. Animals can harbour infectious organisms which are acquired naturally. These infections can give rise to a chronic carrier state, or the agent might persist in a latent non-infective form which can be reactivated periodically or as a result of certain stimuli. If the possibility that such an agent may be excreted by an animal during the course of an experiment cannot be excluded, all those animals should be kept at a containment level appropriate to the risk.

Infectious agents may be transmitted from animals to laboratory workers. This might be the result of the passage of natural infections within the animal, or from microorganisms that they have been inoculated with. Animal blood, urine or feces may be infected, resulting in contaminated bedding. Tissues removed for study may be infected and must be handled accordingly. Examples of pathogens that can be transmitted from laboratory animals to lab workers include (but is not limited to) lymphocytic choriomeningitis, Newcastle disease, vesicular stomatitis, Q fever, and (from primates) cercopithecine Herpes Virus 1 and shigellas. Infection may follow when laboratory workers are bitten, scratched or exposed to body fluids by experimental animals, including arthropods.

Using biohazardous agents in animal models

Research using biological agents in animals may involve:

- deliberately inoculating with microorganisms;
- administrating microbial toxins; or
- introducing tissues (xenographs) and cells (both primary and secondary).

The administration of any biohazardous material into an animal necessitates labelling the cage to communicate the hazards, providing safety information to the facility, and where open caging is used, providing signage to notify personnel of the nature of the hazard prior to entry. This signage must indicate any additional PPE requirements over and above the facility minimum. In the absence of evidence to the contrary, all viable specimens must be (i.e., transformed cells) suspected of containing blood borne pathogens. Under these circumstances, the animals must be kept at the containment level appropriate to the risk of the agent, recognizing that, in some cases, in vivo work may increase that risk.

All experimentation in animal models requires a valid Animal Use Permit from the UBC Animal Care Committee. The ACC Committee will require the completion of training through UBC Animal Care Services and a detailed experimental plan. Where biological agents are used in animal experiments, a Biosafety Permit listing the biological agents used, an experimental outline and a description of safety procedures for mitigating human exposure must be linked to the Animal Use Protocol.

The handling and use of laboratory animals can result in the development of allergic reactions to their dander. These reactions are referred to as Laboratory Animal Dander Allergies (LADA). Approximately 15% of workers whose routine work activities involve the use of animals become hypersensitive to dander. Most of these people (93%) displayed symptoms within 10 minutes of initial contact.

This hypersensitivity to animal dander can also lead to contact sensitivity to animal urine and blood. Persons with LADA are often hypersensitive to more than one species of rodent, but rat seems to be the most predominant.
There are four approaches to reducing exposure or minimizing the effects of exposure:

- Careful pre-employment evaluation
- A well-designed ventilation system
- Use of engineering controls, including cage change stations, biosafety cabinets and filter-top cages
- Use of protective clothing, especially gloves and N95 respirators

When working in animal facilities it is strongly recommended that all personnel work in engineering controls to handle animals or dump animal cages. Workers may also wear/use fit-tested N95 disposable respirators to limit their exposure to dander and other allergens. In all situations, it is the responsibility of the principal investigator and the biosafety committee, in consultation with the animal care authorities, to determine the risk levels inherent in the proposed research. For more information, please see the UBC Lab Animal Allergens: Exposure Control Plan.

Toxins

Microbial toxins are poisonous substances that are a natural product of the metabolic activities of certain microorganisms (Canadian Biosafety Handbook 2016). While they are not, strictly speaking, infectious as they do not replicate and are not transmitted from host to host, they still pose a biological hazard and must be handled with utmost care. "The most likely route of transmission is through accidental inoculation or by the exposure of mucous membranes to aerosols.

Possession of some microbial toxins are regulated by the PHAC. Any amount of these toxins requires a Risk Group 2 UBC Biosafety Permit, a security plan that prevents access by unauthorized personnel and cradle-to-grave inventory tracking. Guidance documents for toxin usage and inactivation are available from the Safety & Risk Services website. A complete list of regulated toxins can be found in the table below and in Schedule 1-5 of the HPTA. A subset of these toxins are described as "prescribed toxins" that require a special license under the HPTR where the inventory held is at or below the trigger quantity.

Table: Regulated Microbial Toxins

Toxin	Microbial Source	Prescribed Yes/No	Trigger Quantity (mg)
Areolysin	Aeromonas hydrophila	No	
Alpha Toxin		**Yes**	**5**
Anthrax Toxins: Lethal Toxin & Oedema Toxin	**Bacillus anthraxis**	**No**	

Bordetella Pertussis Adenylate Cyclase toxin	**Bordetella pertussis**	**No**	
Botulinum neurotoxin	**Clostridium botulinum**	**Yes**	**0.5**
Cholera toxin	**Vibrio cholerae**	**Yes**	**20**
Clostridium botulinum C2 and C3 toxins	**Clostridium botulinum**	**Yes**	**5**
Clostridium difficile toxins A and B	**Clostridium difficile**	**No**	
Clostridium perfringens Epsilon toxin	**Clostridium perfringens**	**Yes**	**5**
Dermonecrotic toxin	**Bordetella**	**No**	
Diphtheria toxin	**Corynebacterium diphtheriae**	**No**	
E.coli toxins: Necrotizing Factor (CNF) Heat-labile E.coli enterotoxin (LT) Heat-stabile E.coli entertoxin (ST) Cytolethal distending toxin (CLDT) Enteroaggregative Shiga-like toxin1 (EAST)	**Escherichia coli**	**No**	
Exfoliative toxin (aka Exfoliatin)	Staphylococcus aureus	No	
Hemolysin	Staphylococcus aureus	Yes	10
Listeriolysin O	Listeria monocytogenes	No	
Pasteurella multocida toxin	Pasteurella multocida	No	
Perfringolysin O	**Clostridium perfringens**	**No**	
Pertussis toxin	Bordetella pertussis	No	
Pneumolysin	**Streptococcus pneumoniae**	**No**	
Pyrogenic exotoxin	Streptococcus pyogenes	No	
Shiga-like toxin (veratoxin)	**Escherichia coli**	**Yes**	**1**
Shigatoxin	Shigella dysenteriae	Yes	1
Staphylococcal enterotoxins	**Staphylococcus**	**Yes**	**1 (Type B)** **10(Types other B)**
Toxic Shock Syndrome Toxin	Staphylococcus aureus	Yes	5
Streptolysin O	**Streptococcus**	**No**	
Tetanolysin	Clostridium tetani	No	
Tetanospasmin (Tetanus toxin)	**Clostridium tetani**	**No**	

Routes of Exposure

There are four major routes of exposure for biological toxins: inhalation, absorption through the skin, needle sticks, and ingestion. The risk of inhalation is at its greatest when performing mechanical agitation such as, shaking, stirring, and blending. Some toxins can be absorbed through the skin, however, the greater risk comes from the use of sharps or needles. Finally, there are some toxins that are toxic when ingested. For instance, C. botulinum is deadly when ingested even in minute amounts with an estimated LD50 of 1-3 ng.

Host Assessment

When assessing the host, their immune status and the risks posed by the infectious biological agent to the host must be considered. The physical, mechanical, and chemical host defenses and the natural flora of a healthy individual can protect against most infectious agents, however if a person is ill or tired, their immune status may be compromised. The Biological Safety: Principles and Practices (3rd Edition) by D. Fleming and D. Hunt goes on to say:

> "Declaration or identification of impaired host defense factors, such as immune deficiencies and extremes of age, is the responsibility of the employees. An evaluation of fitness for duty may need to be obtained from a physician, especially if there is a change in health status that could place that employee at increased risk of infection… Opportunistic pathogens and normal microbial flora that are of no or low risk to healthy adult workers can cause disease in the immunocompromised or immunosuppressed adult… A similar approach is required for pregnant women, because of the risk to the intrinsically immunocompromised fetus".

For reasons of confidentiality, workers should consult with healthcare professionals when making decisions regarding health status, pregnancy, and other medically related topics. Once the host factors are evaluated and a task-based risk analysis is completed, the Biosafety Advisor (in conjunction with the Biosafety Committee) can provide advice to reduce or prevent exposure to the biohazardous agent. UBC's Workplace Health Services provides access to an Occupational nurse and physician.

Other factors to be considered in regards to exposure include an individual's level of experience in a given task. A lack of experience and training can lead to accidents that can put not only the individual at risk but also the other individuals in the laboratory. In addition to this, the complacency of an individual for a given task increases the risk. Complacency results in shortcut taking or a lack of focus on the task that can lead to incidents and accidents. Again this puts both the individual and their colleagues at an increased risk of injury or disease.

Workplace Assessment

How the infectious biological agent is being manipulated must be assessed, including quantity of the agent handled and the handling procedures applied.

Scale

Work with biohazardous materials can be split into 3 categories, research, clinical, or large scale. Large scale is classified as levels greater than 10 litres of the infectious material. UBC has clinical and research spaces. The clinical spaces take and handle patient specimens (bodily fluid and tissue samples) sent for diagnostic purposes. Space is classified as a research space if manipulation of a sample occurs. As the volume and/or concentration of a biohazardous sample increases, so does the risk associated to the work. In clinical samples, the risk is associated to the unknown, meaning that the samples could contain any number of human pathogens. Risk also increases when identifying or isolating pathogens from clinical samples, and culturing pathogens for research. Culturing, or propagating pathogens increases the higher concentration and hence the risk.

Origin

A given biohazard, plant, or animal has an environment in which it is naturally occurring as part of the ecosystem. The term used for this is indigenous. People build natural resistance and immunities to biological agents indigenous to their local environment. When biological materials are non-indigenous, there is not only an increased risk to the individual handling the material, but also to the environment. When exposed to new or non-indigenous organisms, any resultant infections or immune reactions to the organism may be amplified as compared to those from indigenous organisms. The environmental impact of non-indigenous species is not always known nor predictable. There is potential for them to be invasive and cause lasting economic and environmental damage to the area. Special care is required when working with non-indigenous organisms, including disposing of the associated waste to avoid environmental release.

Job Hazard Analysis

A thorough evaluation of the hazard potential of the work practices, procedures, and equipment to be used for the proposed tasks is called a Job Hazard Analysis (JHA). The supervisor or designate is responsible for analyzing the risk of exposure for each task in which a biohazard is handled. The employee or student must be included or trained in this process, as this offers a good learning opportunity and improves compliance with safe work practices. A JHA should include: safe work practices for ALL the hazards, engineering controls, personal protective equipment, any alternatives to the procedure or hazards, training requirements, and any required Standard Operating Procedures (SOP). Some example hazards include the increased risk when working with sharps, pressurized devices, and live animals.

Risk Control

Methods used to control risk include: administrative controls, engineering controls, personal protective equipment, medical surveillance, and facility design. Another term

for risk control is containment. The Containment Level (CL) is determined by the extent of the controls needed for a particular agent. While risk control looks primarily at how to minimize the risk of working with the agent, the risk assessment must also be used to choose the appropriate decontamination and waste disposal procedures.

PHAC and CFIA currently define four containment levels. These, however, do not necessarily correspond to the Risk Group of an organism because the containment level is determined based on a complete risk assessment (Biological, Host and Workplace). It is also possible that an organism can be handled in one type of structural facility (e.g. CL2) but require more stringent operational practices (CL3 operating procedures). The specific requirements are described in Canadian Biosafety Standard, 2nd Edition, 2015 however, these are still dependent on a complete risk assessment.

CL	Containment Level requirements
I	↑ Facility, SOPs, training, engineering controls, PPE
II	↑↑ Facility, SOPs, training, engineering controls, PPE
III	↑↑↑ Facility, SOPs, training, engineering controls, PPE
IV	↑↑↑↑ Facility, SOPs, training, engineering controls, PPE

Administrative Controls

Registration of Biohazardous Materials

Principal investigators working, or proposing to work with biological materials must apply to the UBC Biosafety Committee for a UBC Biosafety Permit that corresponds to the risk group of the most pathogenic material used. This includes research undertaken by UBC appointees in facilities controlled by the University, directed by UBC personnel, or supported by grants processed through the University. All proposals, regardless of funding source, are subject to review by the Biosafety Committee. The Biosafety Permit process is administered through the Office of Research Services.

The Biosafety Committee will determine if the research program falls within the Canadian Biosafety Standard, 2nd Edition and verify the appropriate level of containment. The Biosafety Committee will approve research facilities, confirm that safety equipment, including biological safety cabinets, are certified properly, that procedures are not unduly risky and verify the training required by the faculty and staff conducting the research. A Biosafety Certificate will be issued when all of the prescribed requirements have been met. All biohazardous research as defined by the Biosafety Committee shall be covered by a valid Biosafety Certificate. This certificate indicates the minimum level of containment necessary and is valid for four (4) years unless there are significant changes in the research program, facility, or research personnel, in which case notification must be submitted to the Committee. The University will not release

the granting agencies' funds unless the University "Biosafety Permit Application" form has been completed and approved by the Biosafety Committee.

When a research protocol calls for the use of living human subjects, human remains, cadavers, tissues, biological fluids, embryos or foetuses, the appropriate ethics approval must be obtained. More information with research involving human subjects can be found at: University policy # 89. Any research or teaching conducted at UBC facilities, or by persons connected to the University, involving the use of animals (including fish and invertebrates) must conform to the University Policy on Research and Teaching Involving Animals and must have the approval of the UBC Committee on Animal Care. More information about conducting research with animals can be found at: https://animalcare.ubc.ca/.

The University Biosafety Committee can be reached by contacting the Office of Biosafety, Department of Safety & Risk Services at 604-822-2029 or the Office of Research Services at 604-827-5111.

Standard Operating Procedures and Scientific Procedures

A standard operating procedure (SOP) is a procedure that includes a job hazard analysis. It is more than just the scientific procedure. SOPs offer investigators an alternative to writing detailed procedures in their protocol. Infectious material SOPs must be approved by the Biosafety Committee, just as Animal related SOPs must be approved by the Animal Care Committee. Once approved, the SOP may be referenced in future experimental procedures or protocol. Any deviation from the approved procedures must be clearly described and justified to the appropriate committee. Approval of the protocol indicates approval of the deviation from the SOP for that project only.

All other protocols are considered experimental or scientific procedures. It is required that experimental procedures be documented as well. Ideally, the main protocols would be located in a binder that is accessible by all, however, lab books are also acceptable. Again, this should include more than just the scientific steps, it should include also include precautions and equipment needed to safely work with the materials.

Some general procedures can be found in Appendix A-E.

Inventories, Exporting, Importing, Transfers, and Transportation

Inventories

As part of the Biosafety Permit Application, a very basic inventory is required. This includes the full name of the biohazard, risk group, and locations for use and storage. At the level of the lab, an inventory with this information is all that is required for risk group 1 and 2 organisms and non-prescribed toxins. The inventory requirements for ***risk group 3*** and prescribed toxins include, name, location (room, freezer or cabinet, and shelf), and cradle to grave inventory tracking (number of vials and amount in each vial or container).

Exporting Biohazardous Materials

In 1972 Canada signed the Biological and Toxin Weapons Convention and is therefore required to monitor certain toxicological and biological materials and equipment through the Export Control List. Export permits may be needed to ship certain biohazardous materials outside of the country. For information and advice regarding a specific agent, contact the Department of Foreign Affairs and International Trade Canada, Export Control Division via phone 1-877-808-8838, email eics.scei@international.gc.ca .

Importing Biohazardous Materials

There are two categories for importation of level 2 biohazardous agents, Human Pathogens and Animal Pathogens. A form and a checklist may need to be filled out for each, depending on the agent being imported. Risk group 1 organisms do not require an import permit but do have to be inventoried and a record of the purchase kept on file. It is important to know that this permit allows the applicant access to the agent; it may not be transferred to any other users unless permission from the regulatory body has been obtained.

Human Pathogens: the Public Health Agency of Canada (PHAC) is the regulatory agency for human pathogens.

- Step 1 – ensure that the biological agent and the project that it will be used for is detailed on an approved UBC Biosafety Permit.

- Step 2 – contact the Biosafety Advisor (604-822-2029 or researchsafety@SRS.ubc.ca) with the microorganism, the supplier and the Biosafety Permit number. You will provided with an importation letter and Biosafety Office Confirmation statement (usually by email).

- Step 3 – provide your supplier with copies of the importation letter and confirmation from the Biosafety Advisor that all necessary permissions are in place.

Animal Pathogens and animal tissues/byproducts: the Canadian Food Inspection Agency (CFIA) is the regulatory agency for animal pathogens and products.

- Step 1 – contact the Biosafety Advisor (604-822-2029 or researchsafety@SRS.ubc.ca) with the organism, the supplier and the Biosafety Permit number.

- Step 2 – an SRS Advisor or Associate will come to your site to complete CFIA Containment Level 2 Checklist and Application for Permit to Import Animal Pathogens form with you

- Step 3 – sign the forms and send to the Biosafety Office for submission to CFIA
  Note: if the pathogen or biohazard affects Animals and Humans then both sets of instructions will have to be completed.

Transfers

When a transfer of biological materials occurs, a record must be kept. The U15 has a harmonized material transfer form for this process that requires signatures from the Biosafety Office at both institutions. This form is required for transfers between institutions in Canada – outside of Canada the importation documents serve an equivalent purpose. If it is required, contact the Biosafety Office for the appropriate forms (604-822-2029 or researchsafety@SRS.ubc.ca).

In addition a Material Transfer Agreement (MTA) may be necessary. This form can be found on the University Industry Liaison Office (UILO). A simple form can be filled out for all infectious material transfers to groups outside of UBC, however, if there is any intellectual property involved then further documentation may be required. UILO is able to aid in this process.

Transportation

Transportation within or between Laboratories

- Place the biohazard in a leak proof container, using a screw top cap whenever possible.
- Place this within a secondary leak proof and breakage resistant container.
- Transport either by hand or for heavier samples, a cart.
- If transferring biological agents to another investigator then ensure an appropriate record is kept on file.

Transportation between Buildings within UBC Sites

- Place the biohazard in a leak proof container, as above.
- Place this within a leak proof secondary container containing absorbent material.
- Place the secondary container into a (tertiary) rigid outer box.
- If a cooling material is required place this in a tertiary container
- If transferring biological agents to another investigator then ensure an appropriate record is kept on file.

Transportation to Other UBC sites or Institutions

The transport of biohazardous materials within Canada is regulated by the Transportation of Dangerous Goods Regulations (TDG – SOR/85-77). Internationally they are regulated by the International Air Transport Association (IATA), Universal Postal Union (UPU) and the United Nations Committee of Experts on the Transport of Dangerous Goods. It is prohibited to ship dangerous goods via Canada Post.

All shippers and receivers must be trained in TDG practices to ship and receive risk group 2 or higher infectious materials. Please contact the SRS office (604 822 2029) for more information.

Training

New Worker Training and Orientation

When new personnel join a lab, they must undergo safety orientations and training. This is not only good laboratory practice, but is also a legal requirement under federal and provincial regulations. This regulation applies to any worker that is new to the work site or is under the age of 25. A worker must complete safety orientations for both their facility and lab space and they must be appropriately trained prior to performing any tasks or procedures with an inherent risk. In addition, all safety orientations or training must be documented and kept on file. Training documents are also necessary to meet requirements of CFIA and PHAC.

The necessary items on a training document include the following:

- Name of protocol
- Trainee name and signature
- Trainer name and signature
- Date when the training was completed

Annual Training Needs Assessment

Under the Canadian Biosafety Standard, anyone working with pathogens or toxins must undergo a training needs assessment annually and prior to beginning a new project. This entails a documented discussion between trainee and supervisor to ensure that the trainee has all required competencies to conduct the tasks associated to the project. Review of job hazards in conjunction with medical surveillance enrolment should also be done at this time.

If at any point, the trainer or trainee feels that the training was insufficient, then more training needs to be provided until the trainee is competent in completing the protocol or procedure.

Signage

The following signage must be located on the doors of the laboratory prior to entry.

- Emergency Contact Information (on the door)
- Hazard class information for materials contained within the space
- Site specific instructions for Personal protective equipment

Additional signage may include:

- Restricted Access;
- No Access Except by Authorized Personnel;

The following signage must be located within the laboratory:

- No Eating, Drinking, or Smoking
- Emergency Contact Information
- Biosafety Permit Certificate posted in every approved room

Additional signage may include:

- Emergency signs (like Emergency Shower, First Aid, or Emergency Eye wash;
- Dedicated Hand washing sign;
- Any other site-specific instructions.

Medical Surveillance Program

The University of British Columbia has developed and implemented a comprehensive Workplace Health Service program at the University. This program supports the health and safety of UBC personnel and prevents the spread of infectious materials and disease by establishing best practice activities within the evolving research and occupational environment at UBC.

The fundamental purpose of medical surveillance is to detect and eliminate the underlying causes of occupationally acquired infections of faculty, staff, and students, and thus has a prevention focus. As such, a comprehensive medical surveillance program contributes significantly to the success of worksite health and safety programs by:

- impacting faculty and staff in a positive and meaningful way through well-defined processes of risk reduction
- applying targeted expertise to support the research community in a critical area
- covering all staff at risk regardless of location
- supporting the excellence of research programs; and
- ensuring that the University's duty of care is fulfilled, thereby minimizing reputational risk.

In order to pursue new and vital research into diseases and medical challenges, UBC is engaging in research involving pathogens and life-science protocols that require effective oversight through a well-defined management program. The UBC Workplace Health Services program provides controls and assurances to the workers, the community, and the University that our research is safe, understood and world-class.

Program Description

Medical surveillance is the applied analysis of health information based on risk and hazard assessments that addresses problems that may be occurring in the workplace that require targeted prevention. As such, it serves as a critical feedback loop to ensure the health and well-being of faculty, staff and students. It is a proactive and responsive system of informing and activating the assessment, protection, mitigation, treatment and restoration of employee and student health status related to occupational health risks and exposure (ongoing or episodic). The Workplace Health Services program entails a collaborative partnership with regulators, accreditors, funders, faculty, researchers, laboratories, institutes, and departments at UBC. The Program is consistent with the approach at other Canadian universities and research institutes.

At UBC, the need for such a program exists for animal care workers as well as the many researchers who work with biohazardous materials in research areas. Approximately 1,500 faculty and staff and graduate students are included at various levels of inclusion within the scope the program at UBC. The program provides immunizations, consultations and follow up information to those employees at risk of or exposed to occupational hazards. Clients will be given priority according to their risk of exposure.

Please note that the Workplace Health Services program is for those individuals who are staff, post docs, and faculty of UBC only. Students who have concerns about their exposure risks should contact their area manager or supervisory staff and may be directed to their Family Physician or Student Health Services at UBC for vaccination needs.

Basic information for safely working with human blood and tissue is found in Appendix D. Full information on Blood Borne Pathogens can be found in Blood and Body Fluids: Exposure Control Plan.

For further information on the program or your risks in the workplace, please speak with your direct supervisor or manager or the Workplace Health Services at 604-827-4713.

Engineering Controls

As the term implies, engineering controls provide a line of defense when working with infectious agents. An infectious agent in a capped bottle is, for example, already in a primary container, and risk arises when the integrity of the container is damaged inadvertently or when one intentionally wishes to open or penetrate the container to transfer an aliquot to a new system, host, or vector. During these circumstances, the resulting release of agent must be contained to avoid an exposure or infection of the employee working directly with the agent, of other personnel within the laboratory room, of others in the facility, and of those outside the building. It is more effective and, therefore, "safer" to contain the aerosol as close to the site of release as possible, avoiding the need to install and rely on secondary barriers.

The most common engineering controls are glove boxes, biological safety cabinets, and animal caging equipment. These controls include physical barriers of steel, plastic, glass, or other similar materials; air barriers or air "curtains"; and exhaust and supply barriers such as HEPA filters, charcoal filters, or air incinerators. The general principles applicable to containment include the following:

- Minimize the volume to be contained.
- Provide safe (i.e. non-contaminating) transfer of material into and out of the container without destroying the barrier.
- Provide means for decontamination of the enclosure and effluents.

If the engineering control fails or is inadequate, clothing and other items of personal protective equipment often become an important line of defense against a physical, biological, chemical, or radiological exposure. Such items may be required to prevent introduction of hazardous materials or infectious microorganisms through mucous membranes, broken skin, the circulatory system, the respiratory or the digestive tracts and are often used in combination with biological safety cabinets and other containment devices. The current view is that total containment should be a "system" encompassing clothing, mechanical devices, laboratory design, and work practices.

Centrifuge Safety

One readily recognizable hazard that has been addressed for many years by a simple containment procedure is the microbiological centrifuge, for which the construction of safety cups has provided one method of containment. These containers range from individual sealed tubes to larger screw-capped buckets and sealed rotors. It is important that whenever possible the tubes, buckets or rotors should be loaded and unloaded in another engineering control, a biological safety cabinet (BSC).

Since the tubes used in a centrifuge may be subject to extremely high stresses, careful attention must be paid to the quality of the seal. If an aerosol or fluid containing an infectious agent escapes from a rotor or cup during high-speed operation, the potential for extensive contamination and multiple exposures or infections would be great and the consequences could be severe. Some of the early tube closures that depended on the expansion of o-rings were not satisfactory. Today, most manufacturers produce effective closures that prevent leakage of small-batch materials under low, medium, or even high-speed centrifugation and the user must choose the appropriate tube that is able to withstand the centrifugation speeds it will be subjected to. In addition to selecting the appropriate tubes, the user must ensure that the tubes being centrifuged are appropriately balanced. Despite the improvement in the centrifuge technology, imbalanced tubes can cause severe damage to the centrifuge and it is always good practice to balance the tubes using a mass scale.

Cornell Centrifuge Accident

On December 16, 1998, milk samples were running in a Beckman L2-65B ultracentrifuge using a large aluminum rotor. The rotor had been used for this procedure many times before. Approximately one hour into the operation, the rotor failed due to excessive mechanical stress caused by the "G" forces of the high rotation speed.

The subsequent explosion completely destroyed the centrifuge. The safety shielding in the unit did not contain all the metal fragments. The half-inch thick sliding steel door on top of the unit buckled allowing fragments, including the steel rotor top, to escape. Fragments ruined a nearby refrigerator and an ultra-cold freezer in addition to making holes in the walls and ceiling. The unit itself was propelled sideways and damaged cabinets and shelving that contained over a hundred containers of chemicals.

A shock wave from the accident shattered all four windows in the room. The shock wave also destroyed the control system for an incubator and shook an interior wall causing shelving on the wall to collapse. Fortunately the room was not occupied at the time and there were no personal injuries.

Screw-capped buckets are not available for all models of centrifuges and many of the commercially available plastic tubes and bottles leak. Therefore, when appropriate safety buckets are not obtainable, it is recommended that the chamber remain closed for 30 min after centrifuging infectious materials to allow aerosols to settle before opening the lid. Some units come with HEPA filters built in so the air in the chamber can be filtered while it is evacuated. Large bulk or zonal rotors and continuous-flow centrifuges are particularly difficult to seal, and extreme care should be taken in their use. The simple primary barriers described can be effective, but one must also consider the possibility of a major accident (e.g. rotor rupture). Work with large volumes of infectious agents may merit putting the entire centrifuge in a ventilated enclosure. Regardless of whether or not the centrifuge being used is a microcentrifuge or an ultracentrifuge, the rotors must be cared for appropriately. Every centrifuge manufacturer will have procedures and instructions detailing the proper maintenance and care for rotors. Improper rotor care and maintenance could potentially result in explosions causing severe damage not only to the centrifuge, but also to the surrounding lab and personnel.
In all cases, if there is a biological spill in the centrifuge a protocol must be in place to adequately decontaminate the equipment. The decontaminant chosen must be effective against the biological agent and it also must not damage the parts of the centrifuge.

Blender Safety

Blenders are also well-known producers of aerosols. Without a special sealing design, they can, like centrifuges, rapidly contaminate spaces and spread high levels of surface contamination. An autoclavable safety blender cup is commercially available. This blender cup is autoclavable and is designed to reduce the occurrences of leaks.

Before using a safety blender, always check for cracks or leaks in the blender cup. If possible, the use of glass blender cups should be avoided in favor of the more durable stainless steel cups. Also ensure that the blender's cord is in good functional order. When loading and unloading the blender, do so in a biosafety cabinet (BSC). If possible, run the blender directly in the biological safety cabinet to avoid the spread of aerosols into the open laboratory. When running the blender, place a disinfectant soaked paper towel on the blender's lid to further reduce the chance of aerosol leakage. When run in the BSC, let it sit for 1 minute to allow the aerosols to settle and then open the lid. If running the unit outside of the BSC then let the unit sit for 30 min before opening the lid. The area where the blender was run should be decontaminated with an appropriate decontaminant.

Homogenizer Safety

Homogenizers are very well-known producers of aerosols. They are essentially a blender without the cup and lid. It is for this reason that a homogenizer should be used in a BSC. There are two forms of generators on a homogenizer, open blade and rotor. The open blade generator has the added risk of being a sharp, so extra care should be taken when handling.

For added safety, sample tube seals and chamber assemblies have been developed to limit the production of aerosols. The generators are either a permanent part of the cap or can slide into the cap thus eliminating the open tube. Again, the homogenizer should still be used in a BSC and as with blenders the sample should sit for 1 minute prior to opening the chamber assembly. Once finished ensure that all the components of the homogenizer are decontaminated appropriately.

Lyophilizer Safety

The process of using a laboratory scale lyophilizer presents a number of unique hazards. These hazards include but are not limited to extreme pressure changes, a potential for glassware to explode or implode, and the possibility of aerosols creation. Depending on lyophilizer design, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material should be loaded in a BSC. The vacuum pump exhaust should be filtered to remove any hazardous agents or, alternatively, the pump can be vented into a BSC. After lyophilization is completed, all surfaces of the unit that have been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination. Handling of cultures should be minimized and vapor traps should be used wherever possible. To ensure that there will be no glass breakage, only use glassware that has been designed for the lyophilizer. Also ensure that the glassware is free of any visible defect (cracks, chips, or scratches), no matter how seemingly minor. Any glassware that is defective in this way must not be used under any circumstances.

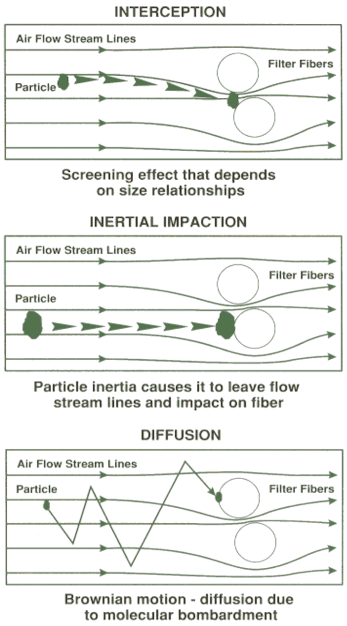
Cryostat Safety

The cryostat with its sharp microtome blade presents a cutting hazard to the user. Since the cryostat is also an expensive, precision piece of equipment, for reasons of both safety and good work practice, all users of the cryostat should be given appropriate training prior to working independently.
Frozen sections of unfixed human tissue or animal tissue pose a risk because freezing tissue does not necessarily inactivate infectious agents. Use of freezing propellants under pressure is not recommended with frozen sections as they may cause spattering

of droplets of potentially infectious material. As such, appropriate gloves should be worn during preparation of frozen sections. When working with human or infected animal tissue, consider the contents of the cryostat to be contaminated and decontaminate it frequently with 70% ethanol. The trimmings and sections of tissue that accumulate in the cryostat should be considered to be potentially infectious and they should be removed during decontamination. The cryostat should be defrosted and decontaminated with a tuberculocidal hospital disinfectant once a week and immediately after use with tissue known to contain bloodborne pathogens, *M. tuberculosis* or other infectious agents. The microtome knives should be handled with extreme care and stainless steel mesh gloves should be worn when changing knife blades. Solutions used for staining potentially infected frozen sections should be considered contaminated and be treated as such.

Animal Safety

Simple but effective engineering controls can be achieved for animal care and use. Spun-molded polyester or polycarbonate filter-top animal cages and ventilated racks are examples of caging systems applicable in rodent housing. Laminar-flow, HEPA-filtered, negative-pressure rack enclosures can also be used in a positive-pressure mode as a laboratory animal clean-air quarantine station. Larger animals can be housed in ventilated cages or in cages within negative-pressure cubicles or rooms with filtered non-recirculating room exhaust air.



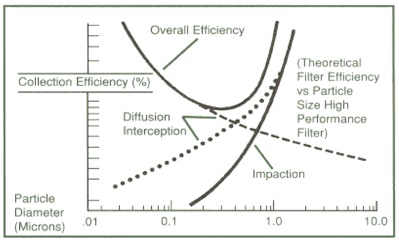


Figure 4

High Efficiency Particulate Air (HEPA) Filters

The HEPA filter is a thin sheet constructed of boron silicate microfibres. The filter paper is pleated, thus increasing its surface area. Corrugated aluminum separators are placed between the pleats to allow the air to penetrate the entire filter surface (Figure 3).

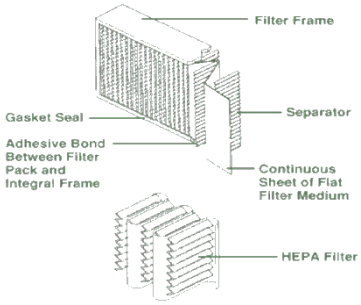


Figure 3

Being a particulate filter, the HEPA filter will retain airborne particles and microorganisms. Gases will, however, pass freely through the filter. Filtration occurs by five distinct mechanisms with HEPA filters (figure 4):

- Sedimentation
- Electrostatic Attraction
- Interception
- Inertial Impaction
- Diffusion

The least effective of these five types of mechanisms are sedimentation and electrostatic attraction. HEPA filters are rated on their ability to retain particles of 0.3 microns in size. Most aerosol droplets are greater than 0.3 um. This is because particles of a single viral or bacterial cell will not exist as an aerosol. The aerosol particles will be made up of clumps of cells and may also be associated with some form of liquid (i.e. the media the cells are cultured in). Therefore, the filtering efficiency of these droplets is actually greater than the rated percentage of the HEPA filter.

Laminar Flow Hoods (Clean Air Benches)

The laminar flow hood (LFH) cannot be thought of as an engineering control as it only provides product protection, not environment or worker protection. The discussion of the LFH is included here as it is a piece of equipment that works to keep a sterile work environment and has specific similarities and differences between a Biological Safety Cabinet (discussed in other sections).
Laminar-flow hoods or clean-air benches were developed from the observation that a stream of air at approximately 100 lfpm (0.5 m/s) forced through a HEPA filter provides a particle-free environment for several feet downstream of the filter if there are no obstructions. This has been termed a laminar flow, essentially non-mixing, air stream and is used in "clean rooms" and in "clean benches" to protect the work product.
These hoods provide product protection only and must NOT be used when working with any form of biohazard or chemical hazard. They provide a sterile work environment across the work surface, by creating a laminar flow of air which has been passed through a HEPA filter (Figure 5). Any potentially infectious aerosol that is created will lead to exposure of the operator and the environment. These hoods are suitable for the preparation of media or products where a sterile work environment must be maintained. However, it does not provide operator protection and, in fact, can expose the worker to aerosols of allergenic or infectious materials.

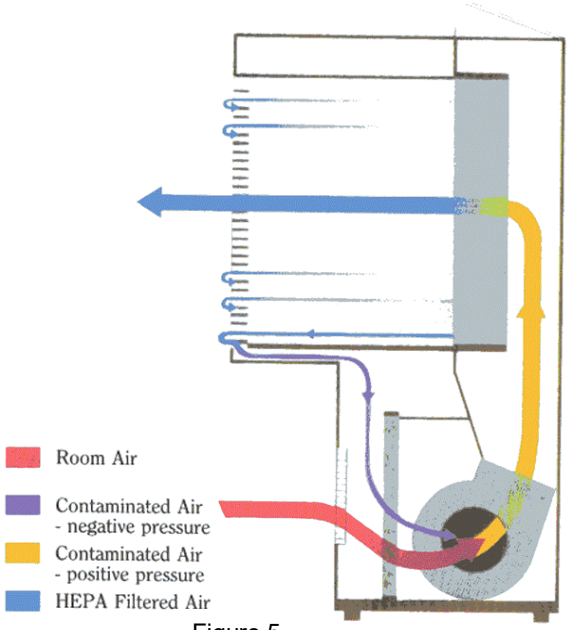


Figure 5

This type of cabinet can be useful in microelectronics fabrication, in the hospital pharmacy laboratories for final preparation of parenteral solutions, for final packaging of reprocessed devices, or for other applications in which the product is unlikely to have any ill effect on the cabinet user. Because clean-air benches blow their air out into the room, they must be differentiated from, and not confused with, biological safety cabinets. In this section, laminar flow will be interpreted to indicate a flow of clean, filtered air over the work surface with minimal mixing with the airstream coming into the cabinet via the work opening.

Biological Safety Cabinets

At present, three general classes of biological safety cabinets are defined. These are:

- Class I, the open-front air inflow cabinet, usually with a fixed height opening and sloped view window.
- Class II, open-front, vertical airflow cabinets, of which there are several subtypes.
- Class III, cabinets hermetically sealed with access through gas-tight air locks and work is accessed through fixed, heavy-duty, arm-length rubber gloves.

The three classes of cabinets are not directly related to the containment level required for agent use. Class I and class II cabinets can be used for work at CL's 1 to 3. Class III cabinets are usually reserved for work at CL 4, although they are found in some CL3 facilities as well.

Information on the selection, installation, and decontamination of biological safety cabinets can be found in Appendix F. Procedures for working in a BSC are found in Appendix C.

UV lamp

UV lamps are often added on to a BSC as a further method of decontamination, but unfortunately it has a number of drawbacks. A UV light loses its intensity as it is being used and it becomes less and less effective over time. Also, anything that is left in the cabinet can create a shadow that prevents the UV light from sufficiently decontaminating the affected area. In addition, the UV light must be cleaned regularly because dust can affect the ability for the UV light to decontaminate. As such, we do not recommend the installation of a UV light in the BSCs.

Certification

Biosafety cabinets should be certified when installed by the manufacturer, by the manufacturer's representative, or by a person who holds an NSF Accreditation status and is approved by the University to perform such work. The biosafety cabinet should be recertified:

- on an annual basis
- when the filters develop excessive pressure loss
- when the cabinet is moved (even within the same room)
- when the motor needs replacement or other repairs are required.

Note that the BSC must be decontaminated before it is repaired or moved.

Class I Cabinets

A Class I cabinet is defined as a ventilated cabinet which provides personnel and environmental protection (Figure 6). Air flow is away from the operator and is not HEPA filtered before entering the work area of the cabinet. Class I cabinets are similar in design to chemical fume hoods except there is a HEPA filter in the exhaust system to protect the environment from possible release of biohazards. The operator is protected

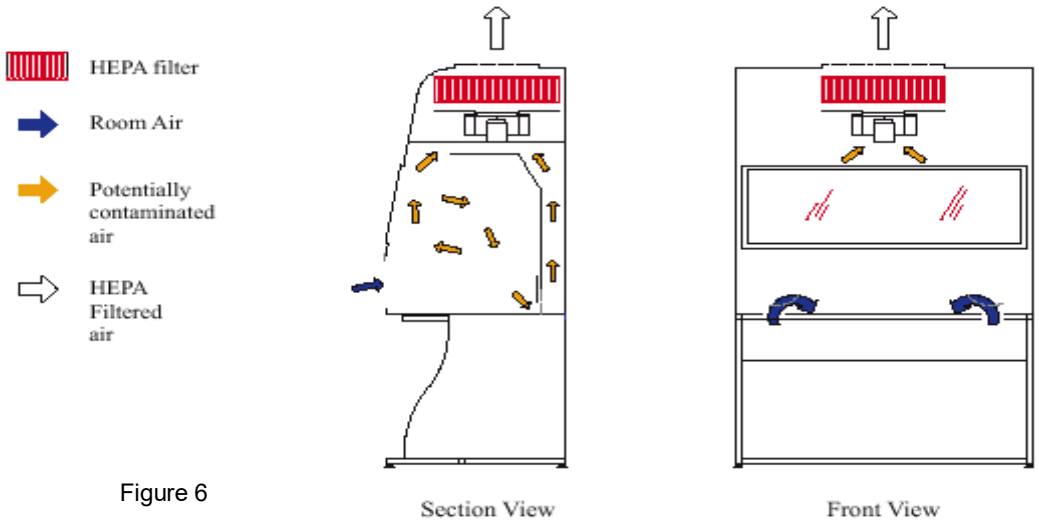


Figure 6

from aerosols created in the work area by an air curtain with an inflow velocity of 75 - 100 linear feet per minute (lfpm). More in Appendix E.

Class I cabinets are limited in their use due to the lack of product protection. In the majority of cases, they are found in animal units where the research animals have been subjected to some form of biohazard, and protection of the workers needs to be maintained.

Class II Cabinets

The Class II cabinet is defined as a cabinet that provides personnel, product and environmental protection. The different types of protection are:

- Personnel protection via inward flow of air creating an air curtain.
- Product protection via downward laminar flow of HEPA filtered air.
- Environment protection via all exhausted air is passed through a HEPA filter as seen in Class I cabinets.

The main difference between the Class I and Class II cabinets of consequence to the user is that the Class II vertical laminar-flow biological safety cabinet offers protection for the operator and the work being performed. There are two main types of Class II cabinets: Class IIA and Class IIB. They are designated IIA and IIB in the Canadian standard (CSA) number Z316.3-95 and in the U.S. National Sanitation Foundation (NSF) standard number 49.

Class IIA	Class IIB
maintains a minimum of 75 lfpm (0.4 m/s) for type A1 and 100lfpm (0.5m/s) for type A2 inflow velocity through the work opening	maintains a minimum of 100 lfpm (0.5 m/s) inflow velocity through the work opening
exhausts approximately 30% of the air traversing the work surface	exhausts either 70% (type B1), or 100% (type B2) of the air traversing the work surface to the outdoors.
often exhausted back into the laboratory or, they may be ducted to the outside environment via a thimble connection	must have a dedicated, sealed exhaust system with an external blower and alarm system

Class II Type A1 Cabinets

Type A1 cabinets are suitable for work with biohazardous agents in the absence of volatile toxic chemicals and volatile radionuclides (Figure 7). It is the simplest of the four subtypes of Class II cabinets and recirculates 70% of the air back through the supply

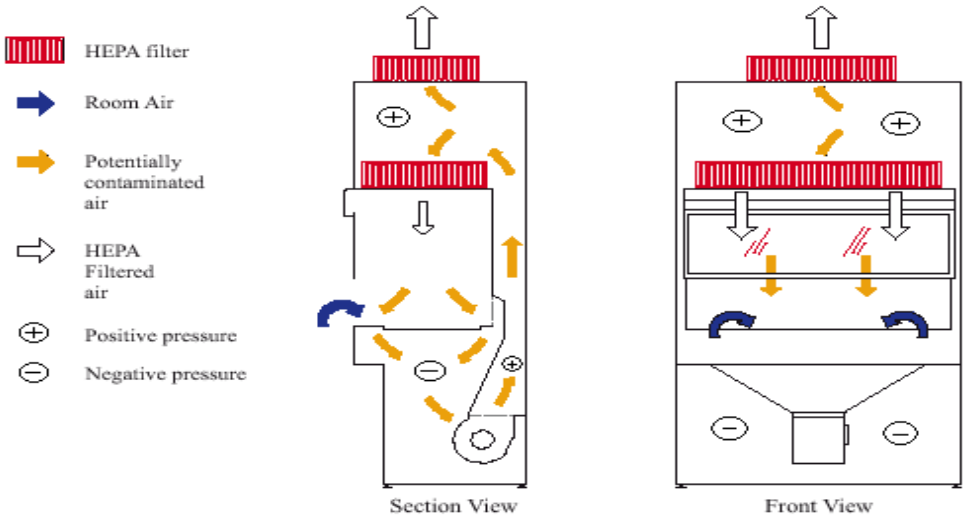


Figure 7 Class II Type A1

HEPA filter (30% of the contaminated air is passed through the exhaust HEPA filter). More in Appendix F.

Class II Type A2 Cabinets

Type A2 cabinets (Figure 8) are suitable for working with biohazardous agents that contain minute amounts of volatile toxic chemicals and radionuclides. In this cabinet, the air may be re-circulated back into the laboratory or can be ducted out of the building with the use of a "thimble connection", which allows the balance of the cabinet to not be disturbed by the fluctuations in the building's exhaust system. When the thimble connection is installed, it must allow for the cabinet to get certified. This cabinet must maintain a minimum average face velocity of 100lfpm (0.5 m/s). Unlike the Type A1,

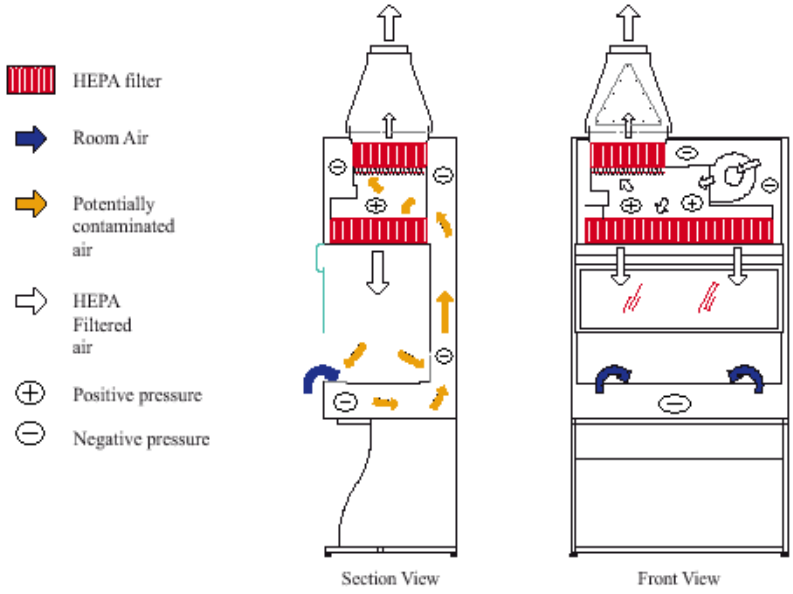


Figure 8 Class II Type A2

this cabinet's ducts and plenums are under negative pressure. This means that air will continue to draw into the cabinet and through the exhaust HEPA filter even if there is a hole in the cabinet.

Class II Type B1 Cabinets

Type B1 cabinets (Figure 9) maintain a minimum average inflow velocity of 100 lfpm (0.5m/s) through the work access opening. In the Type IIB1 cabinet, all of the biologically contaminated plenums and ducts are under negative pressure or are surrounded by negative pressure ducts and plenums. This provides extra protection to

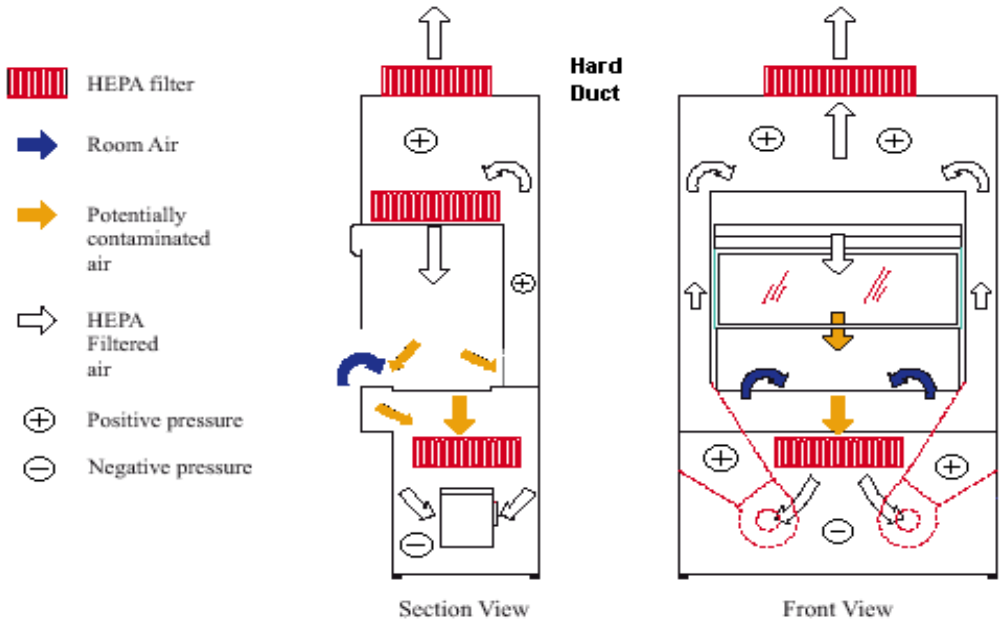


Figure 9 Class II Type B1

the worker in the remote possibility that a leak does occur. There is also a downward HEPA filtered laminar flow of air providing product protection as seen with Type A cabinets. The exhaust air is passed through a HEPA filter prior to entering a dedicated duct to the outside environment. In the case of Type B1 cabinets the amount of exhaust air is 70% (30% of the contaminated air is recirculated through the supply HEPA filter). The cabinet can be useful for microbiological work and for work with low-level radioisotopes and limited amounts of toxic chemicals. However, the degree of air mixing and recirculation in the cabinet requires that use of such materials be restricted to levels not considered toxic to the worker. Further, this class of cabinet will not usually meet the air inflow standards for work with carcinogens in chemical fume hoods.

Class II Type B2 Cabinets

These cabinets are referred to as total exhaust cabinets (Figure 10). There is no recirculation of air within the cabinet work area. All supply HEPA filtered air comes from a dedicated intake. All contaminated air and air brought in the front opening is exhausted directly outside, through a dedicated duct system after passing through a HEPA filter. As with Type B1 cabinets, these also have plenums and ducts under negative pressure.

Type B2 cabinets may be used with biological agents treated with toxic chemicals and radionuclides. Flammables may also be used in these cabinets. More in Appendix F.

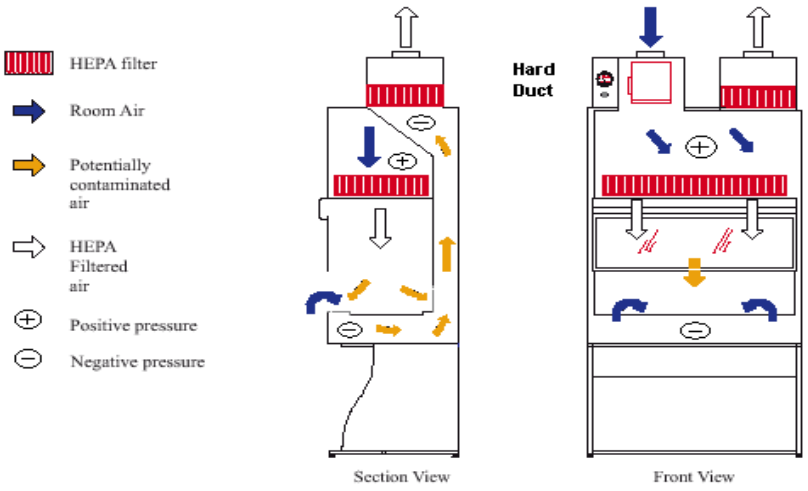


Figure 10 Class II Type B2

Class III Cabinets (Specialized Glove Boxes)

For the most part, the Class III cabinet system comprises a hermetically sealed cabinet system suitable for extremely hazardous work (e.g. usually in the containment laboratories meeting the PHAC Laboratory Biosafety Guidelines CL 4 requirements). The cabinets are gas tight, and all operations within the cabinet are conducted through arm-length rubber gloves (Figure 11). Entry into the cabinet is usually through a sealed air lock, and exit of material may be through an autoclave, a decontamination-type air lock, or a "dunk tank" filled with liquid disinfectant. These cabinets are often built as modules and assembled into specialty lines or systems encompassing a full set of operations in the laboratory. Ideally, one should be able to put all the necessary raw materials into the cabinet system, conduct the work, and remove only waste products. For example, some cabinets have been made for such uses as animal inoculation by

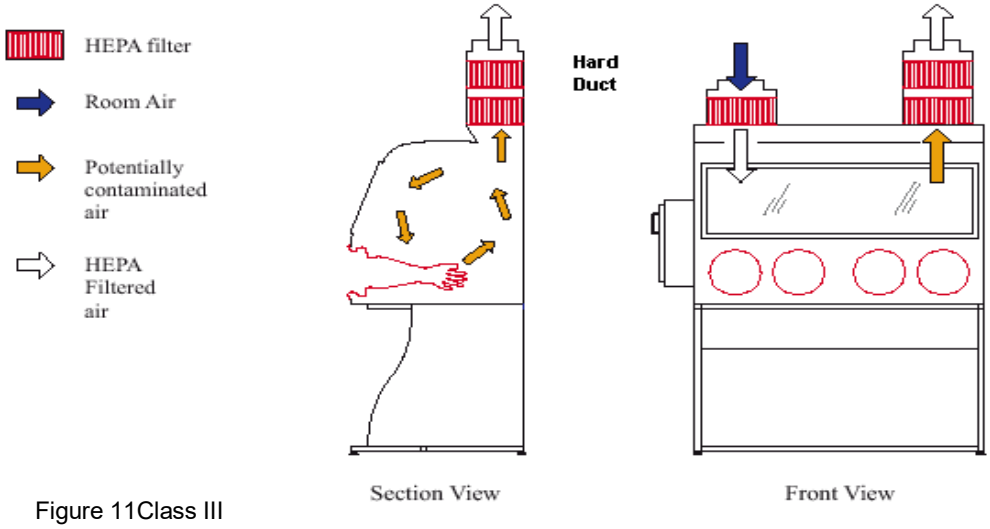


Figure 11Class III

syringe or aerosol challenge; others may accommodate centrifuges, fixed microscopes, incubators, refrigerators, and other equipment. Most such cabinet assemblies are made of stainless steel, although some are made of plastic. The latter are often used for

controlled-atmosphere protective systems (e.g. anaerobic chambers, germ-free animal isolators). Class III cabinets or "glove boxes" may be provided with strippable or removable liners and additional shielding if the work involves the use of high-activity or long-term radioisotopes. More in Appendix F.

Summary table comparing BSC, LFH, and Fume Hoods

Type	Protect Self	Protect Sample	Protect Env.	% of Exh. air
LFH	No	Yes	No	N/A
Class I BSC	Yes	No	Yes	100%
Class II Type A1	Yes	Yes	Yes	30%
Class II Type A2	Yes	Yes	Yes	30%
Class II Type B1	Yes	Yes	Yes	70%
Class II Type B2	Yes	Yes	Yes	100%
Class III Glove Box	Yes	Yes	Yes	100%
Fume hood	Yes	No	No	N/A

The above table describes the type of worker, product and environment protection the BSC types, LFH and fume hood offer. In addition, the % of air that is exhausted out is also described.

Summary table comparing the type II BSCs

Containment Level	Application	Type	BSC Class Exhaust Pattern
2, 3	microorganisms	Type A	Recirculated
2, 3	microorganisms	Type A2	Thimble-ducted
2, 3, 4	microorganisms volatile chemicals and radionuclides	Type B2	Hard-ducted
2, 3	microorganisms volatile chemicals and radionuclides (minute amounts)	Type B1	Hard-ducted

The above table summarizes the products that can be used in each type II BSC and also shows what containment level it is appropriate for.

Personal Protective Equipment

WorkSafeBC states that “the personal wearing apparel of a worker shall be of a type and condition that will not expose them to any unnecessary and avoidable hazards”. It is also important to realize that the use of protective clothing is only a last line of defense against unwanted exposures. The primary line of defense is maintaining good laboratory techniques and procedures. However, if the risk is present then the choice of clothing should be of a type that will not only protect the worker, but also the experiment and the environment.
Personal Protective clothing and equipment (PPE) are designed to protect the laboratory worker from exposure to infectious, toxic and corrosive agents, excessive heat, fire and other physical hazards. Its use also provides some protection to the experiment from unwanted exposures of toxic hazards or contaminants presented by the worker. The wearing of personal protective equipment should be restricted to the laboratory and NOT worn in offices, eating areas or other public areas.

WorkSafeBC legislation makes it mandatory for an employer to furnish employees with a working environment free from the recognized hazards that could cause death, injury, or illness to the worker. Wherever possible WorkSafeBC requires the employer to control the hazard through engineering means or alternatives, however when this is not feasible PPE is a legitimate solution. In assessing the hazards, PPE may not only be necessary but also may be the most practical, cost-effective means available to prevent employee injury or illness.

Laboratory Clothing

Once the hazard has been identified, appropriate protective equipment must be selected for laboratory use. Two criteria should be included:

- The degree of protection that a particular piece of equipment affords under varying conditions.
- The ease with which it may be used. Each Containment Level (CL) requires some type of PPE.

Once the necessary PPE has been determined it is critical that ALL laboratory members follow the policies and procedures set out for its use. It is the responsibility of the Supervisor to ensure that the worker understands and is familiar with the use, fit, and specificity of each piece of protective equipment and clothing.

UCLA Death

In December, 2008, a research technician at UCLA was badly burned when pyrophoric t-butyl lithium sprayed from a syringe. She died in January, 2009 from the burns.

The accident investigation found that the technician was not wearing protective equipment including an appropriate lab coat. The injuries were amplified due to the synthetic materials her clothing was made of. The material literally melted on to her skin.

For this reason, it is highly recommended that synthetic materials not be worn in the lab, unless they are approved PPE.

There are some general clothing requirements for all laboratories. These include: long pants, long hair tied back, and natural fabrics. Nylons and leggings offer little to no protection against hazardous materials, and often react with chemicals to cause more harm. Cotton is one of the best fabrics to wear as it will not react with many hazards. More general information can be found in Appendix B.

Laboratory Coats or Gowns

The laboratory coat can be used to protect street clothing against biological or chemical spills as well as to provide some additional body protection. The degree of protection provided by the common, cost-effective laboratory coat is frequently misunderstood. The specific hazards(s) and the degree of protection required must be known before selecting coats for laboratory personnel. These may include laboratory coats, smocks, gowns, total body suits, coveralls or jump suits, aprons, or two-piece scrub suits, all of which are commercially available. These items come in re-usable or disposable models made from a variety of materials including cotton, Dacron, nylon, polyester, olefin, rayon, vinyl, modacrylic, polyvinyl chloride (PVC), or rubber or trade names such as Tyvek (plain, polyethylene-coated, or Saranex-laminated), Safeguard, Duraguard, and Disposagard. Some materials are designed to protect against specific hazards such as biological, radioactive, chemical, or physical, including heat or cuts. Some materials feature anti-static and flame, caustic, oil, or acid resistance. The selection of the optimum configuration and material depends on the potential hazards, the regulatory requirements, types of operations to be performed, types of decontamination and reprocessing possible and available, the work environment, and personal preferences.

The laboratory coat or gown itself should cover the arms as well as most of the middle body. It is a good laboratory practice to keep the laboratory coat buttoned at all times in the laboratory and to have any loose cuffs taped around the wrists.

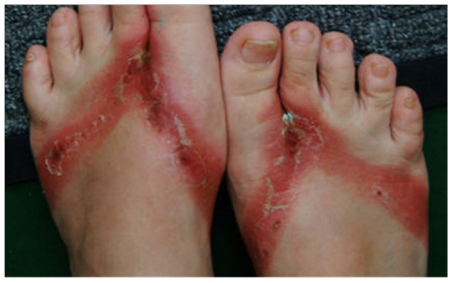
The Canadian Biosafety Standard states:

- **CL 2 Laboratories**: use a laboratory coat, gown, smock or uniform while working.
- **CL 3 Laboratories**: use a solid-front or wrap-around gown, scrub suit or coveralls while working.

An evaluation must be performed to determine whether the laboratory coat or gown is actually sufficient to protect the wearer from the immediate danger. For example, the material must be impervious enough to protect an employee from a spill or splash when

Always keep your Personal Items out of the Lab

In 2004, a graduate student entered his lab and changed from his flip flops and put on appropriate lab wear. He left his flip flops on the floor next to his desk (which was located at the end of a lab bench). During the day a fellow student spilt an acidic buffer on the floor and the flip flops. The spill was cleaned up but the graduate student was never informed that his flip flops had been involved. So when he put his flip flops on at the end of the day, he was unaware that the flip flops could be a danger. The next day he developed the chemical burn seen below.



All personal items should be safely stored outside of the laboratory.

such an event can be expected from the work. When there is a potential for exposure to a flame in the laboratory, the laboratory coat should be made from a fire-resistant material. Because a polyester-cotton blend material is flammable and will melt on the skin after contact with a spark, heat source, or some corrosive materials, a l00% cotton laboratory coat, which is non-reactive to many chemicals as well as flame resistant, may be a better choice.

Scrub Suits

When wearing a scrub suit in a CL 3 laboratory and performing operations that may generate infectious aerosols outside a biological safety cabinet, an additional long-sleeved, solid-front, wrap-around gown can be worn to minimize the contamination of the basic laboratory outfit (i.e. the scrub suit). Examples of such procedures are: inoculating animals with infectious materials, bleeding viremic animals, and otherwise handling or caring for animals that may be shedding hazardous viruses or bacteria in the urine or feces. When exiting the work area, the gown is removed and left in the room for reuse or steam sterilization before reprocessing. Disposable gowns are more frequently used and can be disposed of with the regular biohazardous waste. Heavy-duty rubber aprons or other specialized items of apparel can also be worn if there is a significant possibility of contamination from hazardous chemicals or radioisotopes.

Head Coverings

Head coverings are not usually necessary in biohazardous areas except in those containment areas where a complete clothing change is required or where product protection is also required. In situations in which a total body shower is mandatory on exiting, the option of either washing their hair during the shower or wearing a head covering during the time spent in the containment area can be given. Several styles of head coverings are available: a simple cap, various hood styles, or a bouffant style for long hair. Head coverings come in washable or disposable models in a variety of fabrics including cotton-polyester blends, cotton, polyolefin, or Tyvek.

Shoes and Shoe Covers

For general biological use, comfortable FULL coverage shoes such as tennis shoes or nurses shoes are used extensively. Sandals, clogs and ballet shoes are not allowed in

laboratories using biohazards and chemicals, due to the potential exposure to infectious agents or materials as well as physical injuries associated with the work. A change from street shoes is mandatory for those working in CL 3 areas. This should also be considered for CL 2 areas, especially for work with infected animals in animal rooms. Shoe covers can be used in CL 2 or CL 3 areas when a complete change of clothes and a dedicated pair of shoes is not required. Such shoe covers are available in vinyl, polyethylene, Saranex, or Tyvek and are usually considered disposable items. It is important to test shoe covers under the actual conditions of use to ensure that they provide slip resistance. In animal rooms and other areas where the wearer may encounter the splashing of large amounts of water from the hosing of cages, racks, or floors, the wearing of butyl rubber, neoprene, or PVC boots is strongly advised to reduce slipping hazards. Industrial safety shoes should be worn in any area where there is a significant risk of dropping heavy objects on the foot. When used in containment areas, these shoes, like all others, should be left within the area or decontaminated before removal.

Gloves

Prior to the discussion of gloves and their use it is critical to recognize the necessity for hand washing after gloves are removed. Gloves act as a barrier, but biohazards will still contaminate your hands through microscopic holes, thus proper hand washing essential.

Gloves are used in the laboratory for protection against a wide variety of hazards including heat, cold, acids, solvents, caustics, toxins, infectious microorganisms, radioisotopes, cuts, and animal bites. Unfortunately, there is no ideal glove that will protect against all hazards. The selection of proper gloves is essential when hazardous tasks are to be performed.

For protection of hands, wrists, and forearms against steam or for handling hot objects, insulating gloves or mittens made of Zetex aluminoborosilicate fibers or Kevlar aramid fibers have replaced the traditional asbestos gloves. For handling very cold materials, Zetex or insulated latex or neoprene gloves are available, and for liquid nitrogen, loose-fitting gloves are preferred. Other specialty gloves include gauntlet-type leather gloves for handling monkeys and Kevlar aramid, Kevlar and stainless steel, and stainless steel mesh gloves to be worn during necropsies of infected animals to prevent accidental cuts from contaminated scalpels and surgical saws.

Non-absorbent surgical gloves (usually made of latex or nitrile) should wrap over the cuff and lower sleeve of the laboratory clothing. If working with infectious materials it is recommended that 2 pairs of gloves be worn. It is also important to choose the thickest gloves possible, without sacrificing the touch sensitivity or dexterity needed for the work. For example: when working in Class III cabinets where animals are routinely handled wear 8mil neoprene gloves to reduce penetration from bites.

Respiratory Protection

There are a number of toxic or infectious materials that pose a significant health risk in a laboratory environment. Engineering controls such as, fume hoods, biosafety cabinets, rates of ventilation are all methods for protection, but when these measures are not feasible or adequate PPE becomes mandatory. It is important to note that “dust” or surgical masks are not classified as true respirators. They may be worn to help maintain a sterile surgical field or as a deterrent for hands near the face, but they provide little if any protection from infectious aerosols or toxic fumes.

Respiratory protection equipment can be:

- **Supplied Air** – supplies breathing air inside the face piece via compressed tanks or air lines.
- **Air purifying** – uses filters and/or chemical cartridges or canisters to remove air contaminants. It is critical to use the appropriate canister filter for the given situation.

There are two key styles of face-pieces used for the above respiratory equipment:

- **Tight-fitting** – half face (forming a tight seal around the nose and chin) and full face (forming a tight seal around the entire face and chin). The full facepiece is recommended when the infectious or toxic hazard poses an ocular, as well as, respiration risk. These need to be fit tested on an annual basis.
- **Loose-fitting** – hoods, helmets, bonnets, and full suits. These designs eliminate the need for fit testing and also allow the use of personal glasses and facial hair. Full isolation suits are usually recommended for CL 4 containment areas but may be used under other conditions.

One of the more versatile and newer items of respiratory protection equipment is the airflow hood. These constant-flow air-purifying hoods (PAPR) operate for 8 hours on rechargeable battery packs and can be equipped with a variety of particulate and chemical filters or a combination of both. They are lightweight and comfortable and have the advantage of eliminating fit testing.

Whenever supplied air equipment is used, the respiratory protection program should include a back-up provision in the event of compressor failure. Either an auxiliary compressor or bottled breathing air is recommended. If either of these is not feasible or is unavailable, a combination pressure-demand breathing apparatus should be worn or provided to permit escape from the dangerous atmosphere in case the primary air is interrupted. Such equipment usually incorporates an approved rated 5- to 10-min escape device.

Anyone required to use respirators should be enrolled in the Respirator Program, before using a respirator or ventilated hood. To enroll in the Respirator Program please contact Workplace Health Services at 604-827-4713 or Safety & Risk Services at 604-822-2029.

Eye or Face Protection

Eye protection in the biological laboratory is important for several reasons. Concentrated acids, alkalis, or other corrosive or irritating chemicals are used routinely. Concentrated disinfectants, including phenolics and quaternary ammonium compounds, can cause severe damage and blindness if splashed in the eye. Infection can also occur through the conjunctiva if certain pathogenic microorganisms are splattered into the eye. At least one virus, herpesvirus, has been shown to propagate in the brain after intra-ocular inoculation. Full-face respirators or half-face respirators plus splash goggles are recommended when operations with specific microorganisms or toxins may result in the generation of respirable aerosols or droplets that may enter the eye.

Prescription glasses and contact lenses are both allowed in the laboratory. They both provide a small amount of splash protection but do not replace the need for protective eyewear. Whenever possible the use of contact lenses is discouraged because it has been found that there is an increased risk of an individual touching their eyes when they are worn. If worn an adjustment needs to be made, it is critical that proper hand washing take place first, and then the adjustment must be made outside of the laboratory.

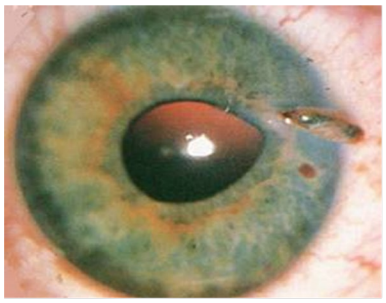
Safety glasses are sometimes requested and used by personnel working in biological laboratories because this work often involves the handling of hazardous chemicals. Safety glasses are intended to provide impact protection against projectiles and broken glass but should not be used to protect against chemical splashes in lieu of approved acid or chemical splash goggles or face shields. Although ordinary eye glasses offer better splash protection than wearing nothing at all, goggles or shields that are designed for this purpose offer maximum eye protection. Such protective devices are relatively inexpensive and are to be readily accessible in all laboratories where such eye hazards as chemicals are used.

UBC Eye Incident

On July 30, 2010, a UBC technician was inventorying his cell lines in the liquid nitrogen storage tanks. He was wearing a lab coat and gloves but no face shield. He would pull out a box of tubes, and then using forceps would read individual tubes and mark them in the inventory. As he read one of the tubes, it exploded in his face.

The result was massive damage to his eye. It required a total of 4 surgeries, the last of which was a cornea transplant on August 3, 2011. He will not be able to play sports, or participate in any activity that can raise the pressure in his eyes for the rest of his life. He now has some limited vision in the damaged eye, but it is still unknown if a full recovery is possible.

His advice to all researchers is why take the risk. Wear the appropriate PPE for the task. Think about what the worst case scenario is and take the appropriate precautions. Even if you go your entire life without injury, it is always a possibility, and is the risk worth it?



Safety shields, or face shields, also provide good protection against a chemical or biological splash and are recommended for workers handling non-human primates

because of the potential for exposure to Cercopithecine Herpes Virus 1. Face shields should also be worn when removing sample tubes from liquid nitrogen tanks.

Hearing Protection

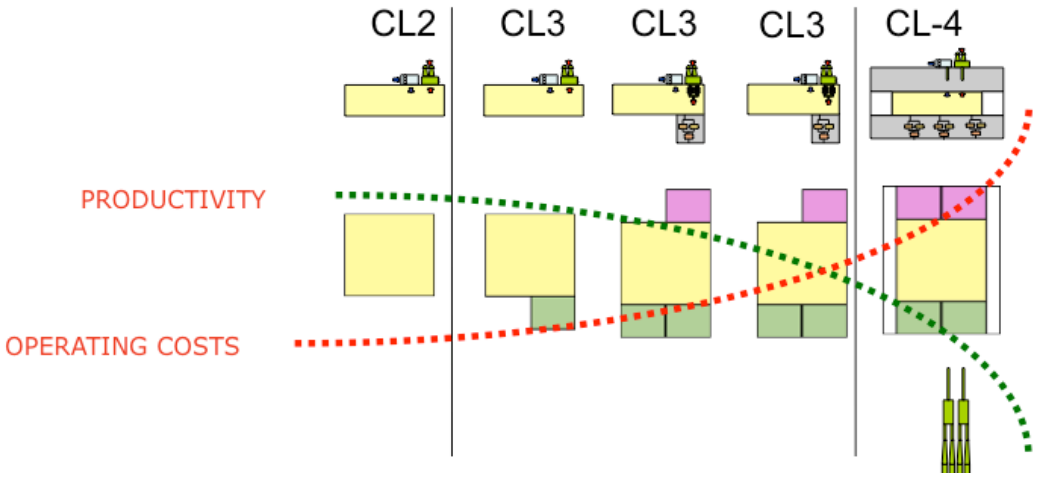
Hearing protection in a biological laboratory is important but rarely needed. If working with heavy machinery or a device that creates loud “bangs” then hearing protection may be necessary. But the most common need is when using a sonicator. Sonicators generate sound waves in the 20,000 Hz range. These sonicator-generated sound waves are outside the normal range of hearing. Often the sound heard while using a sonicator is produced by cavitations of the liquid in the sample container or vibrations from loose equipment. Actions you can take to reduce the hazards include:

- Wear earphone-type sound mufflers to protect your hearing while sonicating
- If possible, have the sonicator located in a "sound-proof" cabinet while sonicating
- Do not sonicate in a room containing people not wearing ear protection
- Shut doors of the room where sonication is taking place

Ensure that the hearing protection chosen is effective for the sound waves generated. Machinery usually creates low Hz as compared to sonicators.

Facility Design

As stated there are four levels of containment as described by PHAC. These levels often correspond to the Risk Group levels. However, the “Containment Level” id dependent on both the risk group of the materials being used and the manipulations and procedures that are being performed. The following section outlines a few key features for containment levels 2-4. For full descriptions and details read the Canadian Biosafety Standard 2nd ed 2015.



Facility design is a secondary barrier, and its primary purpose is to protect the environment and the public from the research occurring within. Facilities should be designed for flexibility, safety, and usability. The more safety features added to a facility, the more difficult the day-to-day operational functionality. The above image shows that the overall footprint of the facility increases as the containment level increases, but the

research space remains the same. This is because of the additional decontamination features, entry/exit features, and ventilation/effluent systems required.

The following are a few key features for each containment level. Each level incorporates the previous levels features. Again, for complete details, consult the Canadian Biosafety Standard. Also, the Department of Safety & Risk Services should be involved in the design of any new facility, or renovations to existing facilities.

Containment Level 1 (Wet Labs): a door separating the laboratory space, a sink, access to emergency eye wash and showers, working surfaces should be chemical and scratch resistance, openable windows have screens, and a separate area for personal items.

Containment Level 2: access limited to authorized personnel, seamless counters and floors, coved floors, all surfaces should be chemically resistant, increased air changes for the ventilation, and access to an autoclave.

Containment Level 3: electronic access, ante room entry, clothing change area, completely sealed surfaces, directional air flows, ventilation alarm system, HEPA filtered exhaust system, pass through autoclave, no external or openable windows, all systems on emergency power, communication system, and may have specialized plumbing and effluent treatment.

Containment Level 4: interlocking door access, suit change and chemical shower area, standalone building, no sharp corners or edges (even for drawers), extensive alarm systems, filtered supply and exhaust air, enhanced communication system, usually have supply air suits for working safely (or extensive Class III BSC banks), effluent treatment system, no floor drains except for showers, and video monitoring system for the perimeter and the laboratory.

Containment Level 2 with Containment Level 3 Procedures

Sometimes a risk assessment shows that the facility design may require a CL2 facility but there is an increased risk to the researcher. This often means that the operational practices for a CL3 need to be followed including some of the documentation requirements, PPE, and decontamination procedures. The following is a the most common set up for this type of facility, however, each facility requires the approval of SRS and the Biosafety Advisor.

Facility Requirements

- Dedicated and isolated CL2 space (usually a cell culture room) with a door
- Ability to restrict access

- Non-absorptive surfaces
- Negative pressure
- Hand-washing sink near the exit
- Ante room or pseudo ante room (created on a case by case situation)
- Seamless flooring
- Access to an autoclave
- Space to contain all the necessary equipment including:
  - Biosafety Cabinet (BSC)
  - Centrifuges (micro, ultra and/or high speed)
  - Incubators (for in vitro work)
  - Vortex (must be located within the BSC)
  - Refrigerator and/or Freezer
  - Microscope
  - Water bath
  - And any other specialized equipment

Animal Facilities

- All of the above for the procedure room
- Isolated housing rooms
- Autoclave access for all the tools and consumables used in the procedure room and for all the bedding, cages etc. in the housing rooms.
- HEPA filtered cage changing stations

Personal Protective Equipment (PPE)

- Isolation gown
- Double gloves
- Shoe covers
- May need Goggles, Hair net and N95 mask depending on the procedures

Risk Decontamination

The initial risk assessment for any project should include an evaluation of the processes to be used to decontaminate the material. This is to ensure that the biohazardous materials involved in the research are inactivated during spill clean-up, before cleaning equipment for re-use, and before final disposal.

Definitions

Sterilization

Any process, physical or chemical, which results in the absence of all life on or in an object. This term applies especially to the destruction of microorganisms, including bacteria, fungi, and their spores, and the inactivation of viruses. The best and most common means of sterilization is the use of saturated steam under pressure or autoclaving.

Decontamination

To destroy, remove, or neutralize living organisms, toxic agents or chemical carcinogens on a surface or object (this does not imply either total destruction or total removal); to make an object safe for unprotected individuals.

Disinfection

To use a chemical agent to kill or inactivate most vegetative bacteria, fungi, and viruses but not necessarily spores. This term applies to a chemical used on inanimate surfaces.

Germicide

Substance used to destroy a specific microorganism.

- Algicide – an agent that kills algae
- Bacteriocide - an agent that kills vegetative bacteria and possibly some less resistant spores (commercial term).
- Fungicide – an agent that kills fungi.
- Sporicide – an agent that kills spores.
- Virucide – an agent that inactivates, destroys or kills viruses.

General Information

There are a number of procedures that should be maintained as a routine protocol when using biohazardous materials:

- All infectious materials and all contaminated equipment or apparatus should be decontaminated before being washed and stored or discarded. Autoclaving is the preferred method and each individual working with biohazardous material is responsible for its decontamination before disposal.
- Biohazardous materials should not be stored in autoclaves overnight for autoclaving the next day.
- To minimize hazards to emergency response personnel, all biohazardous materials should be placed in an appropriately marked refrigerator or incubator and sterilized or otherwise confined at the close of each work day.
- Special precautions should be taken to prevent accidental removal of material from an autoclave before it has been sterilized or the simultaneous opening of both doors on a double door autoclave.
- Dry hypochlorites (bleach), or any other strong oxidizing material, must not be autoclaved with organic materials such as paper, cloth, or oil. The combination of an oxidizer, organic matter and heat may produce an explosion.
- All laboratories containing biohazardous materials should designate two separate areas or containers. One of these containers should be labeled BIOHAZARDOUS (TO BE AUTOCLAVED) the other, NONINFECTIOUS (TO BE CLEANED).
- All floors, laboratory benches and other surfaces in buildings where biohazardous materials are handled should be decontaminated as often as required. After

completion of operations involving plating, pipetting, centrifuging and other procedures that may produce aerosols, the surrounding areas should be disinfected.
- Floor drains should be flooded with water or decontaminant at least once each week in order to fill traps and thus prevent the backflow of sewer gases.
- Floors should be swept with push brooms only. The use of a floor sweeping compound is recommended because of its effectiveness in limiting the generation of airborne organisms. Vacuum cleaners equipped with HEPA filters may also be used. In all laboratories where infectious agents are used, water used to mop floors must contain an appropriate disinfectant.
- Stock solutions of suitable decontaminants should be maintained in each laboratory for disinfection purposes.
- Containers of prepared disinfectants should be labelled with:
  - The name of disinfectant
  - The concentration
  - The date prepared (once diluted, shelf-life is finite)
- WHMIS workplace label
- Use bench coat when there is the potential of aerosol production and/or the bench surface is rough or broken is some way.

Sterilization or Autoclaving

Autoclaving is the most dependable procedure for ensuring the complete destruction of microorganisms. It generally involves heating in a chamber employing saturated steam under a pressure of 103 kPa (15 psi) to achieve a chamber temperature of at least 121-°C for a minimum of 20 minutes (but the time is based on the size of the load and the organism being autoclaved). The time is measured after the temperature of the material being sterilized reaches 121°C. The most critical factor involved in steam sterilization, other than reaching the desired temperature for the correct time, is the prevention of the entrapment of air that is not displaced with steam. The materials being sterilized must come into contact with steam and heat for actual sterilization to result. It is for this reason that the use of some form of efficacy indicator must be done with each cycle.

The use of dry heat sterilization is less effective when compared to wet heat (steam) sterilization. Higher temperatures and longer times are required to ensure complete destruction of the microorganisms. Sterilization by dry heat can usually be accomplished at 160°C - 170°C for periods of 2 - 4 hours. When using dry heat, it is critical to be aware of the heat transfer properties of the material being sterilized as well as the arrangement of the material in the load.

Moist Heat		Dry Heat	
Temperature	Time	Temperature	Time
100°C	20hr	120°C	8hr
110°C	2.5hr	140°C	2.5hr
115°C	50min	160°C	1hr
121°C	20min	170°C	40min
125°C	6.5min	180°C	20min

Indicators

Biological (spore strips)

Contain *Geobacillus stearothermophilus* which, if sterilization occurs, will be unable to grow. It is critical that the strip be placed in the most difficult areas for the steam to reach in your autoclave batch.

Chemical (autoclave tape)

Contain chemicals that change colour if the correct temperature has been reached. Concerns with the use of Autoclave Tape are that the colour change is not time dependant and it does not ensure that all 'difficult to reach areas' of the autoclave load are reaching the required temperature and pressure.

Chemical (vials)

Contain chemicals that change colour if the correct temperature or pressure is not reached. These vials can be placed, like the biological indicators, in the centre of a load.

Autoclave Charts

Take real-time readings for the temperature and pressure within the autoclave. There are two main forms, circular charts and strip charts. It is critical to read the manufacturer's recommendations to ensure proper use of the chart associated to the autoclave.

In-Use Biological Indicators

Contain both a biological spores and chemicals. This means that there is a colour change after the run is completed and the spores can still be tested overnight.

Efficacy Testing

Efficacy testing is determining the functionality of a given autoclave. All autoclaves should be monitored using a combination of charts, chemical indicators and biological indicators. Every load should contain autoclave tape. Any autoclave load where the tape does not change colour needs to be recorded and the load re-run with new tape to determine if there is a problem with the tape or the autoclave. The chart indicator should also be read and kept on file for every load run.

On a monthly basis all autoclaves should be monitored with a biological indicator. It is critical to follow the manufacturer's instructions for the biological system chosen. The results of the test need to be recorded and saved for a minimum of 7 years. If the test shows growth, then the autoclaved must be shut down until it can be repaired.

Prions

Due to the structure of prion molecules it is very difficult to sterilize. Specialized procedures need to be developed. The following are three procedures described by the

World Health Organization and additional comments from the Centre for Disease Control.

- Immerse in a pan containing 1N sodium hydroxide (NaOH) and heat in a gravity displacement autoclave at 121°C for 30 min; clean; rinse in water; and subject to routine sterilization.
  [CDC NOTE: The pan containing sodium hydroxide should be covered, and care should be taken to avoid sodium hydroxide spills in the autoclave. To avoid autoclave exposure to gaseous sodium hydroxide condensing on the lid of the container, the use of containers with a rim and lid designed for condensation to collect and drip back into the pan is recommended. Persons who use this procedure should be cautious in handling hot sodium hydroxide solution (post-autoclave) and in avoiding potential exposure to gaseous sodium hydroxide, exercise caution during all sterilization steps, and allow the autoclave, instruments, and solutions to cool down before removal. An experiment conducted by Food and Drug Administration (FDA) investigators indicated that the use of appropriate containment pans and lids prevents escape of sodium hydroxide vapors that may cause damage to the autoclave (Brown and Merritt. Am J Infect Control 2003;31:257-260).
- Immerse in 1N NaOH or sodium hypochlorite (20,000 ppm available chlorine) for 1 hour; transfer instruments to water; heat in a gravity displacement autoclave at 121°C for 1 hour; clean; and subject to routine sterilization.
  [CDC NOTE: Sodium hypochlorite may be corrosive to some instruments.]
- Immerse in 1N NaOH or sodium hypochlorite (20,000 ppm available chlorine) for 1 hour; remove and rinse in water, and then transfer to open pan and heat in a gravity displacement (121°C) or porous load (134°C) autoclave for 1 hour; clean; and subject to routine sterilization.
  [CDC NOTE: Sodium hypochlorite may be corrosive to some instruments.]

Toxins

Similarly to prions, toxins cannot all be inactivated or neutralized using an autoclave. Depending on the type of toxin and the structure of the molecule, different methods of decontamination are necessary. It is critical to read the Safety Data Sheet (MSDS) associated to a given toxin to determine the appropriate means of decontamination. This may mean autoclaving, however treatment with Sodium Hydroxide is also common. General guidelines for toxin inactivation are also available through SRS.

Effectiveness of Chemical Decontamination

When choosing a product for use, it is important to consider a number of factors that influence a decontaminant's effectiveness. The effectiveness of any disinfectant is limited by a number of factors:

Organic Load

Organic soil (manure, blood, milk, bedding, feed) protects microorganisms from contact with decontaminants and can neutralize many germicides (e.g. sodium hypochlorite).

Removal of bedding, litter, feed etc., and cleaning prior to decontamination will reduce the organic load. All cleaning materials and items removed prior to decontamination must be decontaminated prior to disposal. Cleaning prior to decontamination may not be practical or safe where there is a risk of zoonosis. Under such circumstances, decontaminants that remain active in the presence of considerable amounts of organic material should be selected (e.g. phenolic compounds).

Surface Topography

Uneven, cracked or pitted surfaces, especially wooden surfaces can hide microorganisms and are difficult to decontaminate. High bacterial levels have been recovered from various surfaces in animal units; wood - 22,500 organisms/100 sq. cm.; concrete - 12,500 organisms/100 sq. cm.; brick - 75,600 organisms/100 sq. cm.; metal - 13,900 organisms/100 sq. cm.; plastic - 100 organisms/100 sq. cm.

Method of Application

Surfaces of a building may be treated with decontaminant solution by brushing or spraying. Portable items should be soaked in a tank of decontaminant. Fumigation may be used but is inefficient in buildings with ill-fitting doors and windows, damaged roofs, etc. Waterproof protective clothing and rubber suits can be hosed down with liquid decontaminant.

Concentration of Decontaminant

Generally, the higher the concentration of decontaminant the more rapid the kill. Some chemicals cannot be used in high concentrations because of extreme damage to surfaces or tissues. If the concentration is reduced enough to avoid damage, it is no longer sufficiently germicidal to be useful. Cost should be calculated per litre of use dilution rather than cost of concentrate.

Contact Time

Decontaminants should be effective in a short contact time. Longer contact times may be difficult to achieve due to evaporation. Most chemicals require 10 - 20 minutes contact time, but the manufacturer’s directions should be followed.

Temperature

Generally, elevated temperatures enhance germicidal action and reduced temperatures decrease germicidal action. Elevated temperatures may be hard to achieve and may also enhance evaporation, thus reducing contact time. Again, the manufacturer’s instructions should be followed.

Relative Humidity

Can influence the activity of some decontaminants, particularly formaldehyde. Fumigation using formaldehyde gas requires a relative humidity preferably in excess of 70%.

pH

The activity of some decontaminants is affected by pH (halogens tend to work better at lower pH).

Stability

In-use dilutions of some decontaminants (sodium hypochlorite, alkaline glutaraldehyde) may not be stable over long periods, especially in the presence of heat or light. Therefore, these chemicals must be made fresh on a daily or per use basis. **Diluted disinfectants must be labeled with the date of preparation.**

It is critical to follow the manufacturer's "in-use" instructions to ensure maximum effectiveness.

Microorganisms can be more or less resistant to decontamination. The general order is (most to least resistant):

- Bacterial spores
- Mycobacterium
- Non-lipid or small viruses
- Fungi
- Vegetative bacteria
- Lipid or medium-size viruses

MOST

LEAST

Chemical Decontaminants Classification Table

Class	Recommended Use	How They Work	Advantages	Disadvantages	Examples
Alcohols	Cleaning some instruments and surfaces of BSC's	Cell lysis and Protein Denaturatioin; Presence of water assists with killing action	Fairly inexpensive, easy to use, not corrosive, effective against most micro-organisms.	Evaporates quickly, requires long contact times, flammable, inactivated by organic matter	Ethanol, Isopropanol (70 – 85%)
Formaldehydes	Surface cleaner, as a gas for decontamination of large space's (BSC's & rooms)	Denature proteins and require the presence of water vapour.	Very effective against all foSRS of biohazards (including spores) and the gas can penetrate into small cracks and spaces.	Requires the use of special personal protective equipment.	37% Formalin Paraformaldehyde Cidex 7 Sporociden
Phenolics	When diluted act as an effective bacteriostatic agent.	They have a rapid corrosive action on tissue and cells.	Effective against viruses and vegetative bacteria.	Corrosive, irritant to skin, sticky and strong odour.	Pheno-kill, Phenola, Mikro-Bac (5%)
Quats	Good surface cleaners.	Affect proteins and cell membrane of the micro-organisms.	Contains detergents to aid in cleansing, rapid action, non-corrosive, & non-staining.	May not be active against some bacteria, spores, viruses and is rapidly inactivated by soap & organic matter.	Roccal, Tor, Mikro-Quat
Chlorines	Spills of human body fluids.	Free available chlorine binds with contents within micro-organisms; reaction byproducts cause death to the cell.	Broad spectrum, fast acting, inexpensive.	Corrosive, short use life, inactivated by organic matter, irratates skin and eyes.	Sodium hypochlorite, Javex, Presept, Alcide (10%)
Iodophors	Disinfecting some semi-critical medical equipment.	Free Iodine enters the micro-organism and binds with its cellular components, needs 30 - 50 ppm.	Braod spectrum, cleansing action, built-in colour indicator, inexpensive and few health or disposal problems.	Inactivated by hard water, may stain, weakly corrosive, and reacts with organic matter.	Wescodyne, Mikroclene, Hi-Sine

Categories of Radiation Decontaminants

Ionizing Radiation or Irradiation

There are three different irradiation technologies: gamma rays, electron beams, and x-rays. The first technology uses the radiation given off by a radioactive substance. This can be either a radioactive form of the element cobalt (Cobalt 60) or of the element cesium (Cesium 137). These substances give off high energy photons, called gamma rays, which can penetrate foods to a depth of several feet. These particular substances do not give off neutrons, which means they do not make anything around them radioactive. This technology has been used routinely for more than thirty years to sterilize medical, dental, and household products, and it is also used for radiation treatment of cancer. Radioactive substances emit gamma rays all the time. When not in use, the radioactive "source" is stored down in a pool of water which absorbs the radiation harmlessly and completely. To irradiate food or some other product, the source is pulled up out of the water into a chamber with massive concrete walls that keep any rays from escaping. Medical products or foods to be irradiated are brought into the chamber, and are exposed to the rays for a defined period of time. After it is used, the source is returned to the water tank.

Electron beams, or e-beams, are produced in a different way. The e-beam is a stream of high energy electrons, propelled out of an electron gun. This electron gun apparatus is a larger version of the device in the back of a TV tube that propels electrons into the TV screen at the front of the tube, making it light up. This electron beam generator can be simply switched on or off. No radioactivity is involved. Some shielding is necessary to protect workers from the electron beam, but not the massive concrete walls required to stop gamma rays. The electrons can penetrate food only to a depth of three centimeters, or a little over an inch, so the food to be treated must be no thicker than that to be treated all the way through. Two opposing beams can treat food that is twice as thick. E-beam medical sterilizers have been in use for at least fifteen years.

The newest technology is X-ray irradiation. This is an outgrowth of e-beam technology, and is still being developed. The X-ray machine is a more powerful version of the machines used in many hospitals and dental offices to take X-ray pictures. To produce the X-rays, a beam of electrons is directed at a thin plate of gold or other metal, producing a stream of X-rays coming out the other side. Like cobalt gamma rays, X-rays can pass through thick foods, and require heavy shielding for safety. However, like e-beams, the machine can be switched on and off, and no radioactive substances are involved. Four commercial X-ray irradiation units have been built in the world since 1996.

Non - Ionizing Radiation or UV Light

Ultraviolet (UV) light kills cells by damaging their DNA. The light initiates a reaction between two molecules of thymine, one of the bases that make up DNA. The resulting thymine dimer is very stable, but repair of this kind of DNA damage--usually by excising or removing the two bases and filling in the gaps with new nucleotides--is fairly efficient. Even so, it breaks down when the damage is extensive.

The longer the exposure to UV light, the more thymine dimers are formed in the DNA and the greater the risk of an incorrect repair or a "missed" dimer. If cellular processes

are disrupted because of an incorrect repair or remaining damage, the cell cannot carry out its normal functions. At this point, there are two possibilities, depending on the extent and location of the damage. If the damage is not too extensive, cancerous or precancerous cells are created from healthy cells. If it is widespread, the cell will die.

As a common rule, never allow your eyes or skin to be exposed to UV light in the laboratory. This “laboratory UV light” is heavily concentrated and can cause severe damage with very short exposure periods. Always wear personal protective equipment (PPE) such as gloves, face shields, and lab coats (long sleeves) when using UV light. Thick nitrile gloves are recommended, but latex gloves can be doubled for use. Biological Safety Cabinets (BSCs) are never to be occupied while the UV lamp is activated. Always lower sash and keep away from escaping rays. Mechanical safety devices should be standard on most new cabinets. If there is no safety shield or safety switch, these must be retro-installed in such a way as to prevent exposure and not interfere with the operation of the apparatus. Transilluminators are never to be used without the protective shield in place. A face shield, thick nitrile or double latex gloves along with a lab coat are the recommended PPE. Crosslinkers are not to be used if the door safety interlocking mechanism is not working properly.

Waste Disposal

Hazardous waste is any product, substance, or organism that is dangerous to human health or to the environment, and is no longer used for its original purpose at the time of disposal, or during storage or transportation prior to treatment or disposal. Biological waste may be hazardous due to its quantity, concentration, physical, or infectious characteristics.

SRS operates the Environmental Services Facility (ESF) which manages and handles the hazardous waste generated by UBC core research, education, and operational activities. The facility safely disposes of hazardous waste in accordance with the strict local, provincial, and federal regulations.

All UBC generators need to be aware of the environmental and financial impacts of hazardous waste and actively seek to minimize the amount of waste generated. Principal investigators, supervisors, technicians and students MUST be familiar with current waste disposal procedures for biohazardous and biomedical waste handled in their respective areas. Supervisors are responsible for ensuring that all employees receive the required training and that all laboratory procedures conform to UBC’s requirements.

For more information on hazardous waste disposal at UBC contact the Environmental Services Advisor (604.822.9840) or the ESF Technician (604.827.5389).

Drain Disposal

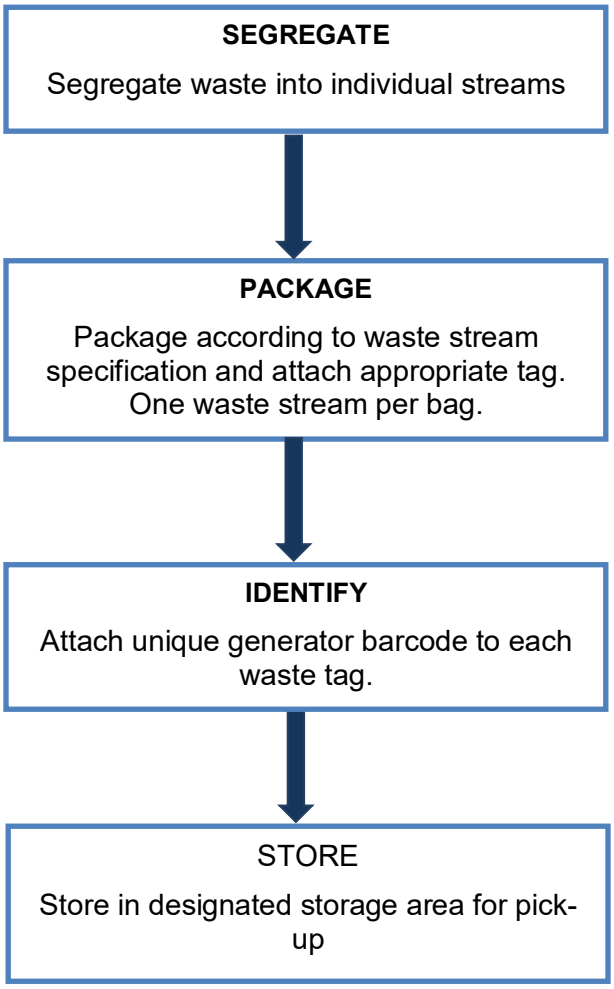
DO NOT pour hazardous materials down the drain! Metro Vancouver Sewer Use By-Law prohibits discharge of contaminants to the sanitary sewer (e.g. corrosives, flammables, toxics, metals, radioactives).

Bleach is corrosive and MUST be **neutralized** (pH = 5.5-10.5) before pouring down the drain with lots of water. Choose an appropriate neutralization agent depending on solution volume or other factors: sodium sulfite, sodium thiosulphate, or hydrogen peroxide are inexpensive additives to neutralize bleach. Note: acids like hydrochloric acid should never be added to bleach due to toxic fume production.

Contact the Environmental Services Advisor (604.822.9840) if you have questions about aqueous solutions disposal.

Hazardous Waste Disposal

There are four critical steps for the proper disposal of biological waste materials.



(Note: biohazardous waste packages need to be decontaminated.)

Segregation

Biological research will commonly result in the following waste streams:

Microbiological Waste

Laboratory cultures, stocks of specimens of micro-organisms, live or attenuated vaccines, human or animal cell cultures used in research, and laboratory material that has come into contact with any of the above

Biohazard Risk Group 1 (RG1): Defined on pg. 18
Biohazard Risk Group 2 (RG2): Defined on pg. 18

Biomedical Waste

Human anatomical: human tissue, organs, body parts
Human blood and body fluids: human blood, blood products, and items saturated or dripping with blood, body fluids contaminated with human blood, human body fluids removed for diagnosis or during surgery, treatment or autopsy

Sharps

Needles, syringes, blades or laboratory glass capable of causing punctures/cuts (contaminated or not contaminated with biohazards)

Toxins

Defined as described on pg 9

Pathological Waste (Uncontaminated and Contaminated)

Animal tissues and carcasses, non-RG2 fungi, and insects (ensure that all other materials have been removed, any tubing, needles or other items)

Non-Indigenous Waste

All materials and samples from outside the UBC or Vancouver area (Includes genetically modified organisms and plants, soil and water samples)

Non-Human Primate Waste

All waste associated with non-human primates

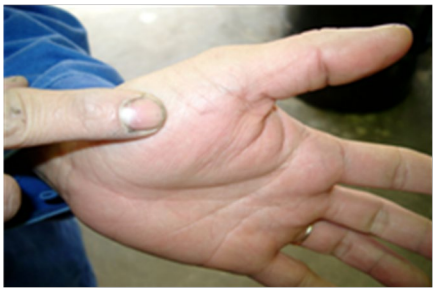
Non-Regulated Waste (Ethidium Bromide)

Solid waste contaminated with ethidium bromide (although not regulated as hazardous waste is not accepted at the landfill)

The Importance of Proper Packaging & Identification

It is extremely important to remember that although the waste is removed from the laboratory, there are still many individuals handling the waste. For this reason, it is **critical** to segregate, package, and identify the waste properly.

A needlestick to an ESF employee in 2006 was the cause of several painful anti-viral injections, and a year's worth of testing to ensure nothing was contracted from the needle.



ESF technicians will not pick up improperly packaged waste.

Pharmaceutical Waste
Any chemical substance used in the treatment, diagnosis, or prevention of abnormal conditions.

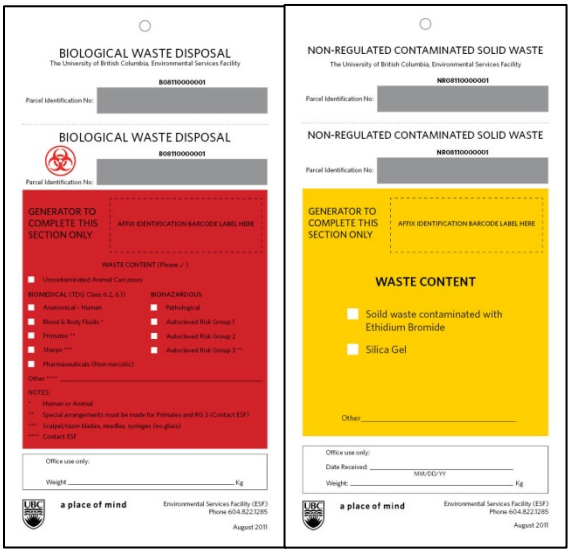
Controlled substances
Are specified under Schedule I, II, III, IV, V of the Controlled Drugs and Substances Act

Laboratory Glass Waste
Disposal of glass that is uncontaminated or contaminated by biohazardous or biomedical waste.

Packaging, Identifying, and Storing

Each waste stream has specific packaging, identification, and storage requirements. A summary of these requirements are located in the table on pg 64. For complete procedures, refer to the UBC Laboratory Pollution Prevention and Hazardous Waste Management Manual.

General guidelines for packaging:
- Double bag
- Not heavier than 10kg
- Ensure that there are no leaks or punctures
- If there is excessive liquid waste to be packaged then the use of an appropriately coloured bucket is recommended
- Off-campus generators use different coloured bags depending on the facility. Please contact your local site manager for site specific procedures



General guidelines for labeling:
- All transport of biological waste at UBC must be accompanied by a serialized Biological Waste Disposal tag (obtained from ESF) attached to each bag. A generator barcode sticker (obtained from ESF) must be affixed to the tag
- ONE waste stream box checked on the tag to identify the type of waste

For storage and pick-up information contact the Environmental Services Facility at 604.827.5389. ESF can help you:


Environmental Services Facility
6025 Nurseries Road, Vancouver, BC V6T 1W4
Date: ___/___/_____
Ticket Number: _____
ESF is unable to pick up this package because of the following reason(s):
- ☐ Weight of package is over 15 Kg
- ☐ Package is oversize
- ☐ Leakage
- ☐ Unsafe for transport
- ☐ Chemical approval form is missing
- ☐ Disposal Tag (Missing / No Barcode / Unidentified Waste Type)
- ☐ Other : _____

Should you have any questions, please contact ESF staff at _____

Thank you for your cooperation,
ESF Staff

- determine the specific location in your department or work area for pick-up of biological waste. All pickup locations must limit access to the general public to prevent the unauthorized removal of the waste, or accidental spillage of materials.
- determine the pick-up day and time at your location and to arrange delivery of supplies, such as waste containers, waste tags and generator barcodes.

If ESF is unable to pick-up packages due to non-compliance with packaging requirements, ESF technicians will leave a tag explaining the reasons for refusal (left hand image).

Refer to the **UBC Hazardous Waste Disposal Information Sheet** for an overview of waste disposal procedures.

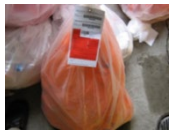
Off-Campus Generators

Generators at off-campus facilities (e.g. research centers located at hospital sites) will follow general procedures as listed in this section with slight modifications, as listed:

- Use ONLY approved colours for waste bags & containers as per off-campus waste disposal procedures
- NO need to use ESF approved generator barcodes
- NO need to use ESF biological waste tags (red) or other tags
- Store biological waste (approved containers with lids) inside labs, in designated storage areas inside or outside off-campus facility
- Facility or hospital staff and contractors will pick-up all your biological waste

NOTE: Generators must contact their off-site facility manager or coordinator for site specific waste disposal procedures.

Summary of UBC Biological Related Waste Procedures

Waste Stream	Examples	Decon	Packaging	Identification	Storage	Picture
Biohazard RG1	Non-pathogenic E. coli, DNA and RNA samples, many animal cell lines	Autoclave	2 x CLEAR bag	RED Biological Waste Tag "Autoclaved Risk Group 1"	Designated area	
Biohazard RG2	S. aureus, many influenza strains, recombinant or wild-type adenovirus, aspergillums, GM plants	Autoclave	1 x ORANGE bag 1 x CLEAR bag	RED Biological Waste Tag "Autoclaved Risk Group 2"	Designated area	
Biomedical	Human tissue, organs, and blood	No action	1 x RED bag 1 x CLEAR bag or 20L RED bucket	RED Biological Waste Tag "Anatomical Human" or "Blood & Body Fluids"	Designated FREEZER	
Toxins	Cholera, Pertussis, and Botulinum toxins	Dependent on toxin. Contact the Environmental Services Advisor (604.822.9840)			Designated area	Dependent on toxin
Sharps	Needles and blades	Seal and tape closed	RED/YELLOW certified sharps container	RED Biological Waste Tag "Sharps"	Designated area	
Pathological	Brain slices, animal tissues, carcasses, insects, and non-RG2 fungi	No action	2 x BLACK bag (6mm)	RED Biological Waste Tag "Uncontaminated Animal Carcass" (uncontaminated) or "Pathological" (contaminated)	Designated FREEZER	

Non-Indigenous	Plants, soils, waster from areas outside Vancouver proper	No action	2 x BLACK bag (6mm)	RED Biological Waste Tag "Uncontaminated Animal Carcass" and write description in "Other" section	Designated area	
Non-Human Primate	Tissues, blood, carcasses from non-human primates	See procedure				
Non-Regulated Contaminated Solid Waste	Ethidium bromide (solid)	No action	2 x CLEAR bag in strong cardboard box	YELLOW Non-Regulated Solid Waste Tag "Solid waste contaminated with Ethidium Bromide"	Designated area	
Liquid Ethidium Bromide Waste	Stock solutions and buffers containing Ethidium bromide	In lab treatment, see procedure				
Pharmaceutical or Controlled Substances	Ketamine, pentobarbital, acetaminophen	See procedure				
Animal Bedding	Wood chips mixed with feces/urine used in cages	See procedure				
Glass waste	Uncontaminated and contaminated glass	See procedure				

End Point of Various Biological Waste Streams

Waste Stream	Treatment	Disposal Company	Destination	Minimization Strategy
Biohazard RG2	Hydroclaved, shredded, and disposed as solid waste	Stericycle	Port Coquitlam, BC	Segregate uncontaminated solid waste
Biomedical	Incineration	Stericycle	Alberta (1500km from UBC)	
Uncontaminated Pathological Waste	Incineration	Forever in Peace	Mission, BC	
Solid Waste Contaminated with Ethidium Bromide	Secure Landfill	Sumas	Alberta (1500km from UBC)	Replace ethidium bromide with: SYBRSafe®, GelRed®

Available Recycling Programs

Laboratory Plastics Recycling Program

Recycle empty non-hazardous material plastic containers in your laboratory through the Laboratory Plastics Recycling Program, sponsored by Corning and Fisher Scientific.

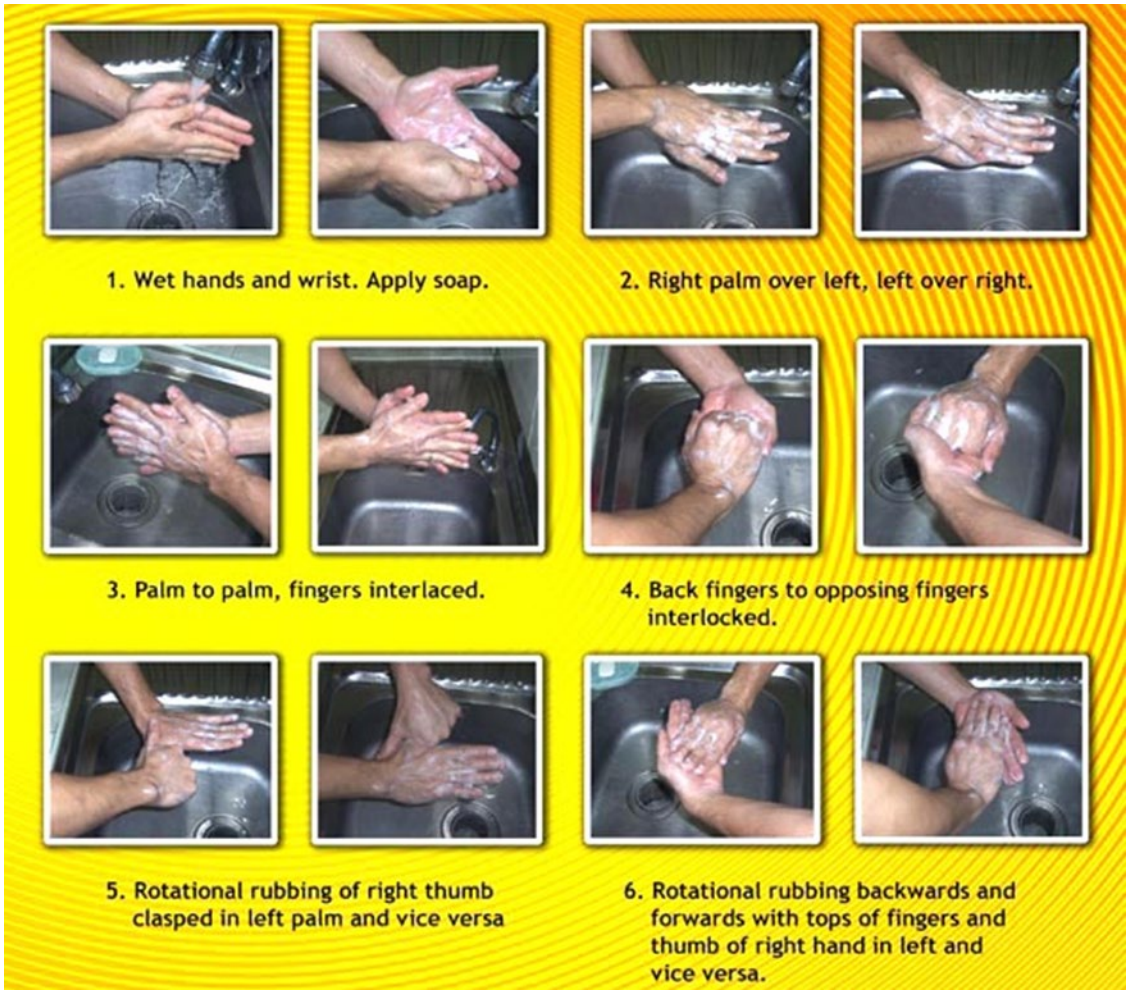
Styrofoam Recycling Pilot Program

The goal of the Styrofoam Recycling Pilot Program is to see no Styrofoam enter our landfills from UBC by providing a convenient and environmentally responsible way to reduce and recycle Styrofoam across the UBC-Vancouver campus. Styrofoam recycling is done through WCS Recycling.

For additional UBC recycling programs refer to Recycling Programs information page.

Appendix A: Hand Washing Procedure for Laboratories

This procedure should be followed when gloves are removed and before leaving the laboratory. 90 seconds is the recommended time for washing your hands, as it has been shown to remove more pathogens. It is also recommended that a surgical scrub brush be used around the nails and when there are cracks from dry hands.



Appendix B: General Lab Practices and Procedures

The following sections describe practices that should be performed regardless of the biological risk groups being manipulated in the lab.

PPE requirements and procedures:

- All lab members, visitors and other individuals entering the laboratory must wear the appropriate PPE in the lab. The PPE must then be removed when exiting the lab
- Appropriate PPE must be worn when there is the known or potential risk of exposure to splashes or flying objects
- Gloves (e.g., latex, vinyl, co-polymer) must be worn for all procedures where there may be direct skin contact with biohazardous materials. Gloves must be removed prior to leaving the laboratory and disposed of appropriately.
- Gloved hands must not touch common equipment and items such as telephones, elevator buttons or door-knobs.

Practices to minimize exposure and spread of biohazards:

- Hands must be washed
  - When gloves are removed
  - Prior to leaving the laboratory
  - When hands have come into contact with contaminated materials.
- No eating, drinking, smoking or mouth pipetting in the lab
- Food, drinks and the storage of personal belongings are not permitted in the lab
- Applying contact lenses and hand creams in the lab is strictly prohibited. Contact lenses can only be worn if other forms of corrective eyewear is not suitable.
- When working in the lab, long hair must be tied back or restrained so that they do not get into contact with equipment, specimens or containers.
- Open wounds, cuts, scratches and grazes should be covered with waterproof dressings
- Laboratories need to be kept clean and tidy and the storage of materials that are not pertinent to the work and cannot be easily decontaminated (e.g., journals, books, correspondence) should be minimized
- Whenever possible, paperwork and report writing should be kept separate from biohazardous materials work areas.
- Only authorized personnel are allowed in the lab
- Doors to the lab must remain shut.
- Leak-proof containers must be used whenever transporting items within facilities.
- Needles must never be re-capped

Decontamination and Disinfection Procedures:

- Work surfaces need to be decontaminated after each working day.
- Materials leaving the lab must be decontaminated and disinfected
- Autoclaves must undergo regular efficacy testing. These tests need to be documented and kept on file. Cycle logs (for temperature and pressure) must also be kept on file.

- Appropriate disinfectants must be accessible in areas where the biohazardous materials are being handled.
- Spills, accidents, exposure and loss of containment must be reported to the supervisor and documented
- Known or suspected contaminated clothing must be decontaminated prior to laundering.

Appendix C: Biosafety Cabinet Use Procedures

The installation of a biological safety cabinet within a laboratory is usually an indication that careful work practices are needed. The cabinets are not substitutes for good practice and can only complement a careful worker.

Preparing for Work within a Class II BSC

PPE requirements:

- The operator should wear a closed-front over garment (e.g. surgical gown with full-length sleeves)
- Gloves (latex or vinyl gloves) must be worn when working in a BSC. The use of a bare hands is not advised.
- Gloves should overlap the cuffs to ensure that aerosols do not contaminate the hands, arms and surfaces.

Planning and Organization:

- Prepare a written checklist of materials necessary for a particular activity prior to starting work.
- Have protocols written out and accessible.
- To minimize the in-and-out motions that could affect the protective barrier of the BSC, determine which materials should be placed in the BSC and which materials should be placed outside.
- Ensure that the BSC you are working with is appropriate for your protocols. For instance, if you are working with radioisotopes or volatile chemicals, ensure that you have selected the correct BSC type.

BSC start up procedure:

The following start up procedures must be followed whenever starting to work in a BSC.

- If a UV light is being employed, turn it off first
- Turn on the BSC and open the sash to the appropriate sash height
- Cabinet blowers should be operated at least ten to fifteen minutes before beginning work to allow the cabinet to "purge". This purge will remove any particulates in the cabinet.
- Ensure that nothing is blocking the front grilles
- The work surface, the interior walls (not including the supply filter diffuser), and the interior surface of the window should be wiped with either
  - 70% ethanol (EtOH)
  - 1:10 dilution of common household bleach (i.e., 0.5% sodium hypochlorite)
  - other disinfectant as determined by the investigator to meet the requirements of the particular activity

Note: When bleach is used, a second wiping with sterile water is needed to remove the residual chlorine, which may eventually corrode stainless steel surfaces. Wiping with

non-sterile water may re-contaminate cabinet surfaces, a critical issue when sterility is essential (e.g., maintenance of cell cultures).

While working in a BSC:

After the BSC has been sufficiently purged and decontaminated, the following practices should be employed to maintain product, personnel and environment protection.

Arm Movements

While working in a BSC, it is imperative that errant air flow velocities are not introduced for the proper functioning of the BSC.

- Once hands/arms are placed inside the cabinet, manipulation of materials should be delayed for approximately one minute. This allows the cabinet to stabilize and to "air sweep" the hands and arms to remove surface microbial contaminants.
- Move arms in and out slowly, perpendicular to the face opening of the cabinet
- Ensure that rapid arm movements in sweeping motions are minimized. This movement will disrupt the air curtain and may compromise the partial barrier containment that is provided by the BSC.

Front Grille

To ensure that the BSC can provide proper product, personnel and environment protection, it is important that the front grilles are not blocked.

- Raise arms slightly to ensure that arms are not resting on the grille.
- Ensure other items are not blocking the grille (i.e. protocols, pipettes etc.)

Placement of materials inside the BSC

Materials or equipment placed inside the cabinet may cause disruption to the airflow, resulting in turbulence, possible cross-contamination, and/or breach of containment.

- The surfaces of all materials and containers placed into the cabinet should be wiped with 70% ETOH to reduce the introduction of contaminants to the cabinet environment. This simple step will reduce introduction of mold spores and thereby minimize contamination of cultures.
- Only the materials and equipment required for the immediate work should be placed in the BSC
- Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet.
- All operations should be performed at least four 4" inches from the front grille on the work surface
- Active work should flow from the clean to contaminated area across the work surface.

Microbiological Techniques

Many common procedures conducted in BSCs may create splatter or aerosols. Good microbiological techniques should always be used when working in a biological safety cabinet. For example, techniques to reduce splatter and aerosol generation will minimize the potential for personnel exposure to infectious materials manipulated within

the cabinet. Class II cabinets are designed so that horizontally aerosol spores will be captured by the downward flowing cabinet air within fourteen inches of travel.

- Keep clean materials at least one foot away from aerosol-generating activities. This will minimize the potential for cross-contamination.
- The general work flow should be from "clean" to “contaminated” (“dirty”). Materials and supplies should be placed in such a way as to limit the movement of "dirty" items over "clean" ones.
- Opened tubes or bottles should not be held in a vertical position. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impaction of downward air.
- Bottle or tube caps should not be placed on the toweling.
- Items should be recapped or covered as soon as possible.

Biohazard bags and other waste containers

The frequent inward/outward movement needed to place objects in biohazardous bags and pipette collection trays is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. The following describes specific practices to use when working with either of these items:

Biohazard bags

Typically used when contaminated waste is going to be autoclaved.

- Ensure that the correct type of bag is used for the correct biohazard
- To minimize the chance of leaks, double bag
- The bag should be placed to one side of the interior of the cabinet and not taped to the outside of the cabinet.
- Water should be placed within the bag to allow steam to be generated during the autoclave cycle
- Materials that are contaminated must be placed into the bag and the bag must be sealed prior to it being removed from the cabinet.
- The bag should be transported and autoclaved in a leak proof tray or pan.

Discard trays or pans

Only horizontal pipette discard trays or pans should be used within the cabinet. Upright pipette collection containers should not be used in BSC’s nor placed on the floor outside the cabinet.

- Practices to use when discard trays and pans are decontaminated using chemical disinfectants:
  - Discard pipette trays should be placed to one side of the interior of the cabinet.
  - Items should be introduced into the pan with minimum splatter, and allowed appropriate contact time as per manufacturer's instructions.
  - The discard pan should be covered and surface decontaminated in the BSC prior to removal out of the cabinet.

- Practices to use when discard trays and pans are decontaminated using the autoclave:

- Discard pipette trays should be placed to one side of the interior of the cabinet.
- Water should be added to the bag or tray prior to autoclaving, to allow for steam to be generated through the autoclave cycle.
- Items should be introduced into the pan with minimum splatter.
- The tray needs to be sealed prior to removal from the cabinet.

Absorbent Toweling

Plastic-backed absorbent toweling can be placed on the work surface (but not on the front or rear openings). This toweling facilitates routine cleanup and reduces splatter and aerosol formation during an overt spill. It can then be folded and placed in an autoclavable biohazard bag when work is completed.

Aerosol generating equipment

Aerosol-generating equipment (e.g., vortex mixers, tabletop centrifuges) should be placed toward the rear of the cabinet to take advantage of the air split that occurs in the BSC. The downward moving air "splits" as it approaches the work surface; the blower draws part of the air to the front grille and the remainder to the rear grille.

Open Flames

Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current that prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence that disrupts the pattern of air supplied to the work surface. When deemed absolutely necessary, touch-plate microburners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable sterile loops can also be used.

Aspirator bottles or suction flasks

Aspirator bottles or suction flasks must be connected to an overflow collection flask containing appropriate disinfectant, and to an in-line HEPA or equivalent filter. The flasks and aspirator bottles, if kept in the BSC, must be kept to one side of the cabinet. This combination will provide protection to the central building vacuum system or vacuum pump, as well as to the personnel who service this equipment. Inactivation of aspirated materials can be accomplished by placing sufficient chemical decontamination solution into the flask to kill the microorganisms as they are collected. Once inactivation occurs, liquid materials can be disposed of appropriately as noninfectious waste.

Power Failure while working in the BSC

When a power failure occurs while you are working in the BSC, the following procedures must be employed.

- Seal all open containers
- Dispose of gloves within the BSC
- If the BSC has a movable sash, bring it down to the closed position.

BSC shut down procedures:

After work is completed in the cabinet, the following procedures should be followed:

- Allow the cabinet to run for 5 minutes with no activity
- All containers and equipment should be surface decontaminated prior to removal
- Remove gloves and dispose of them as appropriate. Wash your hands.
- Put on clean gloves and ensure that all contaminated materials have been appropriately disposed of in the biohazardous bag or discard tray. Seal and surface decontaminate biohazardous bags and waste containers prior to their removal.
- Decontaminate the work surface using an appropriate disinfectant (ie. 70% ethanol)
- At the end of the workday, the final surface decontamination of the cabinet should include a wipe-down of the work surface, the cabinet's sides and back, and the interior of the glass.
- Remove gloves and gowns and wash hands.

Appendix D: Working with Human Blood and Body Fluids

As human blood and body fluids could potentially contain pathogens that could infect the user, it should be worked with under containment level 2 requirements and practices. In addition, the following specific practices should be followed when handling human blood.

General Practices

- Wear appropriate PPE (at a minimum, this should include: lab coats, long pants/skirts, appropriate footwear and gloves)
- Work in containment level 2 facilities with containment level 2 practices
- Use the appropriate biohazardous waste bags
- Decontaminate all wastes appropriately either through autoclaving or chemical disinfection
- Wash your hands after removing gloves and handling contaminated or potentially contaminated materials
- Obtain Hepatitis B vaccination and other medical surveillance as deemed appropriate
- Always treat all needles and sharps as if they have been contaminated
- Never recap or purposely bend, shear, or break needles
- Always dispose of needles and sharps in a in a secure, leak-proof, puncture-resistant container

Acceptable sources for blood

When doing experiments with human blood, the samples can only be obtained from the following sources

- Commercial sources
- Volunteers who fit the following criteria:
  - Not yourself
  - Not from your own lab or others who have access to your lab space

Note: When using volunteers, the appropriate ethics approval must be completed.

First Aid measures when working with blood

Needlestick/Sharp injury:

- Apply first aid
- Wash thoroughly with soap and water (do not use any other caustic solutions like bleach)
- Obtain medical attention immediately (Emergency Care)
- Obtain the appropriate medical screening
- Report the incident to the supervisor and fill out the appropriate accident/incident forms. (CAIRS)

Eye splash:

- Flush the eye out at an eye wash station for 15-20 minutes
- Obtain medical attention immediately (Emergency Care)

- Obtain the appropriate medical screening
- Report the incident to the supervisor and fill out the appropriate accident/incident forms. (CAIRS)

Skin exposure:

- Wash thoroughly with soap and water (do not use any other caustic solutions like bleach)
- Obtain the appropriate medical screening
- Report the incident to the supervisor and fill out the appropriate accident/incident forms. (CAIRS)

Appendix E: Spills of Biological Agents

Contact with spills is second only to aerosol generation as a health hazard associated with working with biohazardous agents.

Every laboratory working with infectious biological agents must have written spill control and safety procedures appropriate to the hazards and characteristics of the agents in use. Spills or other accidental releases of biohazards can be large or small, confined within equipment such as centrifuges or biological safety cabinets, or unconfined, and they may be liquid or dried. Spills may also involve other hazards such as isotopes, chemical, electrical equipment and aerosol generation. These other hazards must be considered when planning your response. Identification of all risks, both potential and actual, as well as the various factors listed above, must be taken into consideration before spill clean-up begins.

Basic Spill Clean-up Procedure (Biohazards)

What follows is a generic spill clean-up procedure only. The spill control procedure you follow in your laboratory must be appropriate for your agents, your lab, and your equipment and procedures. Your clean-up procedure must consider the safety of all personnel involved.

1. Immediately notify other individuals in the area that there has been a biohazard spill.
2. If there is any hazard associated with aerosol release, everyone should immediately leave the area. If necessary, block access to the area and mark with a Biohazard Spill Notice sign. Allow at least 30 minutes for the aerosols to settle before re-entering. Notify the supervisor and the Biosafety Officer (822-2029). If the spill is greater than 1 litre phone the Hazardous Materials Response Unit of the Vancouver Fire Department at 822-4567.
3. Individuals involved in the spill should check for contamination of clothing, footwear, and skin and take the appropriate action according to their specific spill control protocol prior to attempting spill clean-up.
4. Put on the appropriate personal protective equipment.
5. Identify the area requiring clean-up and decontamination, allowing sufficient area for any splattering or drying which may have occurred.
6. Set up a disposal bag to allow easy discarding of contaminated clean-up materials.
7. Move slowly and carefully while gently pouring the appropriate decontaminant around and not on the spill. This will avoid the creation of new aerosols.
8. Use absorbent materials (i.e. paper or cloth towels) to work the decontaminant into the area of the spill.
9. Cover the entire spill area with absorbent material soaked in decontaminant, and allow the decontaminant to remain in contact with the spill for an appropriate amount of time (usually 20 - 30 minutes).
10. Place the used absorbent material into the disposal bag and repeat the decontamination procedure.
11. Repeat steps 7-9 two more times.

12. Carefully remove gloves and place with the other contaminated materials in clearly marked Biohazard containers for further decontamination or disposal. DO NOT autoclave bags containing organic matter and oxidizing agents such as bleach.
13. Wash hands thoroughly with mild soap and water.
14. Complete an “Incident/Accident” Report form on CAIRS.

Basic Biological Spill Clean-up Kit

- Written spill clean-up procedure
- Gloves, protective clothing, and safety glasses
- Tape or marking pencil to mark off spill area
- Biohazard Spill Notice (Keep out) sign
- Appropriate chemical disinfectant (check expiry date and dilution) – 5% Wescodyne or 5-10% hypochlorite (bleach) are most common
- Absorbent material (paper towel, incontinent pads, cloth rags or absorbent carbon pads
- Disposal bags – leak proof, autoclavable, and labeled (biohazard tags)
- Sharps collector and forceps for picking up broken glass or sharps
- Paper, Incident/Accident Report form and pencil to document the spill and any possible personnel exposure.

Additional recommendations for a spill kit include:

- Scrubs
- Towel
- Rubber boots or disposable shoe cover
- Privacy curtain for if someone needs to use the emergency shower.
- Bar of soap (because the soap from the dispenser is awkward in an emergency)

Appendix F: Additional Biosafety Cabinet Information

Class I Cabinets: Additional Information

The Class I depend on a flow of air into the front work opening, across the work surface, and out through a decontamination device, usually a high-efficiency filter, via an exhaust blower. The cabinet can provide good protection of the operator from the work and allows the use of electronic incinerators, small gas (i.e. Touch-A-Matic) burners, small centrifuges, and other equipment without seriously degrading the containment effectiveness. The cabinets may be constructed of stainless steel or fire-resistant reinforced plastic, with glass or clear optical-grade plastic for view windows. Materials and equipment may be moved in and out through the front opening, through a hinged view window, or via air-lock doors added to the cabinet end. There is general agreement that the cabinet should have an interior rear baffle to provide a smooth airflow across the work surface while permitting some air to be removed from the upper section. The front opening design is also important, and the user should ensure that this aspect of design has been resolved satisfactorily.

Class I cabinets offer no protection of the work from the operator or the environment. In a laboratory that does not supply clean air or in a cell culture operation in which contamination from the worker may affect the work product, the Class I cabinet may be contraindicated. However, to their advantage, Class I cabinets are simple and economical, easily installed, can be used with radioisotopes and some toxic chemicals, and can be adapted in various forms to meet the unique needs of special processes.

Class II Cabinets: Additional Information

Class II Type A1 Cabinets

The air is drawn into and over the blower and then, under pressure, up to the recirculating or exhaust filters and through the exit filter is contaminated both from the work and from the room. Therefore, this air plenum must be airtight and leak proof. Because a substantial fraction of the air in the cabinet (up to 70%,) is re-circulated through the supply filter, the Type A1 cabinet is generally not considered suitable for use with high-activity radioactive materials or with toxic or carcinogenic chemicals. The essential elements of Class II Type A1 cabinets are HEPA-filtered laminar-flow recirculated air, traveling downward over the work surface, air inlet into the front with immediate conveyance away from the work surface, and discharge of excess air from the cabinet to the room or outdoors via a HEPA filter. The blower in the cabinet forces the air both through the recirculating air filter and the exhaust air filter, and thus a careful balance must be achieved to obtain the expected performance.

The concept of sealing the Type A1 positive-pressure plenum Freon gas-tight is good, but testing it is a difficult task. An alternative to guaranteeing that the positive-pressure plenum is leak tight is to surround the plenum with a negative-pressure area. This is referred to as a Class II Type A2 cabinet and is discussed in the next section. The performance characteristics of this type of cabinet are equal to those of the conventional Class II Type A1 cabinet.

Class II Type B2 Cabinets

The Type IIB2 "total exhaust" cabinet is similar in design, but all air entering the cabinet makes only one pass through the cabinet before being discharged through a HEPA filter to the outdoors. The work opening inlet air velocity averages 100 lfpm (0.5 m/s) or higher. This air is prevented from contaminating the work by a protective flow of HEPA-filtered room air entering the top of the cabinet.

Class IIB2 cabinets are designed to be used for work with limited quantities of toxic chemicals or radionuclides required in microbiological studies. Cabinets of this design meet NSF 49 standards for biocontainment and product protection. If air velocities (downward and inward) are maintained similar to those in the IIB1 configuration, the containment performance should be equal.

Class III Cabinet: Additional Information

Disadvantages of Class III cabinets include the initial expense of the equipment, as well as the installation and maintenance. The preparation before actual work in the cabinet line is extensive, and the work is made more difficult by the use of the relatively thick arm-length gloves. However, the cabinets are extremely useful when a very high level of protection is required for the operator and the environment. With appropriate training, the operator can become accustomed to the limitations afforded by working through fixed gloves. The gloves provide both the aerosol containment and protection from hand and arm contamination, which can be a main source of contamination release from Class I or II cabinets. However, these gloves can be punctured, and thus they constitute the weakest part of the Class III cabinet system protection.

Selection of Class I and II Biosafety Cabinets

When selecting one of the Class I or Class II biosafety cabinets, there are several factors to be considered:

- operations to be conducted in the cabinet
- classification of the etiologic agents to be used
- protection required for the work product
- possibility of use of radioisotopes or toxic or carcinogenic material in the course of the work
- funds available to use cabinets that meet accepted standards for housing the work to be performed.

Biosafety Cabinet Installation Recommendations

Installation of Class I and II Cabinets

Class I and II open-front cabinets are more frequently used in CL 2 and 3 laboratories under a variety of conditions. They are basic tools for use in the microbiology laboratory. The best location for such cabinets is at the end of a U-configuration, where there will be a minimum of cross-traffic in front of the work surface to interrupt the airflow or to disrupt the operation, and at the same time work bench space will be available at either end for materials. Positioning the work-space against an outside wall permits ready installation of duct work to the outside. An inside wall adjacent to service chases can permit connection to ventilation exhausts or a duct to the roof. In addition, the room

where the cabinet work is being planned should contain a hand-washing sink and eyewash station.

Leakage, both into and out of the cabinets, has been shown to be proportional to the velocity of air crossing in front to the cabinet. To minimize the introduction of the high-velocity air draft from disrupting the proper functioning of the BSC, it is important that the BSC be installed away from swinging doors in low traffic areas away from air conditioning vents or fans.
With space at a premium in most microbiology laboratories, adequate room for removal and exchange of the cabinet filters from the Class I and II cabinets may be overlooked. Provisions for ready access for periodic maintenance and re-certification must be made.

Special considerations for the Class I cabinet

Class I cabinets can be exhausted through a HEPA filter to the laboratory; however, a direct outdoor duct will permit use of the cabinets for chemicals and radioisotopes and is the preferred installation.

Special considerations for the Class IIA cabinet

Class IIA cabinets do not specifically require ventilation of the exhaust directly to the outside. These cabinets, if they have blowers and exhaust filters incorporated within them, may be exhausted through the filter into the laboratory. However, this is not always the best practice. Exhaust filters occasionally develop leaks; furthermore, discharge and ventilation of the waste formaldehyde used to decontaminate the cabinet is considerably easier when the cabinet has an exhaust duct to the outdoors. The cabinet should have its own exhaust blower and be exhausted directly outside, with an anti-backflow damper in the exhaust to ensure that there is no flow back through the filter and cabinet into the "negative pressure" laboratory when the cabinet is shut off. The exhaust should be run to an area clear of and at least 10 ft (approx. 3.05 m) above the roof line, so that workers do not come near the outlet. It should not discharge out into a courtyard or where it may be drawn into other parts of the building. In some cases, it may be possible to exhaust the cabinet into a building exhaust system that does not re-circulate to other parts of the building. This is frequently done with a loose connection to the exhaust called a "thimble piece" or variation thereof. Decontamination will require that the cabinet exhaust be substantially blocked during decontamination gassing; thus a hinge on the thimble or a flexible ducting will be needed for access to the exhaust filter area to be sealed. If necessary, even a "hard" connection can be used if the ductwork system is dedicated to a limited number of cabinets or exhaust systems. Suitable provisions must be made to prevent backflow and to shut down the cabinet in the event that exhaust flow is lost.

Special considerations for the Class IIB cabinet

Class IIB cabinets require connection to a separate exhaust system because many such cabinets do not have an internal exhaust blower. Even if the cabinet has been installed with a dedicated exhaust fan, the use of radioactive or toxic chemicals requires the discharge of the exhaust to be clear of occupied spaces. The most obvious installation problem for IIB series cabinets is the requirement to provide sufficient inflow

of air and lack of cross-drafts. This, and the effect on room ventilation balance are similar to the requirements for the Class I cabinet.

A major drawback to the Class IIB cabinet is the requirement for relatively large quantities of room air and subsequent discharge to the atmosphere. As is done with fume hoods, some unconditioned air may be supplied directly from outdoors by separate ducting. This can be expensive and difficult to accomplish. Another drawback common to most Class IIB cabinets is that they must have at least two fans (supply and exhaust) operating in balance (e.g. with the exhaust always exceeding the input to provide the necessary work opening inflow and negative pressure within the cabinet). Considering that the exhaust and supply filters are subject to differing rates of dirt loading, airflow at the inlet can vary with usage. This added complication in installation and setup should be examined by the prospective users of these cabinets.

Installation of class III cabinets

The installation of Class III cabinets is highly specialized. Although it is possible to use only a single element of Class III modular cabinetry, such equipment is usually installed as a "system," and specialized design requirements often include use of continuous spaces for animal holding and other activities, as described below. The space within which a Class III cabinet system is used must be suitable for containment in the event of failure of the cabinet.

Biosafety Cabinet Decontamination

Biosafety cabinet decontamination involves sealing off the cabinet, including both inlets and exhausts, and vaporizing dry paraformaldehyde (0.3 g/ft^3) to provide a concentration of 10,000 ppm. (Recently, vapor-phase hydrogen peroxide has been used as an alternate method.) The overall volume of the cabinet is calculated to allow for take up in the supply and exhaust filters. The formaldehyde vapor is held in the cabinet for 4 hrs or overnight. It is very important to ensure that the temperature remains in the 20 to 25°C range and humidity is at least 60%, for maximum effectiveness. After sufficient contact time, the formaldehyde gas may be discharged through the exhaust filter to the outdoors, or neutralized with ammonium bicarbonate (0.3 g/ft^3) or other appropriate agent. Decontamination effectiveness can be estimated by placing Bacillus subtilis spore strips (10^6 to 10^8 per strip) in the cabinet before decontamination. These spore strips are then incubated on Trypticase soy agar to validate spore kill, as a worst case scenario.

Although formaldehyde is the best choice for most cabinet or space decontamination procedures, the effectiveness of any vapor-phase decontaminant against the specific agents used in the cabinet should be ensured. For example, formaldehyde is not effective against many of the so-called slow viruses such as the agents of scrapie or the prion responsible for Creutzfeldt-Jakob disease. In such cases, vigorously applied liquid decontaminants may be required. It may be desirable to wet down, remove and autoclave or incinerate filters in such cases. In fact, considering the relatively poor penetrating capability of formaldehyde vapor, it is prudent to autoclave or incinerate HEPA filters after use in certain infectious disease laboratories.

The initial risk assessment for any project should include an evaluation of the processes and/or disinfectants to be used. This is to ensure that the biohazardous materials involved in the research are inactivated during spill clean up, before cleaning equipment for re-use and before final disposal.