THE UNIVERSITY OF BRITISH COLUMBIA

Health, Safety and Environment

Laboratory Biosafety

Reference Manual

Okanagan version

1st Edition, 2010

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Health Safety and Environment (Biosafety)
Okanagan Version Edited by Cherie Michels, 2010

Emergency Numbers

UBC Campus - Okanagan

Fire, Police, Ambulance	**911**
Emergency / First Aid / Security	**250 80(7-8111)**
Health, Safety and Environment	**250 80(7-8111)**
Hazardous Materials Response	**911**
Poison Control	**1-800-567-8911**

Non-Emergency Numbers

Parking, Transportation and Campus Security (Non-Emergency)	**250 80(7-9236)**
UBC Health, Safety and Environment, Okanagan	**250 80(7-9236)**
UBC Health, Safety and Environment, Vancouver	**604.822.2029**
Facilities Management	**250 80(7-9272)**
Health & Wellness (students)	**250 80(7-9270)**
R.C.M.P. (Non-Emergency)	**250.762.3300**
Fire Department (Non-Emergency)	**250.469.8801**
Kelowna General Hospital Emergency Department	**250.862.4485**
Employee and Family Assistance program (EFAP)	**1-800-663-1142**

Forward

This manual has been developed by the Office of Biosafety, Department of Health, Safety and Environment, UBC, with the endorsement of the University's Biosafety Committee. In the Okanagan, the principals contained within have been revised to reflect the local needs by Health, Safety and the Environment who administer the program locally. 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 well being 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 Principle Investigator (PI) 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 Health, Safety and Environment are available. In the Okanagan, the advisor for Biosafety in Health, Safety and Environment can be reached at 250 80(7-8656).

University Safety Policy

Approved: March 1994
Vice President Administration & Finance

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 WorkSafe BC, Public Health Agency of Canada 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.

University Safety Policy Continued

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 properly and care for adequately personal protective equipment provided by the University; participate, if elected or appointed, on departmental or building safety committees.

Detailed Procedures

The University Health and Safety Committee works to achieve these objectives by providing education and reviewing policies and procedures.

Local Safety Committees carry out the safety programs within their areas and make recommendations to ensure that the safety objectives of the University can be achieved. (Terms of Reference for these committees available through the Department of Health, Safety and Environment.)

The Department of Health, Safety and Environment and the Department of Parking and Security Services 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 Health, Safety and Environment and/or the Department of Parking and Security, Services.

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Introduction

The University of British Columbia is committed to providing a safe and healthful workplace for its faculty, staff and students and to ensure the protection of the community and the environment. To meet this commitment Health, Safety and Environment 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 UBC 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.

I. 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 Environment.

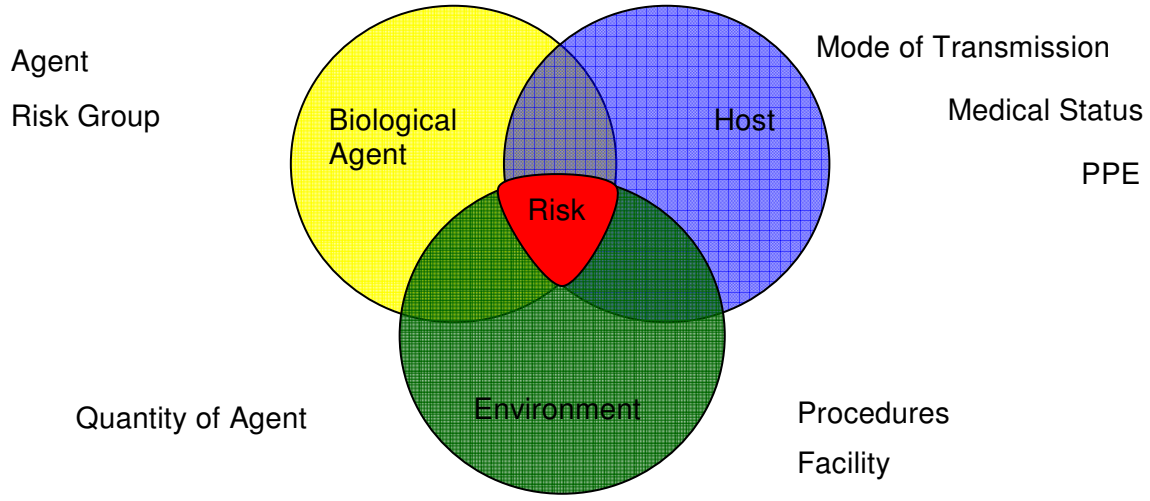
I: Risk Assessment

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

Host: When assessing the host, their immune status and the risks posed by the 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 biological agents, however if a person is ill or tired, their immune status may be compromised.

Environment: When assessing the environment, the manner in which the biological agent is being manipulated must be examined. For this part of the assessment, specifics such as quantity of the agent and procedures applied to the agent must be considered.

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



The following sections define the agents that are considered to be a biohazard and describe the risk factors that are used to have the agents organized into the 4 different risk groups.

1.1. Definition of a Biohazard

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. The risk can be direct through infection or indirect through damage to the environment. Biohazardous agents can be classified into 7 groups:

Cultured animal cells: While cultured animal 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.

Primate body fluids and other potentially infected clinical specimens: When handling other items such as human blood and body fluids, workers must be aware that they may contain other pathogens such as influenza, HIV and hepatitis B. Therefore, it is imperative that when working with these specimens, they should always be handled as if they are potentially infected. To obtain more information on practices, procedures and universal precautions to take when working with these products, please see the section on "Working with human blood and body fluids" in section 2-7 (Standard Operating Procedures).

When working with non-human primates or old world monkeys, there is the potential for exposure to a naturally occurring virus called **Herpes B virus** (*Cercopithecine herpesvirus 1, herpesvirus simiae, B virus*). Herpes B virus is the endemic simplexvirus of macaque monkeys. B virus is an alphaherpesvirus, which consists of a subset of herpes viruses that travel within hosts using the peripheral nerves. As such, this neurotropic virus is not found in the blood. In the natural host, the virus exhibits pathogenesis similar to that of herpes simplex virus (HSV) in humans. Conversely, when humans are zoonotically infected with B virus, patients can present with severe central nervous system disease, resulting in permanent neurological dysfunction or death. Severity of the disease increases for untreated patients, with a mortality rate of approximately 80%. As such, when working with non-human primates, it is imperative that workers obtain special training and use appropriate personal protective equipment.

Microorganisms: This group includes organisms such as bacteria, viruses, fungi, rickettsia and chlamydiae. Each organism poses a different type of risk to the worker. As such, the worker must be aware of the precautions that must be taken to safely work with the agent.

Parasites: Parasites are organisms that can only survive if they live within a host. They usually increase their fitness by exploiting host for food, habitat and dispersal. As a result, the host is usually harmed, but not killed by the parasite.

Animals: Animals that are used in a research setting or those found in the field can pose a number of different risks. All animals contain animal dander which after continuous exposure can result in allergies or other adverse reactions. Animals, such as sheep and birds, could harbor zoonotic diseases such as Q fever and psittacosis respectively. Consequently, when working with animals, the worker must be aware of the pathogens and other risks that the animal poses to humans.

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.

Recombinant DNA: Recombinant DNA is formed when DNA from different organisms are combined together. 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.

I: Risk Assessment

1.2. Registration of Biohazardous Materials

Principal investigators working, or proposing to work with cultured animal cells, microorganisms, primate body fluids, animals, toxins, parasites or recombinant DNA must consult with the UBC Biosafety Committee. 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 this review.

Forms for the Biosafety Approval process can be found at
http://www.ors.ubc.ca/ethics/biohazard.htm

The Biosafety Committee will determine if the research program falls within the Public Health Agency of Canada (PHAC), Laboratory Biosafety Guidelines (3rd Edition 2004) and propose the appropriate level of containment.

The Biosafety Committee in collaboration with HSE will approve research facilities, confirm that safety equipment, including biological safety cabinets, are functioning properly and advise on 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 (*#HSE-04/2/F1*). 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.

Application for research grants, regardless of funding source, must be reviewed by the Biosafety Committee to determine the nature of the research, the most appropriate level of containment and whether the laboratory facilities are adequate. **The University will not release the granting agencies' funds unless the University "Biosafety Project Approval" form (*#HSE-04/2/F2*) has been completed and approved by the Biosafety Committee.**

The location and working volumes of all biohazardous materials must be registered with Health, Safety and Environment in order to ensure that appropriate precautions are taken to protect personnel, research and the environment from possible exposure to such materials. The registry of biohazardous materials allows for rapid response by appropriate personnel in case of spills or other accidental exposures.

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: http://web.ubc.ca/okanagan/provost-research/ethics.html and 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: is http://web.ubc.ca/okanagan/provost-research/ethics.html.

The University Biosafety Committee can be reached by contacting the advisor for Biosafety in Health, Safety and Environment at 250-80(7-8656).

1.3. 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 poses risks which vary with each agent and the way it is used. PHAC has developed the Laboratory Biosafety Guidelines which can be used to assist in classifying biological agents into different Risk Groups. Pathogen risks are determined by weighing a number of factors associated with the biological agent. The factors used are described below:

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

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

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.

Concentration: When working in the laboratory, research protocols often call for large volumes or concentrations of the biological agent to be cultured and manipulated. By increasing the concentration of the biohazardous material, the likelihood of exposure increases and could pose a risk to the worker. This must be considered when performing a Risk Assessment. In certain cases, increased controls, precautions, equipment and training may be needed to safely handle the larger or more concentrated amounts of the agent.

Route of Exposure: Route of exposure is defined as the way the biological agent can infect a host. Typically, biological agents can infect a host through the following routes: airborne, ingestion, direct inoculation, mucous membrane and skin contact. This risk factor is a very important to consider as it helps determine the precautions that must be taken when the agent is being manipulated in the laboratory. See more under Laboratory Acquired Infections (Pg. 8).

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 pertusis* 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 University Medical Surveillance Programme Personnel at Health, Safety and the Environment (250-80(7-8621)) prior to performing work with biohazardous materials.

1.4. Risk Groups

The PHAC Laboratory Biosafety Guidelines uses the above risk factors to classify biohazards into four distinct risk groups. A fifth group which includes agents that are not indigenous to Canada will not be discussed in this manual.

Risk Group 1 Agents: “Any biological agent that is unlikely to cause disease in healthy workers or animals” (PHAC 2004 Guidelines). These are agents that have a **low** individual and **low** community risk. Examples in this group include recombinant DNA, non-pathogenic strains of E. coli, most mouse cell lines and recombinant DNA work.

Risk Group 2 Agents: “Any pathogen that can cause human disease but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventive measures are available, and the risk of spread is limited” (PHAC 2004 Guidelines). These agents have a **moderate** individual risk and **low** community risk. Examples include Haemophilus influenza, Salmonella, Hepatitis B virus and HIV that is non-cultured.

Risk Group 3 Agents: "Any pathogen that usually causes serious human disease or can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that cause diseases treatable by antimicrobial or antiparasitic agents" (PHAC 2004 Guidelines). These agents have a **high** individual risk, but a **low** community risk. Examples include Bacillus anthracis, all species of Brucella, Hantavirus and HIV that is being cultured.

Risk Group 4 Agents: "Any pathogen that usually produces very serious human disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice-versa, directly or indirectly, or by casual contact" (PHAC 2004 Guidelines). These agents have a **high** individual risk and a **high** community risk. All of the agents in this group are viruses. Examples include Monkeypox, Lassa virus, Herpes simian B virus and Ebola.

Manipulation of the Biological Agent: Once the risk group has been assessed and identified, the conditions under which an infectious agent is used in a laboratory is also of concern. When using agents in unusual or untried research settings, the associated risks can be affected and it is imperative that this is reflected in the risk assessment. In addition, the quantity of the agent that is being manipulated must also be examined for the risk assessment.

1.5. 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.

Thus, it is required that all spills, possible exposures and incidents involving biohazardous materials be documented and reported to the Principal Investigator, Health, Safety and Environment and the University Occupational Health Nurse.

1.5.1 How Laboratory Infections Are Acquired

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 reasonable 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. In Chapter 4 of Biological Safety Principles and Practices (3rd edition, Fleming and Hunt *eds.*), Harding and Byers summarized laboratory infection rates and reported that from 1979 to 1999 laboratory infection rates ranged from 223 cases of *M. tuberculosis* to 2 cases of *Mycoplasma pneumonia* reported in the scientific literature. However, they also estimates that laboratory infection rates are frequently under reported.

Accidental Inoculation

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 needlesticks 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 Materials Management website http://web.ubc.ca/okanagan/hse/environment/hazardousmaterials.html.

Accidental Ingestion/Exposure to Mucous Membranes

Microorganisms may be ingested as a result of:

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. It is for this reason that eating, drinking, smoking or mouth pipetting are **NOT** permissible in any laboratory, as per the Worksafe BC Regulation 4.84.

Splashing into 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.

Spillage and direct contact: It is good practice to assume that all work surfaces and equipment may be contaminated. When material containing micro-organisms 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. Organisms may then be transferred by the fingers 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 an aerosol. If these droplets contain bacteria or other forms of infectious material they are referred to as infected air-borne particles, or Bioaerosols. 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 and deeper lung penetration.

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) (see page 23). 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.

1.5.2 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. It is these dilute solutions that present the greatest hazard because the droplets formed contain very few 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 micro-organisms can therefore be transferred to the mouth and eyes, making it essential that lab personnel wear the appropriate Personal Protective Equipment (PPE) and wash their hands prior to leaving the laboratory.

1.6. 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 (see page 49 for spill clean-up procedure).

1.7. 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 which oversees 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. There are no specific procedures required by a foreign granting agency to ensure compliance with that country's legislation.

In some provinces there is legislation which controls certain aspects of animal use, particularly the procurement of dogs and cats (e.g. The Animals for Research Act in Ontario). In the absence of legislation, it behooves every person who uses animals within a Canadian institution to treat them with the respect they deserve and to ensure that they do not suffer needlessly. The tenets of Russell and Burch should always be borne in mind (i.e. to refine, reduce and replace the use of animals in teaching, testing and research)[1].

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

[1] From Zurlo, Rudacille and Goldberg. (1996) The Three Rs: The Way Forward. *Environmental Health Perspectives*. Volume 104, No. 8. Accessed June 3, 2010. http://ehp.niehs.nih.gov/members/1996/104-8/zurlo.html

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.

Animals may also be deliberately inoculated with viruses or organisms in each of the four risk groups or with viable materials (i.e., transformed cells) suspected of containing these infectious agents. Under these circumstances, the animals should 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.

Another concern when work involves the use of laboratory animals is the development of an allergic reaction 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
- Use of filter-top cages
- Use of protective clothing, especially gloves and HEPA filtered respirators
- A well-designed ventilation system

When working in animal facilities it is recommended that all personnel wear/use fit tested N-95 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

II. Risk Control

Methods used to control the risk include: facility design and construction, engineering controls, personal protective equipment, training and standard operating procedures. 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.

II: Risk Control

2.1 Training

When new personnel join a lab, they must undergo safety orientations and training. This is not only good laboratory practice, but it also a part of WorkSafe BC's New and Young Worker Regulation. 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 work duties. In addition, all safety orientations or training must be documented and kept on file. Training documents are also necessary to meet requirements of the Canadian Food Inspection Agency (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

If 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.

For more information about the New and Young Worker regulation, please go to the following website:
http://www.worksafebc.com/regulation_and_policy/public_hearings/assets/pdf/2006_fall_public_hearings/Part%203_approved.pdf

2.2 Inventories and Transfers

All risk group 1, 2, 3 agents must be inventoried. The documentation must include:

- Name of the organism
- Biohazard level
- Where they are located in the lab

Transfer of risk group 1, 2 and 3 agents between labs must be documented. The documentation must include:

- Date of transfer
- Lab donating the agent
- Lab accepting the agent
- Amount of agent transferred

2.3 Biosecurity 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, is access to pathogens restricted somehow (ie. kept under lock and key) and does the facility have specific security protocols in place to minimize the entry of unauthorized personnel (ie. Key card access, identity badges, protocols for locking doors).

Inventory of Pathogens: When working with pathogens, labs must have an inventory of all vials, plates and tubes that are being stored. Working solutions of the agents that are currently being used for on-going experiments do not need to be inventoried. 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.

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 (ie. Supervisor, security, law enforcement agencies, HSE etc.).

2.4 Containment Facilities

There are four levels of containment as described by the Public Health Agency of Canada (PHAC) Laboratory Biosafety Guidelines (3rd Edition, 2004) http://www.phac-aspc.gc.ca/ols-bsl/lbg-ldmbl/. These levels often correspond to the Risk Group levels (i.e. if working with Risk Group 2 level organisms you must be working in a minimum of Containment Level II). However, the "Biosafety Level" of containment is dependent on both the Risk Group of the materials being used and the manipulations and procedures that are being performed. The HSE Advisor (Biosafety) must be consulted prior to starting any new research or protocols involving different microorganisms than those which have been registered with the Office of Biosafety. In the Okanagan, the advisor for Biosafety can be reached at 250-80(7-8656).

The following section, which outlines the general requirements for all laboratories handling infectious substances and the requirements for Containment Levels 2, 3 and 4, are from the PHAC Laboratory Biosafety Guidelines (3rd Edition, 2004). Specific requirements for laboratories located at the University have also been outlined in this section.

2.4.1 General Requirements

The following general practices are required for all laboratories handling infectious substances.

1. A documented procedural (safety) manual must be available for all staff, and its requirements followed; it must be reviewed and updated regularly.
2. Personnel must receive training on the potential hazards associated with the work involved and the necessary precautions to prevent exposure to infectious agents and release of contained material; personnel must show evidence that they understood the training provided; training must be documented and signed by both the employee and

supervisor; retraining programs should also be implemented.

3. Eating, drinking, smoking, storing of either food, personal belongings, or utensils, applying cosmetics, and inserting or removing contact lenses are not permitted in any laboratory; the wearing of contact lenses is permitted only when other forms of corrective eyewear are not suitable; wearing jewelry is not recommended in the laboratory.
4. Oral pipetting of any substance is prohibited in any laboratory.
5. Long hair is to be tied back or restrained so that it cannot come into contact with hands, specimens, containers or equipment.
6. Access to laboratory and support areas is limited to authorized personnel.
7. Doors to laboratories must not be left open (this does not apply to an open area within a laboratory).
8. Open wounds, cuts, scratches and grazes should be covered with waterproof dressings.
9. Laboratories are to be kept clean and tidy. Storage of materials that are not pertinent to the work and cannot be easily decontaminated (e.g., journals, books, correspondence) should be minimized; paperwork and report writing should be kept separate from such biohazardous materials work areas.
10. Protective laboratory clothing, properly fastened, must be worn by all personnel, including visitors, trainees and others entering or working in the laboratory; suitable footwear with closed toes and heels must be worn in all laboratory areas.
11. Where there is a known or potential risk of exposure to splashes or flying objects, whether during routine operations or under unusual circumstances (e.g., accidents), eye and face protection must be used. Careful consideration should be given to the identification of procedures requiring eye and face protection, and selection should be appropriate to the hazard.
12. Gloves (e.g., latex, vinyl, co-polymer) must be worn for all procedures that might involve direct skin contact with biohazardous material or infected animals; gloves are to be removed when leaving the laboratory and decontaminated with other laboratory wastes before disposal; metal mesh gloves can be worn underneath the glove. Gloves should be chosen by relating specific glove characteristics to the hazards involved.
13. Protective laboratory clothing must not be worn in non laboratory areas; laboratory clothing must not be stored in contact with street clothing.
14. If a known or suspected exposure occurs, contaminated clothing must be decontaminated before laundering (unless laundering facilities are within the containment laboratory and have been proven to be effective in decontamination).
15. The use of needles, syringes and other sharp objects should be strictly limited; needles and syringes should be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles; caution should be used when handling needles and syringes to avoid auto-inoculation and the generation of aerosols during use and disposal; where appropriate, procedures should be performed in a BSC; needles should not be bent, sheared, recapped or removed from the syringe; they should be promptly placed in a puncture-resistant sharps container (in accordance with Canadian Standards Association [CSA] standard Z316.6-95(R2000)) before disposal.
16. Hands must be washed after gloves have been removed, before leaving the laboratory

and at any time after handling materials known or suspected to be contaminated.

17. Work surfaces must be cleaned and decontaminated with a suitable disinfectant at the end of the day and after any spill of potentially biohazardous material; work surfaces that have become permeable (i.e., cracked, chipped, loose) to biohazardous material must be replaced or repaired.
18. Contaminated materials and equipment leaving the laboratory for servicing or disposal must be appropriately decontaminated and labelled or tagged-out as such.
19. Efficacy monitoring of autoclaves used for decontamination with biological indicators must be done regularly (i.e., consider weekly, depending on the frequency of use of the autoclave), and the records of these results and cycle logs (i.e., time, temperature and pressure) must also be kept on file. For more information, please refer to the Autoclave section (3-2) of this manual.
20. All contaminated materials, solid or liquid, must be decontaminated before disposal or reuse; the material must be contained in such a way as to prevent the release of the contaminated contents during removal; centralized autoclaving facilities are to follow the applicable containment level 2 requirements.
21. Disinfectants effective against the agents in use must be available at all times within the areas where the biohazardous material is handled or stored.
22. Leak-proof containers are to be used for the transport of infectious materials within facilities (e.g., between laboratories in the same facility).
23. Spills, accidents or exposures to infectious materials and losses of containment must be reported immediately to the laboratory supervisor; written records of such incidents must be maintained, and the results of incident investigations should be used for continuing education.
24. An effective rodent and insect control program must be maintained.
25. An emergency door sign (fig. 1) must be affixed on the outside of the laboratory door. This sign should clearly indicate the chemical hazards, radiation hazards and biohazards contained within the space. Emergency contact information for two senior members of the lab should also be listed on the door. The phone numbers listed must allow for the personnel to be contacted during non-working hours.

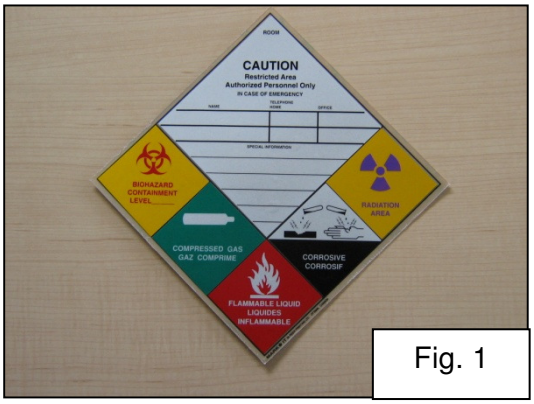


Fig. 1

26. The laboratory must be equipped with a safety shower and eyewash stations. The safety showers and eyewash stations must be in good working order and they must be tested on a monthly basis. The shower and eyewash station tests must be documented and kept on file in the laboratory.

2.4.2 Containment Level 2

In addition to the general practices required for all laboratories handling infectious substances, the following describe the minimum operational practices required for containment level 2.

1. Good microbiological laboratory practices intended to avoid the release of infectious agents are to be employed.
2. Biosafety Cabinets (BSC) must be used for procedures that may produce infectious aerosols and that involve high concentrations or large volumes of biohazardous material. Laboratory supervisors[2] should perform a risk assessment to determine which procedures and what concentrations and volumes necessitate the use of a BSC. These procedures will be approved by the Biosafety Committee prior to offering a Biosafety Approval.
3. Appropriate signage indicating the nature of the hazard being used (e.g., biohazard sign, containment level) must be posted outside each laboratory; if infectious agents used in the laboratory require special provisions for entry, the relevant information must be included on the sign; the contact information of the laboratory supervisor or other responsible person(s) must also be listed.
4. Entry must be restricted to laboratory staff, animal handlers, maintenance staff and others on official business.
5. All people working in the containment area must be trained in and follow the operational protocols for the project in process. Trainees must be accompanied by a trained staff member. Visitors, maintenance staff, janitorial staff and others, as deemed appropriate, must also be provided with training and/or supervision commensurate with their anticipated activities in the containment area.
6. Emergency procedures for spill clean-up, BSC failure, fire, animal escape and other emergencies must be written, easily accessible and followed. A record must be made of other people entering the facility during an emergency.

2.4.3 Containment Level 3

In addition to the operational practices for all laboratories handling infectious substances and those minimum requirements for containment level 2, the following describe the minimum operational practices required at containment level 3:

1. There must be a program for the management of biological safety issues in place with appropriate authority to oversee safety and containment practices.
2. Everyone entering the containment laboratory must have completed a training course in procedures specific to the containment laboratory and must show evidence of having understood the training; training must be documented and signed by the employee and supervisor.
3. Employees working in the containment area must have knowledge of the physical operation and design of the facility (e.g., air pressure gradients between zones, directional airflow patterns, alarm signals for air pressure failure, containment perimeter).

[2] Consultation with the Biological Safety Officer/Institutional Biosafety Committee is available if desired.

4. A protocol specific to the operation of the laboratory must be developed and read by personnel; employees must certify in writing that they have understood the material in the protocol. This should include entry and exit protocols for people, animals, equipment, samples and waste. General protocols must be supplemented with protocols specific to each project in progress.
5. Personnel must have demonstrated proficiency in microbiological practices and techniques.
6. Smoke testing (i.e., using a smoke pencil held at the door between the anteroom and the containment facility, and other doors as required) should be done periodically by laboratory staff to verify correct airflow; a containment check must be performed before entering the containment laboratory (e.g., verify correct reading on the pressure monitoring device).
7. People entering a containment facility must be well prepared and bring all materials they will need with them; if something has been forgotten, established traffic patterns must still be adhered to (i.e., do not go back to get it; either phone for someone to bring it or exit using proper protocols).
8. Routine laboratory cleaning must be done by personnel using the containment facility or by specific personnel dedicated and trained for this task.
9. The containment laboratory must be kept locked.
10. Infectious agents should be stored inside the containment laboratory; agents stored outside of the zone must be kept locked, in leak proof containers; emergency response procedures are to take into account the existence of such infectious agents outside of the containment level 3 laboratory.
11. Personal items such as purses and outdoor clothing must not be brought into the containment laboratory.
12. Drainage traps must be filled with liquid (i.e., through regular sink usage, automatic primers or by filling traps in areas that are not frequently used).
13. Laboratory samples and supplies may be carried into the containment laboratory or passed in through a pass-box; if the barrier autoclave is used to pass materials into the laboratory, the autoclave must have been cycled before the outer "clean side" door is opened.
14. Personnel entering the containment laboratory must remove street clothing and jewellery, and change into dedicated laboratory clothing and shoes; dedicated laboratory clothing and shoes must be removed before leaving the containment laboratory in a manner that minimizes any contamination of the skin with the potentially contaminated dedicated laboratory clothing. The use of full coverage protective clothing (i.e., completely covering all street clothing) is an acceptable alternative. When a known or suspected exposure may have occurred, all clothing, including street clothing, requires appropriate decontamination. Laboratories manipulating organisms, such as HIV, that are not infectious via inhalation, are not required to remove street clothing.
15. An additional layer of protective clothing (i.e., solid-front gowns with tight-fitting wrists, gloves, respiratory protection) may be worn over laboratory clothing when infectious materials are directly handled and should be removed after completion of work (e.g., dedicated for use at the BSC).

16. Centrifugation of infectious materials must be carried out in closed containers placed in sealed safety cups or rotors that are unloaded in a BSC.
17. Animals or arthropods that have been experimentally infected must remain in the laboratory or appropriate animal containment facility.
18. When a known or suspected aerosol exposure may have occurred, protocols based on a local risk assessment must be in place to determine whether showering is required on exit from the laboratory.
19. All activities with infectious materials are conducted in a BSC; if this is not possible, other primary containment devices in combination with personal protective clothing and equipment must be used; no work with open vessels containing infectious materials is conducted on the open bench.
20. Heat-sensitive materials that cannot be autoclaved out of the containment laboratory must be decontaminated at the containment barrier (e.g., fumigated with formaldehyde, vaporized hydrogen peroxide or a suitable alternative; disinfected using liquid chemicals; or subjected to other technology proven to be effective).
21. Emergency procedures for failure of air handling systems and other containment emergencies must be written, easily accessible and followed.
22. In the event of life-threatening emergencies, personal health and safety are a priority; exit protocols must be established whereby routine procedures might be bypassed; a reporting area must be identified where further steps must be taken (e.g., disinfecting footwear, changing, showering).

2.4.4. Containment Level 4

In addition to the operational practices for all laboratories handling infectious substances and those minimum requirements for containment level 2 and 3, the following describe the minimum operational practices required at containment level 4:

1. Protocols must be established for emergencies, including damage to positive pressure suits, loss of breathing air, and loss of chemical shower.
2. Employees must immediately notify their supervisor of any unexplained febrile illness; supervisors must contact any employee with unexplained work absences.
3. The employer must establish liaison with the local hospital/health care facility to ensure that in the event of an employee's accidental exposure to containment level 4 agents the hospital/health care facility is fully aware of the infectious agents involved and that the appropriate procedures are in place for the treatment of the employee (patient).
4. A record of containment laboratory usage is to be maintained (i.e., log book of all entry and exits) with date and time.
5. Cultures and stocks of infectious agents must be stored in a secure area inside the containment laboratory and an inventory of pathogens maintained.
6. A daily check of containment systems (e.g., directional airflow, disinfectant level in chemical shower, critical containment points for a class III BSC line) and life support systems (e.g., back-up breathing air) must be carried out before entering the laboratory.

7. Personnel entering the laboratory must remove street clothing (including undergarments) and jewelry, and change into dedicated laboratory clothing and shoes.
8. Positive pressure suits must be worn (for level 4 suit mode); the integrity of the suit must be routinely checked for leaks.
9. A chemical shower of appropriate duration is required for personnel in suits who are leaving the containment laboratory; the disinfectant used must be effective against the agents of concern, be diluted as specified and prepared fresh as required; this is not applicable for class III BSC line level 4 containment facilities.
10. A body shower is required on exit from the containment laboratory.
11. Material can be removed from the containment laboratory only after appropriate decontamination or after specific approval from the Biological Safety Officer or other appropriate authority.
12. A competent person must be available outside the containment level 4 laboratory when work is being conducted within the laboratory, to assist in case of emergency.
13. Small laboratory animals, primates or insects infected with level 4 agents are to be housed in a partial containment system (e.g., cages placed in HEPA filtered containment enclosures).
14. All laboratory procedures are to be conducted within a BSC in conjunction with a positive pressure suit or within a class III BSC line.
15. Large animals require specialized care and handling not dealt with by these *Guidelines*. For details, please refer to the current edition of *Containment Standards for Veterinary Facilities*, by the Canadian Food Inspection Agency. This office can be contacted by calling the Biohazard Containment and Safety Division directly at (613) 221-7088 or accessing their Web site: http://www.inspection.gc.ca/english/sci/lab/convet/convete.shtml

2.5 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.

2.5.1 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 (Fig. 2). It is important that whenever possible the tubes, buckets or rotors should be loaded and unloaded in another engineering control, a biosafety cabinet (BSC). BSCs will be discussed in section 2.5.9.

Because 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 0-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

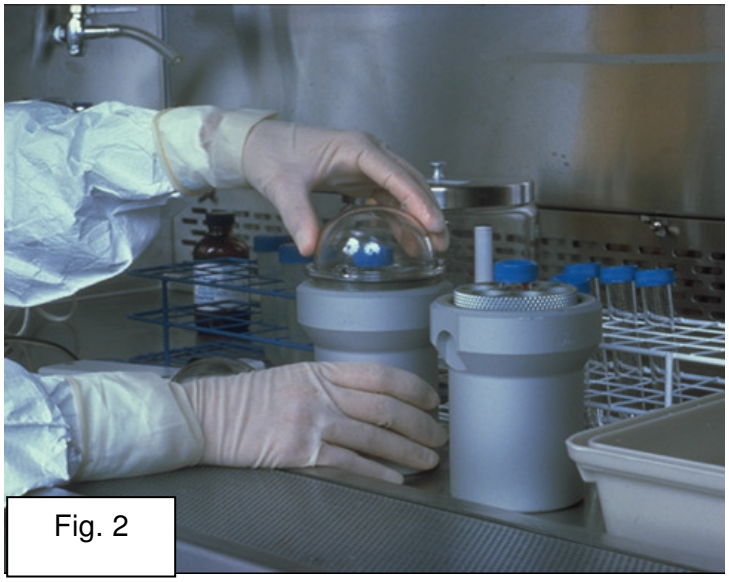


Fig. 2

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.

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 be evacuated after centrifuging infectious materials before the centrifuge is opened. This can be accomplished by means of a vacuum pump and Tygon tubing hose inserted into one of the available capped ports located on the side of some models or by drilling an access hole into the chamber in the side of models not so equipped. A disinfectant trap and/or in-line HEPA filter should be used to protect the pump from contamination. 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.

2.5.2 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, marketed by Waring Products Division, 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. Prior to opening the blender, let it sit for 1 minute to allow the aerosols to settle and then open in a BSC. The area where the blender was run should be decontaminated with an appropriate decontaminant.

2.5.3 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.

2.5.4 Lyophilizer Safety

The process of using a laboratory scale lyophilizer presents a number of unique hazards. These hazards include but are not limited to the following:

- extreme pressure changes,
- a potential for glassware to explode or implode, and
- the possibility of aerosol 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 vapour 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**.

2.5.5 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 infected with an etiologic agent 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. For further information on the maintenance and decontamination of a cryostat, please see sections 1.4 and 1.5 of A Practical Guide to Frozen Section Technique[3].

2.5.6 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.

2.5.7 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 (Fig. 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 (Fig. 4):

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

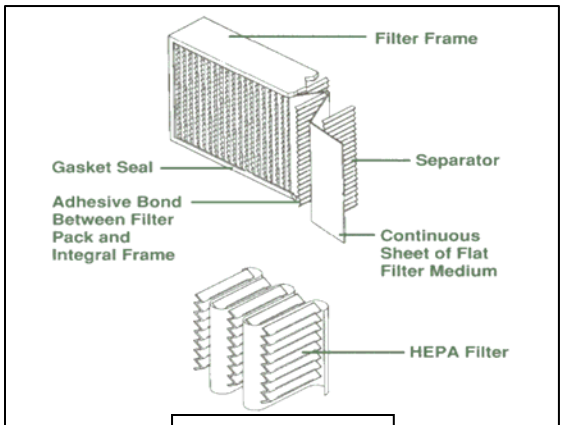


Fig. 3

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

[3] From Peters, S R. (2009) A Practical Guide to Frozen Section Technique. Springer. P. 193

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.

Leak test – BSC certification procedure

HEPA filters are generally tested by the use of Polyalpha Olefin (PAO). When PAO is impinged on by an air-operated nebulizer, such as a Laskin nozzle, an aerosol is generated with an average particle size of 0.3 um. The downstream side of the HEPA filter is then scanned with a photometer to determine if penetration of these particles occurs. If a filter allows 1 or less droplets to penetrate with an initial concentration of 10,000 particles, then the filter is rated at 99.99% efficient.

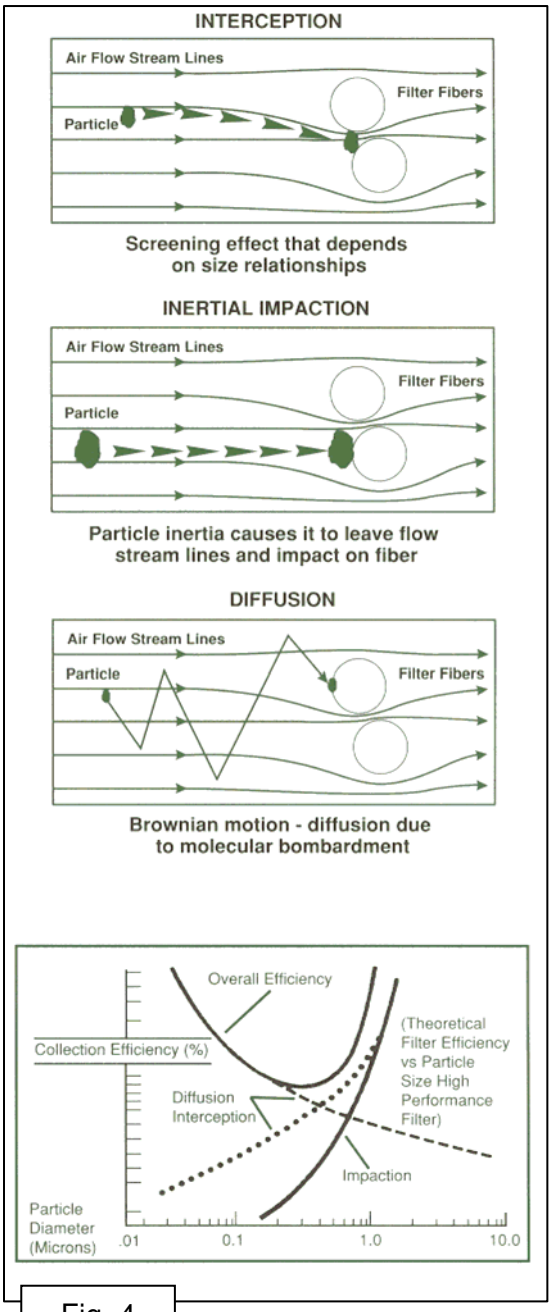


Fig. 4

2.5.8 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 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 (Fig. 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.

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 just as horizontal-flow clean-air benches do, 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.

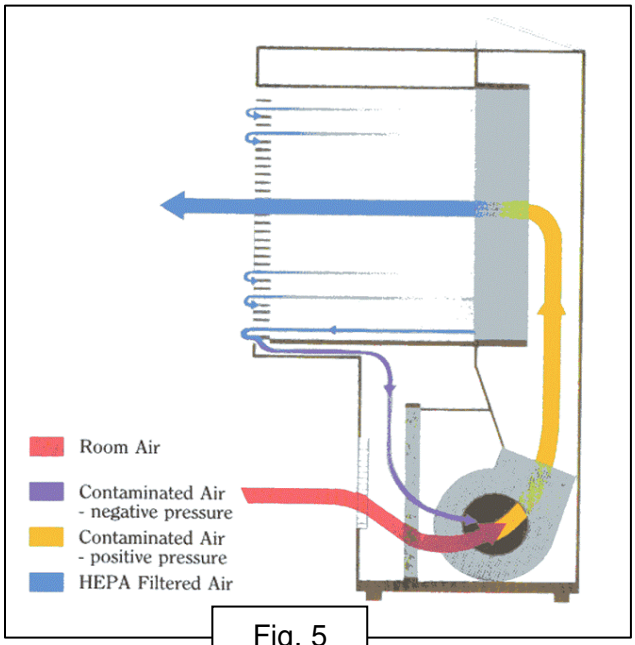


Fig. 5

2.5.9 Biological Safety Cabinets

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

- **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 access through fixed, heavy-duty, arm-length rubber gloves.

The three classes of cabinets are not directly related to the biosafety level (BSL) required for agent use. Class I and class II cabinets can be used for work at BSL's 1 to 3. Class III cabinets are usually reserved for work at BSL 4, although a Class II cabinet may be used for that purpose if the worker is provided protection such as the use of a ventilated suit

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. As such, we do not recommend the installation of a UV light in the BSCs.

Class I Cabinets

A Class I cabinet is defined as a ventilated cabinet which provides personnel and environmental protection (Fig. 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 from aerosols created in the work area by an air curtain with an inflow velocity of 75 - 100 feet per minute (fpm).

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.

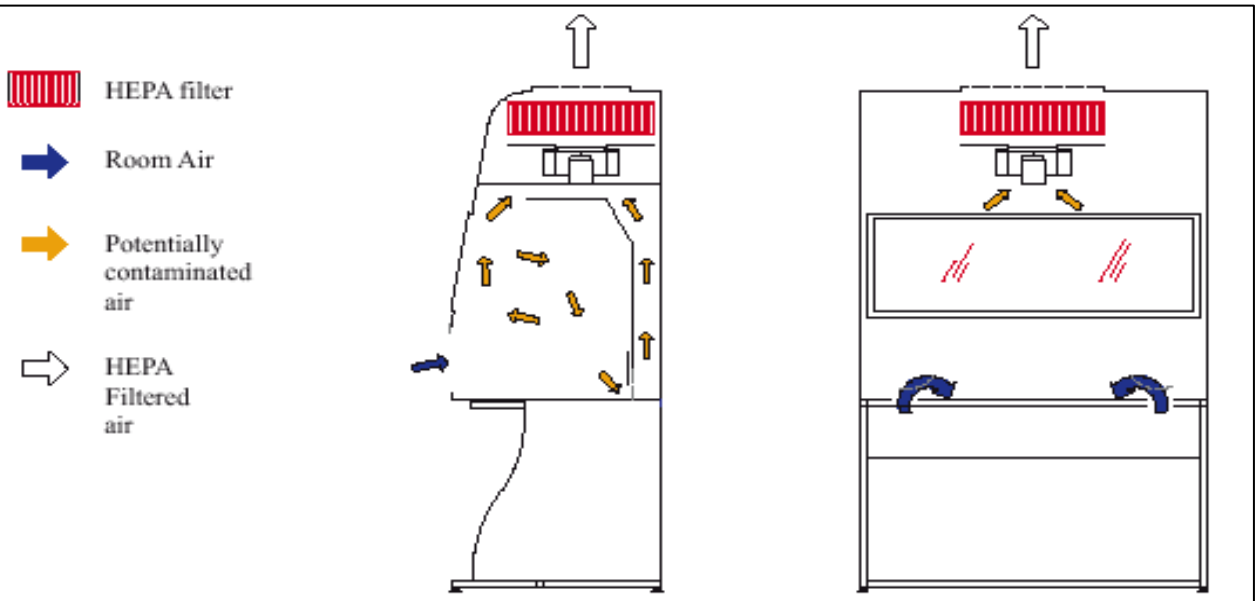


Fig 6

The Class I cabinets 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

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 as 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.

Types of Class II Cabinets

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.

The table below describes the basic differences between the Class IIA and Class IIB cabinets:

Class IIA	Class IIB
maintains a minimum of 75 lfpm (0.4 m/s) inflow velocity thorough the work opening	maintains an inlet flow velocity of 100 lfpm (0.5 m/s)
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 canopy connection	must have a dedicated, sealed exhaust system with an external blower and alarm system

The class IIB cabinets are further broken down into 3 subtypes: Class IIB1, Class IIB2 and Class IIB3. The functional differences between the four types of Class II cabinets (Class IIA, Class IIB1, Class IIB2 and Class IIB3) are in the amount of air that is re-circulated within the cabinet and whether or not the plenum is under positive or negative pressure.

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 (Fig. 7). It is the simplest of the four subtypes of Class II cabinets and re-circulates 70% of the air back through the supply HEPA filter (30% of the contaminated air is passed through the exhaust HEPA filter).

The air that 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 re-circulated 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 re-circulating 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.

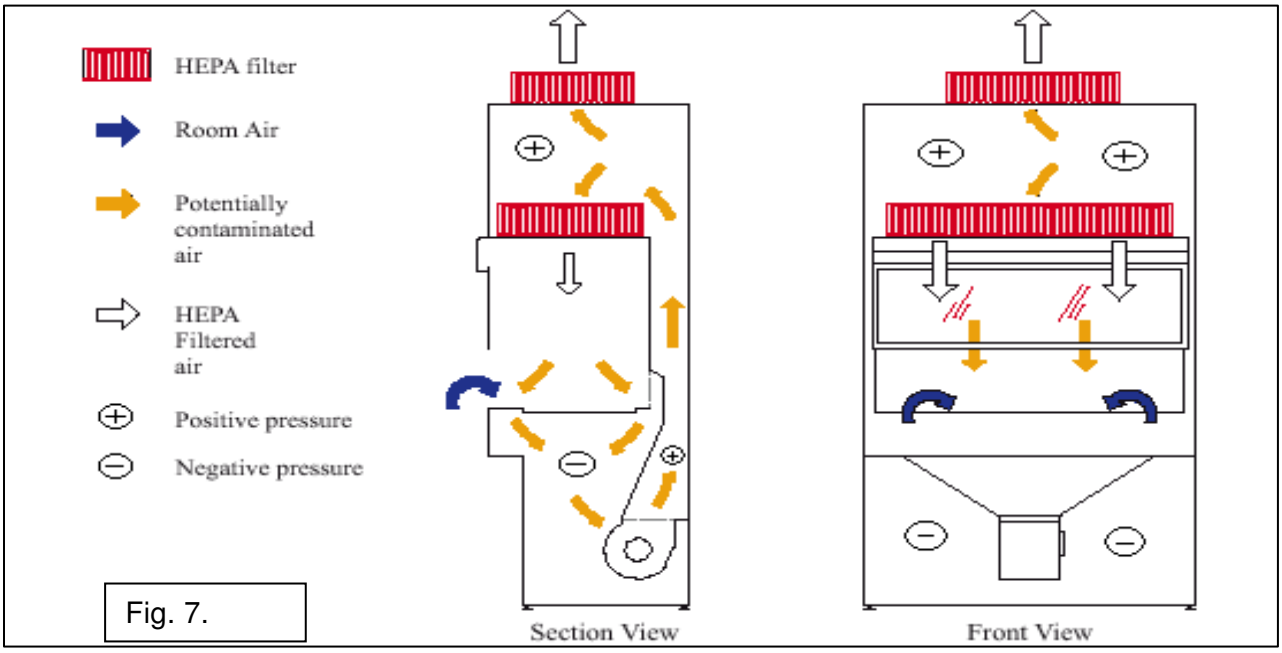


Fig. 7.

Class II Type A2 Cabinets

Type A2 cabinets (Fig 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 0.5 m/s. Unlike

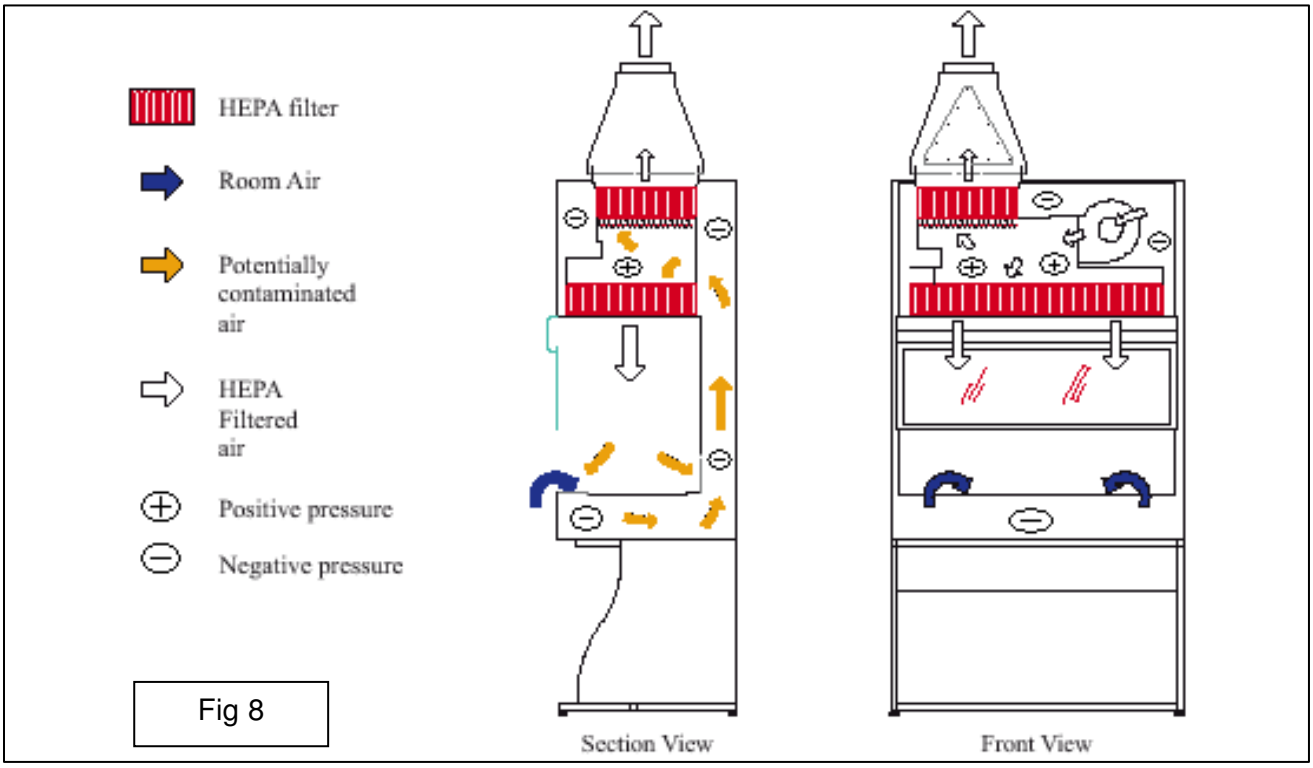


Fig 8

the Type A1, this cabinet's ducts and plenums are under negative pressure and it exhausts 100% of the contaminated air through the exhaust filter.

Class II Type B Cabinets

Class IIB Biosafety cabinets maintain a minimum average inflow velocity of 100 lfpm (0.5 m/s) through the work area access opening and must be hard-ducted to a dedicated external exhaust that discharges outside the building at a height and location that permit no recirculation.

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.

The former Class II Type B3 cabinet definition by NSF is essentially that of a Type IIA cabinet. In use, it must be considered equivalent to the front portion of the IIB1, where the down flow air is coming to the front perforated grill. It is described later in this section but is now (as of 2002) designated as a Class II Type A2 cabinet described previously in this section.

Class II Type B1 Cabinets (Overview)

Type B1 cabinets (Fig 9) maintain a minimum average inflow velocity of 100 fpm 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 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 re-circulated 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 work product. Further, this class of cabinet will not usually meet the air inflow standards for work with carcinogens in chemical fume hoods.

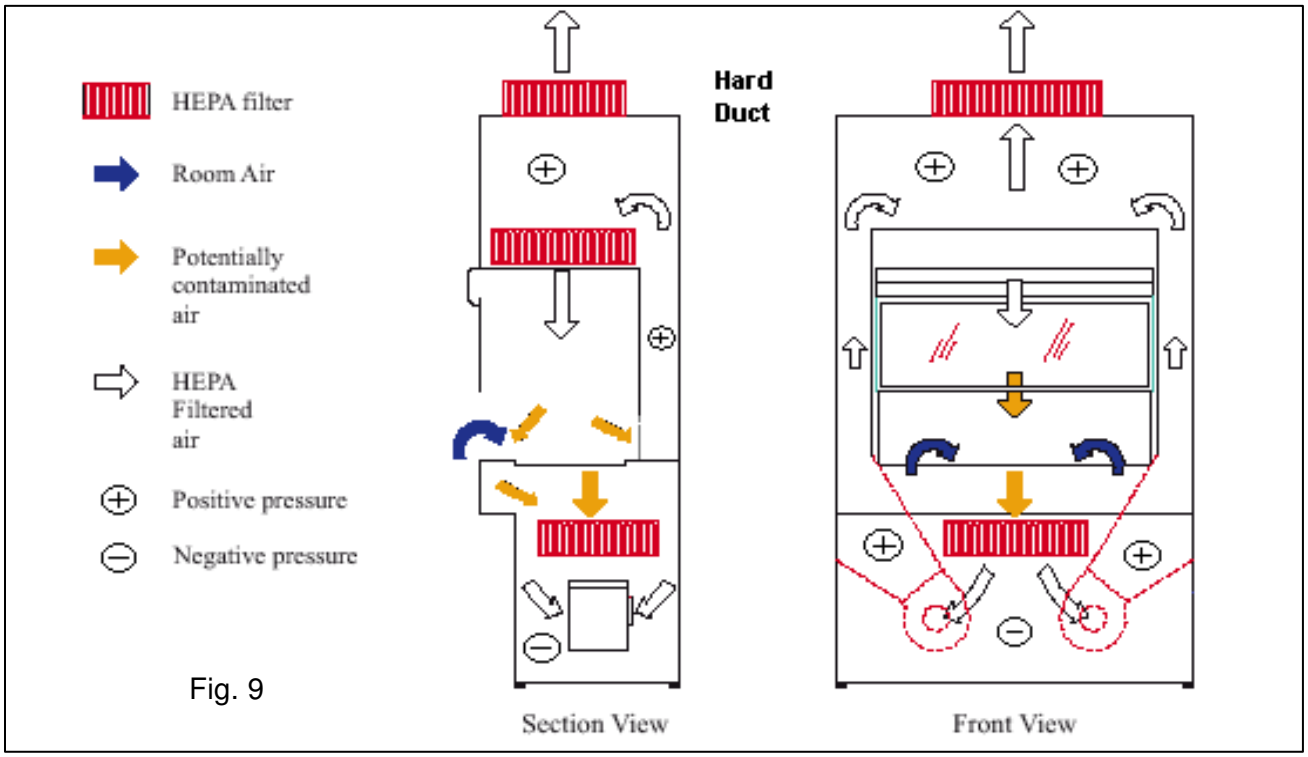


Fig. 9

Class II Type B2 Cabinets (Overview)

These cabinets are referred to as total exhaust cabinets (Fig. 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 work access 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.

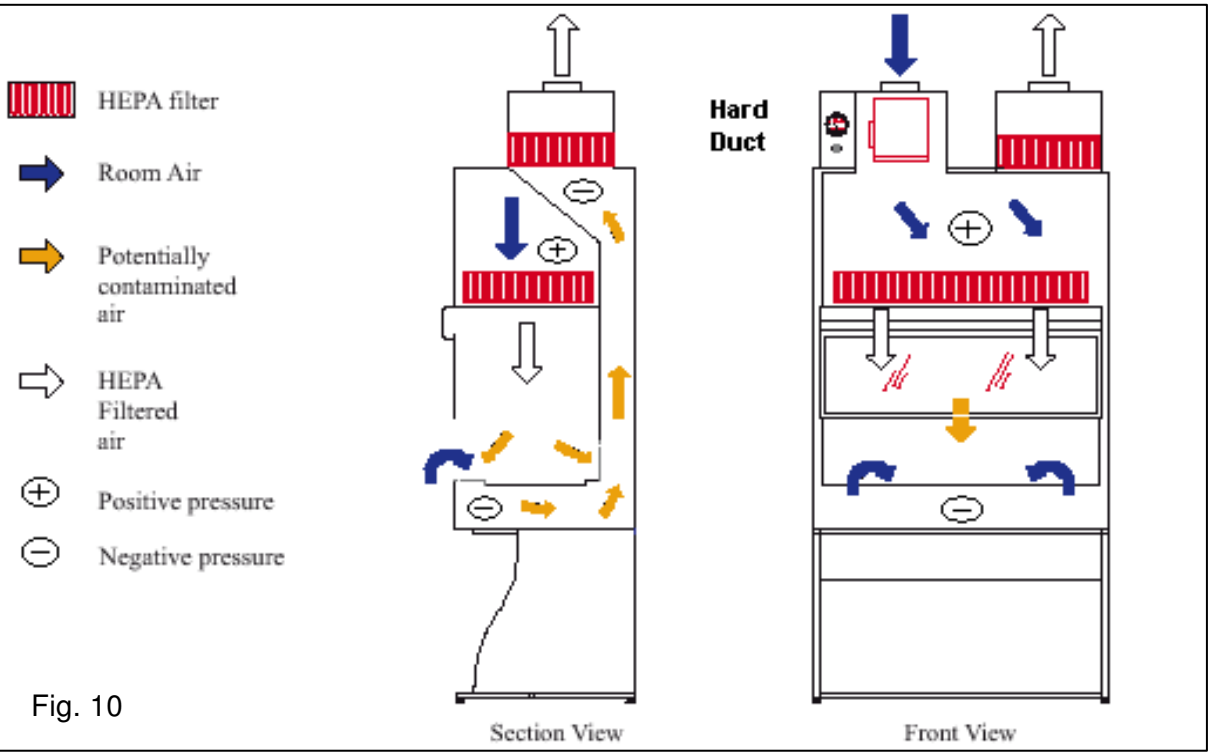


Fig. 10

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.

Type B2 cabinets may be used with biological agents treated with toxic chemicals and radionuclides and are generally associated with pharmacies preparing compounds involving the use of antineoplastic or cancer fighting drugs.

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
- need to use cabinets that meet accepted standards for housing the work to be performed.

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 BSL 4 requirements). The cabinets are gas tight, and all operations within the cabinet are conducted through arm-length rubber gloves (Fig 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 syringe or aerosol challenge; others may accommodate centrifuges, fixed microscopes, incubators, refrigerators, and other equipment. Most of 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.

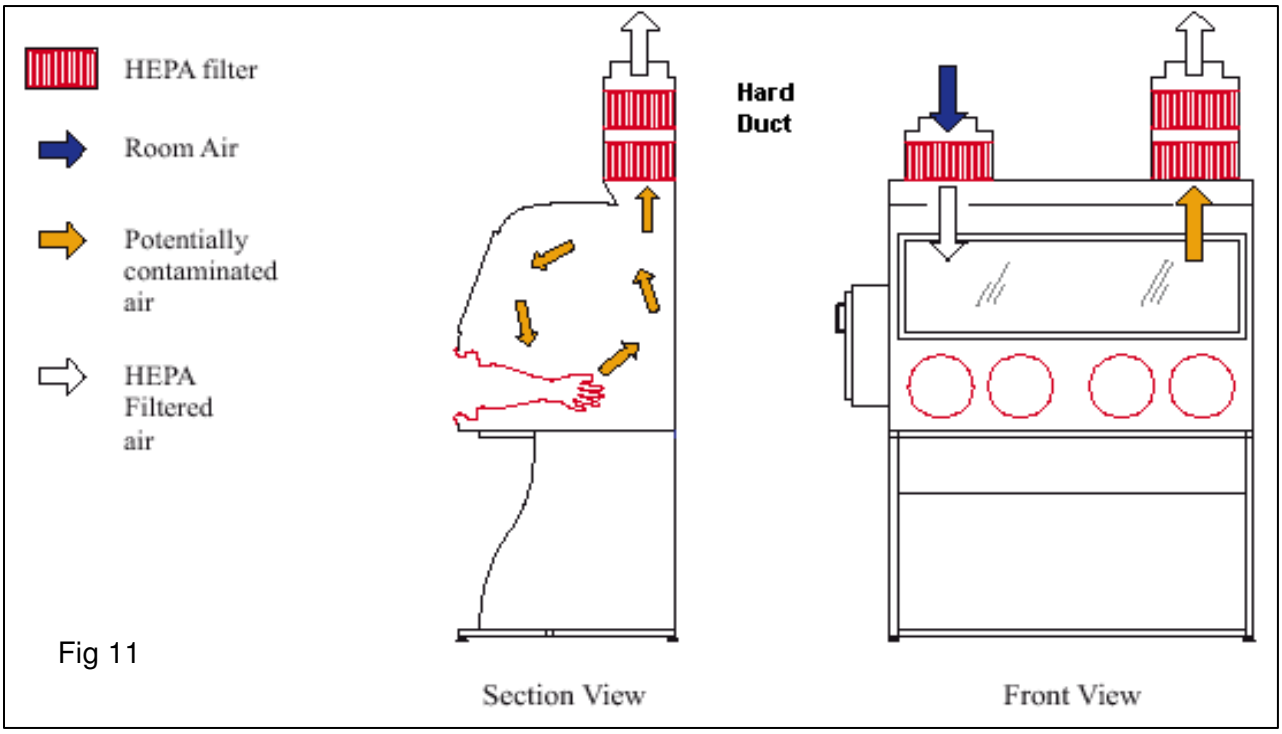


Fig 11

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.

SUMMARY TABLE COMPARING BSCS AND LFHS

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 fumehood 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

From CFIA - http://www.inspection.gc.ca/english/sci/lab/convet/convet4-5e.shtml#5
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

Biosafety Cabinet Installation Recommendations

Installation of Class I and II Cabinets

Class I and II open-front cabinets are more frequently used in BSL 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 an 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.

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 and Recertification

Biosafety cabinets should be certified in place initially 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.

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 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.

2.6 Medical Surveillance Program

The Okanagan Campus has a growing medical surveillance program. 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 a 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.

II: Risk Control

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 Medical Surveillance Program provides controls and assurances to the workers, the community and the University that our research is safe, understood and world-class.

Program Description

Focus on People, Strategy #1 identified the implementation of a comprehensive Medical Surveillance Program for faculty and staff as an institutional priority. The fundamental purpose of medical surveillance is to detect and eliminate the underlying causes of occupationally acquired infections and thus has a prevention focus.

Starting in 2008/2009 UBC Okanagan, began offering immunizations and respirator fit testing as an early prevention and intervention strategy. In January 2011, HSE will have a occupational health nurse on campus to support the Medical Surveillance Program.

For the most current information on these programs in the Okanagan, please visit the HSE websites at http://web.ubc.ca/okanagan/hse/health/medsurv.html (Medical Surveillance) or http://web.ubc.ca/okanagan/hse/health/medsurv/immunizations.html (immunizations). Alternately, please contact us at 80(7-8621).

2.7 Standard Operating Procedures (SOPs)

SOPs, or a protocols manual, must be available for all lab personnel. The SOPs manual should include written procedures for:

- Experimental protocols
- Proper use of lab equipment
- Decontamination of lab equipment, biohazardous waste and lab surfaces
- Emergency procedures for biohazard spills, accidental inoculation and containment failure

The SOPs manual can be a good tool to assist with training all laboratory personnel. As changes are made to the protocols, the changes must be documented and re-training must take place periodically. Version numbers and dates on SOPs help to ensure that the most recent copy is being used.

As SOPs are designed to be the standard of operation in a lab, there can only be a single copy of an SOP in use at a time. Therefore, SOPs must not be copied or removed from a lab. By only having only one copy of the SOPs and re-training personnel, when new versions are created, the creator can be certain that most recent SOP is being used.

2.7.1 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 Personal Protective Equipment (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.
- Wearing jewellery is not recommended in the lab.
- 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.

2.7.2 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 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 (ie 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 towelling.
- 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.
    - T he 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 Towelling: Plastic-backed absorbent towelling can be placed on the work surface (but not on the front or rear openings). This towelling 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 should 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.

Biohazardous Spills: Small contained spills on the work surface can be handled as outlined in the Biohazardous spill section (page 49). Spills large enough to result in liquids flowing through the front or rear grilles require more extensive decontamination.

- All items within the cabinet should be surface decontaminated and removed.
- After ensuring that the drain valve is closed, decontaminating solution can be poured onto the work surface and through the grille(s) into the drain pan.
- Twenty to thirty minutes is generally considered an appropriate contact time for decontamination, but this varies with the disinfectant and the microbiological agent.

Manufacturer's directions should be followed.

- The spilled fluid and disinfectant solution on the work surface should be absorbed with paper towels and discarded into a biohazard bag.
- The drain pan should be emptied into a collection vessel containing disinfectant. A flexible tube should be attached to the drain valve and be of sufficient length to allow the open end to be submerged in the disinfectant within the collection vessel. This procedure serves to minimize aerosol generation.
- The drain pan should be flushed with water and the drain tube removed.
- Gloves should be disposed of and hands must be washed.

Note: Should the spilled liquid contain radioactive material, a similar procedure can be followed. Radiation safety personnel should be contacted for specific instructions.

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.

2.7.3 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.

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.

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.

2.7.4 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.

- Immediately notify other individuals in the area that there has been a biohazard spill.
- 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 80(7-8656). If the spill is greater than 1 litre, phone the Hazardous Materials Response Unit of the Kelowna Fire Department at 911.
- 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.
- Put on the appropriate personal protective equipment.
- Identify the area requiring clean-up and decontamination, allowing sufficient area for any splattering or drying which may have occurred.
- Set up a disposal bag to allow easy discarding of contaminated clean-up materials.
- Move slowly and carefully while gently pouring the appropriate decontaminant *around* and **not** on the spill. This will avoid the creation of new aerosols.
- Use absorbent materials (i.e. paper or cloth towels) to work the decontaminant into the area of the spill.
- 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).
- Place the used absorbent material into the disposal bag and repeat the decontamination procedure.
- 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.

- Wash hands thoroughly with mild soap and water.
- Complete an "Incident/Accident" Report form from http://web.ubc.ca/okanagan/hse/safety/firstaid/accidents.html

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 labelled (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.

2.8 Personal Protective Equipment

WorkSafe BC 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 defence against unwanted exposures. The primary line of defence 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 or eating areas.

WorkSafe BC 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 WorkSafe BC 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.

2.8.1 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 Biosafety Level (BSL) 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.

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.

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 PHAC Laboratory Biosafety Guidelines state the following requirements:

- **BSL 2 Laboratories**: use a laboratory coat, gown, smock or uniform while working.
- **BSL 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 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 BSL 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; they can be treated and 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 BSL 3 areas. This should also be considered for BSL 2 areas, especially for work with infected animals in animal rooms. Shoe covers can be used in BSL 2 or BSL 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, wearing 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, making 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.

Gloves are made of such a variety of materials, including rubber, neoprene, neoprene-latex, Viton, polyurethane, fluoroelastomer, nitrile, polyethylene, PVC, polyvinyl alcohol, and for such special uses that the worker should seek advice regarding the best glove for the task to be performed. For example, chemical solvents such as xylene, toluene, benzene, perchloroethylene, dichloroethane, and carbon tetrachloride normally degrade rubber, neoprene, and PVC; it is

important to avoid these materials in favour of polyvinyl alcohol or Buna-N.

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.

Microbiological hazards

Due to the need for dexterity, surgical gloves are usually used when working with biological hazards. It is important to understand that gloves are the weakest point in the PPE spectrum and that frequent handwashing is necessary. Surgical gloves do not provide any protection against needlesticks, sharps, animal bites, and possibly some viruses.

Nonabsorbent surgical gloves (usually made of latex or nitrile) should wrap over the cuff and lower sleeve of the laboratory clothing. If working with viruses 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.

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.

See **www.bestgloves.com** for suggestions of the right glove material for a given hazard.

2.8.2 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 fumehoods, biosafety cabinets, and adequate 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, dusts or toxic fumes.

Respiratory protection equipment can be:

- **Supplied Air** – supplies breathing air inside the facepiece 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 BSL 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 operate for 8 hr 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 must be enrolled in the Respirator Program, administered through the Health, Safety and Environment, before using a respirator or ventilated hood.

2.8.3 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[4] 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. It is believed that the contact lens may act as a barrier to an irritant, suggesting that an irritant will cause lid spasm to occur, causing the lens to tighten against the cornea, thereby effectively sealing off the area under the lens. This theory is supported by actual cases described by Rengstorff and Black. However, Rowe cited the observation that rigid lenses are soluble in or swollen by many organic solvents and aqueous chemical solutions are readily soluble in the water phase of contact lenses. Ennis and Arons argued that lenses will increase the concentration of chemicals in contact with the eye because the chemicals become trapped underneath the lens and prevent thorough irrigation of the eye, thus accentuating corneal

[4] Conjunctiva – the mucus membrane that lines eyelids and the white part of the eyeball

damage. 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; however if worn and an adjustment needs to be made, it is critical that proper hand washing take place first.

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. 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.

2.8.4 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.

III: Risk Decontamination

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.

III: Risk Decontamination

3.1 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. Examples of decontaminants and their use will be discussed in detail in the following sections.

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 not to living tissues.

Germicide: substance used to destroy a 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.

3.2 Autoclave

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^{O}C for a minimum of 15 minutes. The time is measured after the temperature of the material being sterilized reaches 121^{O}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.

3.2.1 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.

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^{O}C - 170^{O}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^{o}C	20hr	120^{o}C	8hr
110^{o}C	2.5hr	140^{o}C	2.5hr
115^{o}C	50min	160^{o}C	1hr
121^{o}C	15min	170^{o}C	40min
125^{o}C	6.5min	180^{o}C	20min

3.2.2 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.

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 2 years. If the test shows growth, then the autoclaved must be shut down until it can be repaired.

3.2.3 General Procedures

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 person working with biohazardous material is responsible for its decontamination before disposal.

- Biohazardous materials should not be placed 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 until it is sterilized in an autoclave or other using another sterilization method. All wastes should be sterilized by the end 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.
- Use bench coat when there is the potential of aerosol production and/or the bench surface is rough or broken is some way.

3.3 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.

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.

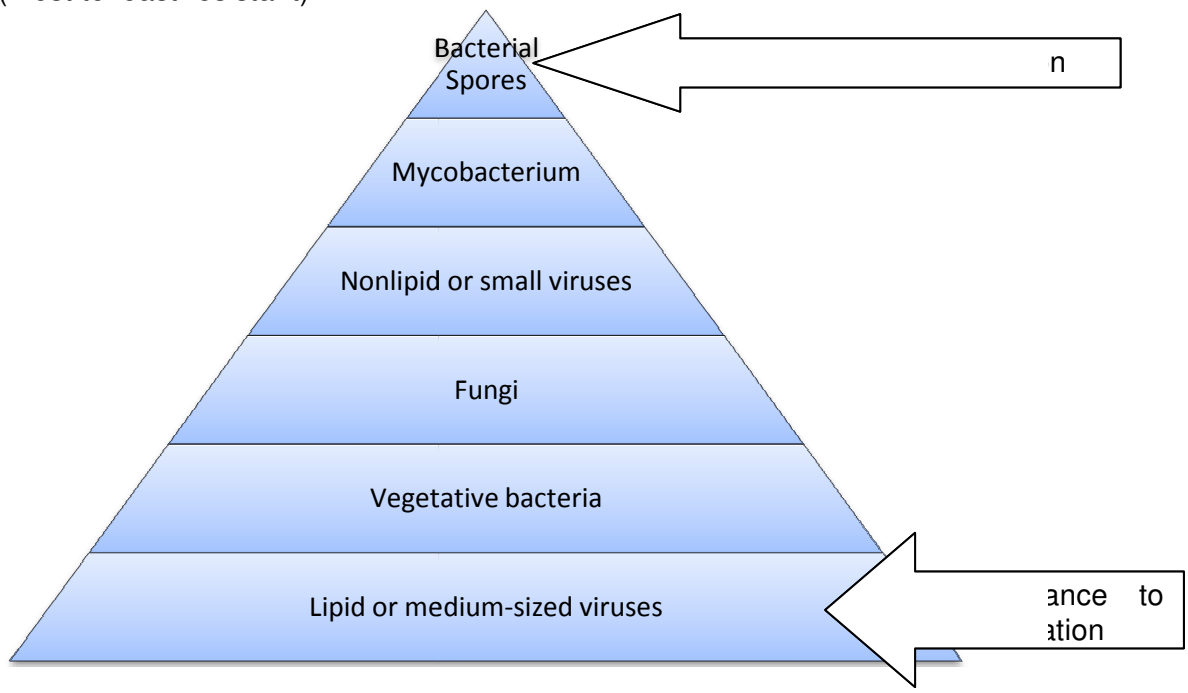
Relative Humidity: Can influence the activity of some decontaminants, particularly formaldehyde. Fumigation using formaldehyde gas requires a relative humidity preferably in excess of 70%.

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.

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 below (most to least resistant):



3.4 Categories of Chemical Decontaminants

Chemical decontaminants are usually supplied as either liquid concentrates or solids which must be diluted or hydrated prior to use. Once diluted, there is generally a limited time period in which they maintain acceptable levels of antimicrobial activity. This period is called the "use life" and it varies from hours to weeks depending on the disinfectant.

Microorganisms are affected by chemical decontaminants through:

- Cell lysis
- Protein coagulation or denaturation
- Enzyme denaturation or inactivation
- Destruction of enzyme substrates

The ideal decontaminant would be a broad spectrum agent able to act effectively against all biohazards. It would be fast acting, not easily inactivated, non-toxic to the user and non-corrosive. It would also be economical, easy to use, easy to dispose of and have a long "use life". Unfortunately, the ideal decontaminant does not exist. There is no 'one' chemical agent that can fulfill all of these requirements.

There are several categories of liquid decontaminants, each of which has different characteristics and different ranges of usefulness. Manufacturers often mix compatible active ingredients in their products, so it is important to read the label to determine what active ingredients are actually being used in the different products. Some agents are not compatible, so always follow the

manufacturer's instructions regarding mixing agents, dilution, storage and use of the decontaminant.

Alcohols

Ethyl or isopropyl alcohol in a concentration of 70 - 85% by volume is often used. Alcohols denature proteins and are somewhat slow in their germicidal action. However, they are effective decontaminants against lipid-containing viruses, enveloped viruses and vegetative bacteria. Longer contact times are required for activity against fungi and mycobacteria. There is variable activity against non-enveloped viruses and no activity against bacterial spores. Also, they are easy to use and non-corrosive.

Disadvantages include the fact that they evaporate easily, are extremely flammable, are easily inactivated by organic matter and have no cleansing properties.

Formaldehydes

Formaldehyde for use as a decontaminant is usually marketed at about 37% concentration of the gas in water solution (referred to as formalin), or as a polymerized compound called paraformaldehyde. Formaldehyde, in a concentration of 5% active ingredient (18.5 g/l formaldehyde), is an effective liquid decontaminant. However prolonged contact times are required (at least 30 minutes and up to 3 hours for activity against bacterial spores). Formaldehyde at 0.2 - 0.4% is often used to inactivate viruses in the preparation of vaccines. It has a broad spectrum of activity against all classes of microorganisms and is less susceptible to inactivation by organic material.

Disadvantages include loss of considerable decontaminant activity at refrigeration temperatures. Its pungent, irritating odour requires that care be taken when using formaldehyde solutions in the laboratory. It can be expensive, cause hypersensitivity and has a short "use life". From a human health perspective, formaldehydes are carcinogens.

Examples: Cidex 7, Sporocidin, Sonacide

Phenolic Compounds

Phenol is not often used as a decontaminant. The odour is somewhat unpleasant and a sticky, gummy residue remains on treated surfaces, especially during steam sterilization. Although phenol itself may not have wide spread use, phenol homologs and phenolic compounds (in combination with detergents) are basic to a number of popular decontaminants. The phenolic compounds are effective decontaminants against enveloped viruses and vegetative bacteria, but have variable activity against fungi and mycobacteria (depending on product), limited activity against non-enveloped viruses, and no activity in ordinary usage against bacterial spores.

Other disadvantages include its toxicity and possible irritation to skin, as well as neutralization by hard water. The actual toxic effects from phenolic compounds are specific to chemical; however, phenol itself is easily absorbed through the skin causing severe burns, numbness, and convulsions. Inhaled phenol fumes irritate and damage the respiratory tract which can ultimately lead to an inability to breathe. If splashed in the eyes, blindness can result. Through all routes of entry into the body, phenols can be lethal in small doses. There is presently not enough

information available to confirm or deny that phenol causes cancer. Due to the risks of working with phenols, you should consult the MSDS of your specific compound to ensure that you thoroughly understand the compound.

Quaternary Ammonium Compounds or Quats

After 40 years of testing and use there is still controversy about the efficacy of the "Quats" as decontaminants. These cationic detergents are strongly surface-active and this detergent property makes them good surface cleaners. The Quats will attach to protein; therefore dilute solutions of Quats will lose effectiveness in the presence of protein. They also tend to clump microorganisms and are neutralized by anionic detergents such as soap. Low concentrations are bacteriostatic, tuberculostatic, sporostatic , fungistatic and algistatic. At medium concentrations they are bactericidal, fungicidal, algicidal and virucidal against lipophilic viruses. However, even at high concentrations they are not tuberculocidal, sporocidal or virucidal against hydrophilic viruses. They do have advantages of being odourless, nonstaining, non-corrosive to metals, stable, inexpensive and relatively nontoxic.

Disadvantages include the fact that these compounds are inactivated by anionic soaps and organic materials (protein) and are ineffective against gram negative bacteria, spores, mycobacteria and many viruses. Regarding toxicity, these compounds are listed by the US EPA as highly toxic; however, little information regarding specific effects is currently available. As such, care should be taken until lack of harm is proven otherwise.

Examples: Roccal, Tor, Mikro-Quat

Chlorine Compounds

This halogen is a universal decontaminant active against all microorganisms including bacterial spores. Chlorine combines with protein and rapidly decreases in concentration in its presence. It is a strong oxidizing agent, which is corrosive to metals. Chlorine solutions will gradually lose strength; therefore it is important to prepare fresh solutions frequently. Sodium hypochlorite is usually used as a base for chlorine decontaminants. An excellent decontaminant can be prepared from household bleach. These bleaches usually contain 5.25% available chlorine (52,500 ppm). If the solution is diluted 1 to 100, giving 525 ppm active chlorine, a very good decontaminant is created.

Corrosiveness, short use life, inactivation by organic matter, irritation to skin and eyes and the fact that diluted solutions tend to be unstable are the primary disadvantages of chlorine compounds. It is also important for people working with ^{14}C not to use sodium hypochlorites as their decontaminant of choice because of the release of radioactive CO_2 as a byproduct.

Examples: Bleach, Javex, Presept, Alcide

Iodine Compounds

The characteristics of chlorine and iodine are very similar due to their position on the periodic table. Iodine compounds are available as aqueous solutions, as tinctures (solution in alcohol) and as iodophors. One of the more common groups of decontaminants used in laboratories are the iodophors. Complexed with a carrier molecule results in increased solubility and provides

sustained release of the halogen. The recommended range of concentration of active iodine to be present in solution is 25 - 75 ppm. At 75 ppm, the concentration of free iodine present is 0.0075%. This small amount can rapidly be taken up by excessive protein present. Clean surfaces or clear water can be effectively treated by 75 ppm available iodine, but difficulties may be experienced if any appreciable amount of protein is present. For washing hands or for use as a sporicide, it is recommended that a solution of 1600 ppm available iodine be made in 50% ethanol. This concentration of available iodine will provide relatively rapid inactivation of all microorganisms. There is also the presence of a built-in colour indicator due to the properties of iodine. When the solution is no longer active, it turns from a brown colour to yellow. Also, because it is a natural element, there are few health and disposal problems.

Disadvantages include that it is weakly corrosive, is inactivated by extraneous organic matter and that with continual use it may stain surfaces.

Glutaraldehydes

Glutaraldehydes are available as a 2% solutions supplied with a bicarbonate compound to activate the product. It has a broad spectrum of activity against all classes of microorganisms, including non-enveloped viruses and mycobacteria (requires a contact time of at least 20 minutes), and bacterial spores (with a prolonged contact time of at least 3 hours). Glutaraldehyde compunds are less sensitive to organic soils but do not readily penetrate organic material, they are non-corrosive and are rapidly bactericidal. The activated product has a limited shelf-life.

Disadvantages include adverse health effects including mucous membrane irritation, contact dermatitis, and occupational asthma.

Hydrogen peroxides

Hydrogen peroxide is available as a concentrated 30% solution in water. In-use solutions should be diluted to 6% hydrogen peroxide. At this concentration it is effective against vegetative bacteria, mycobacteria, fungi and viruses, with limited sporicidal activity. Hydrogen peroxides can NOT be used on aluminum, copper, zinc or brass materials. It is also unstable when exposed to heat and light.

Chlohexidine compounds

Available as a 4% solution of chlorhexidine gluconate in a detergent base (used undiluted) and as concentrated alcohol-based solutions requiring dilution prior to use. Alcoholic solutions show superior activity to aqueous solutions. They are effective against fungi, mycobacteria, non-enveloped viruses and have no sporicidal activity. Chlorhexidines are generally used as skin disinfectants and handwash products.

Examples: Wescodyne, Mikroclene, Hi-Sine

3.5 Categories of Radiation Decontaminants

3.5.1 Ionizing Radiation or Irradiation

There are 3 different irradiation technologies that use three different kinds of rays:

- gamma rays
- electron beams, and
- x-rays.

The first technology (gamma rays) 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.

3.5.2 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.

The use of UV light as a means of decontamination is discouraged by Health, Safety and Environment, the Public Health Agency of Canada, the Canadian Food Inspection Agency, the Centre for Disease Control and the National Institutes of Health.

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	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 forms of biohazards (including spores) and the gas can penetrate into small cracks and	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
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	Broad spectrum, fast acting, inexpensive.	Corrosive, short use life, inactivated by organic matter, irratates skin and eyes.	Sodium hypochlorite, Javex, Presept, Alcide
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

IV – Risk Disposal

The risk assessment of a hazard should include detailed procedures for waste disposal. If performed improperly the hazards pose a risk not only to the researchers, but to the maintenance, cleaning and environmental service people as well. For detailed information regarding a specific hazard refer to the Hazardous Materials Management Guide at http://web.ubc.ca/okanagan/hse/environment/hazardousmaterials.html or contact HSE at 80(7-8821).

IV: Risk Disposal

4.1 Waste Disposal Procedures

Waste disposal requires well defined procedures to prevent exposure to hazardous materials. Improper disposal of sharps and needles, glass and biohazardous waste puts the custodial staff and waste handlers at risk as well as jeopardizes the University's access the Glenmore landfill.

Principal investigators, supervisors, technicians and students must be familiar with current waste disposal procedures for biohazardous, chemical, pathological and radioactive substances handled in their respective areas[5].

Supervisors are responsible for ensuring that all employees are trained and familiar with the disposal procedures and that all laboratory procedures are in conformance with these requirements.

4.1.1 General Information

Hazardous waste is produced as a result of chemicals being left over from, or the products of, an experiment. A concerted attempt should be made to minimize hazardous waste generation by reducing, reusing or recycling where possible.

Examples of minimization methods include using diluted solutions rather than concentrated; using micro or semi-micro techniques; considering the substitution or elimination of extremely hazardous materials for less hazardous materials; using films, videotapes or demonstrations rather than individual experiments.

Consult with the administrator or chair of your local safety committee to determine what, if any, are the departmental procedures for handling and disposal of chemical waste.

If you have questions regarding the pick-up and disposal of hazardous wastes, please contact HSE in the Okanagan at 250-80(7-8821). For hazardous waste pick-up or to obtain supplies or information related to hazardous materials management, please visit the HSE Okanagan Hazardous Wastes website at http://web.ubc.ca/okanagan/hse/environment/hazardousmaterials.html

4.2 Transportation of Biohazardous Materials

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 use a cart.
- Ensure the Internal Transfer of Materials has been filled out and submitted.

[5] See http://web.ubc.ca/okanagan/hse/environment/hazardousmaterials.html for further information.

IV: Risk Disposal

Transportation between Buildings within UBC Sites

- Place the biohazard in a leak proof container, as above.
- Place this within a secondary leak proof and breakage resistant container.
- Place absorbent material around the primary container inside the secondary container.
- If a cooling material is required place this in a tertiary container.
- Ensure the Internal Transfer of Materials has been filled out and submitted.

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 biohazardous materials. Please contact the HSE office 80(7-8821) for more information.

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 or go to their website:
http://www.dfait-maeci.gc.ca/eicb/menu-en.asp.

Importing Biohazardous Materials

There are two categories for importation of level 2 biohazardous agents, Human Pathogens and Animal Pathogens. A form and a checklist need to be filled out for each, depending on the agent being imported. For containment level 1 agents, it is important that a letter stating a permit was not necessary be included in the package. 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.

1. **Human Pathogens:** the Public Health Agency of Canada (PHAC) is the regulatory agency for human pathogens.
    - Step 1 – fill out an Application for Permit to Import Human Pathogens form and Containment Level 2 Checklist.
      http://www.phac-aspc.gc.ca/ols-bsl/pathogen/howto_e.html.
    - Step 2 – fax completed forms to the HSE office for approval 80(7-9591). If the lab is unknown to HSE then a HSE Associate will come and ensure the form has been

completed accurately.
- Step 3 – send a copy of signed forms to PHAC. Fax: 613-957-1779

2. **Animal Pathogens:** the Canadian Food Inspection Agency (CFIA) is the regulatory agency for animal pathogens.
    - Step 1 – fill out an Application for Permit to Import Animal Pathogens form and CFIA facility certification form. http://www.inspection.gc.ca/english/sci/bio/anima/animae.shtml
    - Step 2 – fax completed forms to the HSE office for approval 80(7-9591). If the lab is unknown to HSE then a HSE Associate will come and ensure the form has been completed accurately.
    - Step 3 – send a copy of signed forms to CFIA. Fax: 613-228-6129

Note: if the pathogen or biohazard affects Animals and Humans then both sets of forms will have to be completed.

Glossary

absolute barrier/containment - has the capacity for the complete retainment of any specified substance including biohazards. A Class III biological safety cabinet can offer absolute containment.

actinomycetales - an order of bacteria that includes the families *Mycobacteriaceae, Actinomycetaceae, Actinoplanaceae, Dermatophilaceae, Micromonosporaceae, Nocardiaceae* and *Streptomycetaceae*.

actinomycete - any bacterium of the order *Actinomycetales*.

aerosol - a colloid of liquid or solid particles suspended in a gas, usually air. Biological agents can be aerosolized during many common laboratory procedures and inhalation or contact with these aerosols is the main cause of laboratory acquired infections

air balance - meaning, as it applies to Class II cabinets, to maintain the cabinet air flow so that air volume exhausted is equal to air intake, thereby eliminating (or minimizing) movement of outside air into the cabinet work area and vice versa through the face opening.

air barrier ("air curtain") - the unidirectional movement of air past and parallel to the plane of an opening and at a velocity greater than that on either side, which creates an air flow barrier to the movement of airborne particulates through the opening.

air change rate - the number of times the total air volume of a defined space is replaced in a given unit of time ordinarily computed by dividing the total volume of the subject space (in cubic feet) into the total volume of air exhausted from the space per unit time (e.g. a containment room would have 10 room changes of air per hour).

algice - an agent that destroys algae.

algistatic - an agent that is active against algae.

allergen - any substance that causes manifestations of allergy (e.g. dusts, pollens, fungi, smoke, perfumes or odours).

antibiotic - an antimicrobial agent used to prevent microbial replication in living organisms, tissues, or cells.

antimicrobial - an agent that destroys or prevents the development of microorganisms.

antiparasitic - an agent that destroys parasites.

antiseptic - a compound that prevents the multiplication of microorganisms (generally bacteriostatic in action, not bactericidal). This term applies to agents used on tissues rather than on inanimate surfaces (e.g. Hibitane).

aseptic technique - The performance of a procedure or operation in a manner that prevents the introduction or spread of contamination.

assessment of risk - in the present context, the process of defining the biological hazard

associated with a microbial, biological, or antigenic entity.

autoclave - an apparatus for sterilization by steam pressure, usually at 121°C for a specified length of time.

bacteria - microscopic, single-celled, prokaryotic organisms.

bactericidal - an agent that has bactericide properties.

bactericide - an agent that kills vegetative bacteria and possibly some less resistant spores (commercial term).

bacteriostatic - an agent that stops the growth and multiplication of bacteria but does not necessarily kill them. Usually growth resumes when the agent is diluted or removed.

biohazard - a contraction of the words biological and hazard: for the purpose of this text, the term refers to those microbial or antigenic entities presenting a risk or potential risk to the well--being of man, other animals, or plants, either directly through infection or indirectly through disruption of the environment.

biohazard area - any area in which work has been, or is being performed with biohazardous agents or materials.

biohazard control - any set of equipment and procedures utilized to prevent or minimize the exposure of workers and their environment to biohazardous agents or materials.

biohazardous material - any substance which contains or potentially contains biohazardous agents.

biological safety cabinet - a ventilated containment device intended to protect the user and the environment from the hazards of handling infected material and other dangerous biological materials (excluding radioactive, toxic and corrosive substances). Any air discharged to the atmosphere shall be filtered. Some types of cabinets may also protect the materials being handled in them from environmental contamination. Laminar flow biological safety cabinets may be used for the containment of in vitro procedures involving the use of chemical carcinogens providing that

- the exhaust air flow is sufficient to provide the inward air flow at the face opening of the cabinet equal to 100 linear fpm,
- contaminated air plenums that are under positive air pressure are leak-tight and,
- the cabinet exhaust air is discharged outdoors.

cabinet classification -

- **CLASS I:** a ventilated cabinet for personnel and environmental protection which may be operated with an open front, or with a glove port panel in place, with or without gloves attached. The cabinet exhaust air may be filtered through a HEPA and charcoal filter before being discharged to the outside atmosphere. This cabinet is suitable for work with low and moderate risk biological agents where no product protection is required.

- **CLASS II:** a ventilated cabinet for personnel and product protection having an open front with inward air flow (for personnel protection) and a mass recirculated HEPA filtered air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. This cabinet is suitable for work with low to moderate risk biological agents. Vapours and gases which are toxic, radioactive, or flammable should not be used in cabinets which recirculate all or part of the air. Consideration of use of such materials should be evaluated carefully from the standpoint of build-up (which may reach dangerous levels) and decontamination problems.

- **CLASS III:** a closed front ventilated cabinet with negative pressure and gas-tight construction which provides total personnel and product protection from contaminants contained within the cabinet. Supply and exhaust air are suitably treated to protect the environment. This cabinet, fitted with rubber gloves, provides the highest containment reliability of the three cabinet classes and is used for all activities involving high risk agents.

cabinet certification - measurement and/or correction of safety cabinet air velocities, patterns of air flow, balance, leakage and filtration system efficiency by a qualified technician to assure that the unit meets standard performance specifications. The Medical Research Council of Canada Laboratory Biosafety Guidelines require that biological safety cabinets be certified annually, when newly installed, repaired and when relocated.

cancer - a general term frequently used to indicate any of various types of malignant neoplasms.

capture velocity - the minimum air velocity necessary to overcome opposing air currents and cause contaminated air to follow the speed and direction of the exhaust air stream.

carcinogen - any cancer producing substance.

certification - see "cabinet certification".

charcoal filter - activated charcoal barrier which will adsorb organic material attempting to pass through it.

chlamydia - small, coccoid, Gram-negative microorganisms that resemble rickettsia.

chemical carcinogen - those chemicals designated as posing a potential occupational carcinogenic hazard.

clean room - a room in which the concentration of airborne particles is controlled to within specified limits. A dust free manufacturing facility.

conjunctivae - the mucus membrane that lines eyelids and the white part of the eyeball.

containment - prevention of agent transmission from one point to another (see primary, secondary, absolute, partial, total).

containment level - the combination of physical and operational requirements necessary to work with a particular agent or to perform a particular procedure or manipulation safely. The four levels of containment (I - IV) generally coincide with the four risk groups of infectious agents.

contamination - any foreign substance which makes an unwanted incursion. In the present context, usually viable airborne particulates.

controlled access area - an anteroom, change room, air lock, or any other double door arrangement which separates the laboratory from areas of unrestricted traffic flow.

dander - small scales from the hair or feathers of animals that may cause allergy in sensitive individuals.

decontaminate - 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.

diffuser - a device, often a screen, used to distribute air flow evenly.

diffusion - a phenomenon of HEPA filtration by which Brownian motion causes particles to diffuse across airstream lines impacting them on a filter fibre.

disease - a pathological condition of the body that presents a group of symptoms peculiar to it and that sets the condition apart as an abnormal entity differing from other normal or pathological body states.

dioctyl phthalate - an oil that can be aerosolized to an extremely uniform size (i.e. 0.3 mm for a major portion of the sample), this aerosol is used to challenge HEPA filters.

disinfectant - a chemical agent that kills or inactivates most vegetative bacteria, fungi, and viruses, but not necessarily spores. This term applies to a chemical used on inanimate surfaces as opposed to living tissues.

DOP - see dioctyl phthalate.

droplet - an air-borne particle consisting primarily of liquid. While some settle out quickly, many dry to become droplet nuclei and can add significant numbers of microorganisms to the air.

droplet nuclei - air-borne particles that originate as liquid droplets, but have dried and left behind the solid material that may have been contained within them. Because of their small mass, they may remain suspended in the air for long periods of time.

environmental protection - protection of the general working environment and the exterior environment from contamination originating within the work space. This means that any aerosol generated from manipulations performed within the unit is removed from the air or inactivated (such as by incineration) before the air from the cabinet is discharged either inside or outside the facility.

erythema - the temporary reddening of the skin produced by exposure to ultraviolet energy and other types of radiation.

etiologic agent - a viable microorganism or its toxin which causes, or may cause disease.

exhaust - the air which leaves a cabinet by means of a blower or fan; that portion of the cabinet air that is discharged after filtration, either to the room or into a ventilation system.

exhaust rate - the volumetric rate of exhaust air flow.

face velocity - air velocity at the cabinet work opening, velocity of the air entering the cabinet at the work opening.

filter - a device used to remove particulates, including microorganisms, from air, liquids or other gasses.

filter efficiency - the efficiency of various filters can be established on the basis of entrapped particles (i.e. collection efficiency), or on the basis of particles passed through the filter (i.e. penetration efficiency).

fungicidal - an agent that kills Fungi.

fungus - a member of the Fungi; a plant-like organism feeding on organic matter, e.g. mushrooms, yeasts, and moulds.

germicide - an agent able to destroy bacteria, fungi and viruses. The term is often misused commercially.

glove box - a sealed enclosure in which the handling of items inside the box is carried out through long rubber, or neoprene gloves, sealed to ports in the walls of the enclosure. The operator places their hands and forearms in the gloves from the room side of the box so that the operator is physically separated from the environment within the box but is still able to manipulate items inside with relative freedom. The manipulation can be viewed through a window.

gloves - primary barrier protection used to protect the hands of a worker against direct contact with, or exposure to, hazardous materials. Different types and thicknesses are appropriate for different tasks.

hard ducting - permanently installed ductwork not intended to be disassembled for normal cabinet servicing or testing.

HEPA filter - High Efficiency Particulate Air filter. A disposable extended-pleated dry-type filter with:

- a rigid casing enclosing the full depth of the pleats
- a minimum particle removal efficiency of 99.99% for cold polydispersed generated DOP or PAO smoke particles and

- a maximum pressure drop of 1 in. w.g. when clean and operated at its rated air flow capacity

high efficiency particulate air filter - see HEPA filter.

hood - a shaped enclosure designed to capture and retain contaminated air and conduct it into the exhaust duct system.

horizontal laminar flow hood (bench) - a ventilated cubicle with solid sides having a table-height work surface and unidirectional, minimum turbulence air entering from a vertically mounted HEPA filter at one side and leaving the cubicle at the opposite (open) side.

impaction - a HEPA filter phenomenon by which particles, because of their inertia, resist change in direction and continue on their original path to collide with filter fibres regardless of the changing path of the air.

in-place test - penetration test of filter units made after they are installed.

lyophilization - the process of rapidly freezing a substance at an extremely low temperature and then dehydrating in a high vacuum. SYN: freeze drying.

parasite - an organism that lives within, upon, or at expense of another organism, known as the host, without contributing to the survival of the host.

pathogen - a microorganism or substance capable of producing a disease.

pathogenicity - the state of producing or being able to produce pathological changes and disease.

pathological - diseased; due to a disease.

personnel protection - protection of the person performing work on or with a hazardous agent. This means that any aerosol generated within the cabinet is kept away from the face of the operator. Personnel protection is provided by an inflow of room air at the work opening where it is quickly entrained in a recirculating air stream and removed through an exhaust grill at the leading edge of the work area.

photometer - instrument used for measuring the concentration of DOP smoke particles up and downstream of a HEPA filter.

physical containment - a term that describes those methods used in the microbiology laboratory to contain infectious agents in the environment where the agent is being handled or maintained.

pipetting aid - a device which, through the application of suction, is employed in filling a pipette and which makes unnecessary the contact of the operator's mouth with the end of the pipette.

plenum - an enclosure for flowing gases in which the static pressure at all points is relatively uniform.

positive pressure - pressure in a space which causes an outflow of air.

primary containment device - an engineering system, a fully enclosed container, or a laboratory-type hood which is designated to contain hazardous materials so as to reduce or eliminate the potential for worker exposures to the contained hazardous materials.

principal investigator - the person who plans and directs research projects or other activities in the laboratory.

product protection - protection of the material, culture, etc., being worked on from contamination. This means that the air at the work surface of the cabinet has been filtered so that it is free of airborne particles and organisms which could contaminate the work. A contamination-free work zone is provided by supplying air through HEPA filters downward towards the work surface at a uniform velocity. Air flow equal to the inflow of room air is exhausted through HEPA filters incorporated in the cabinet or the exhaust system.

protection (Class II cabinets) - means that any aerosol generated within the cabinet is kept away from the face of the technician doing the work.

protective clothing - clothing designed to protect a worker against contact with or exposure to hazardous materials.

psittacosis - an infectious disease, caused by Chlamydia psittaci, of parrots and other birds that may be transmitted to humans.

Q-fever - an acute infectious disease characterized by headache, fever, malaise, myalgia, and anorexia. Caused by the rickettsial organism, Coxiella burnetii. Contracted by inhaling infected dusts, drinking unpasteurized milk from infected animals such as goats, cows or sheep.

radionuclide - a radioactive nuclide, or atom.

recombinant - a microbe or strain that has received chromosomal parts from different parental strains.

respirator - a personal protective device designed to protect a wearer against inhalation of:

- material dispersed in air as distinct particles
- gases and vapours.

risk levels -

- **Risk Group 1** (low individual risk, low community risk) - This group includes those microorganisms, bacteria, fungi, viruses and parasites which are unlikely to cause disease in healthy workers or animals (requires containment level I).
- **Risk Group 2** (moderate individual risk, limited community risk) - This group includes pathogens that can cause human or animal disease but, under normal circumstances, are

unlikely to be a serious hazard to healthy laboratory workers, the community, livestock, or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventive measures are available and the risk of spread is limited (requires containment level II).

- **Risk Group 3** (high individual risk, low community risk) - This group includes pathogens that usually cause serious human or animal disease, or can result in serious economic consequences but, do not ordinarily spread by casual contact from one individual to another, or that can be treated by antimicrobial or antiparasitic agents (requires containment level III).
- **Risk Group 4** (high individual risk, high community risk) - This group includes pathogens that usually produce very serious human or animal disease, are often untreatable, and that may be readily transmitted from one individual to another, or from animal to human or vice-versa, directly or indirectly or by casual contact (requires containment level IV).

rickettsiae - a genus of bacteria consisting of small organisms which do not grow in cell-free media.

sanitize - to eliminate microbial contamination which might be aesthetically objectionable.

secondary containment - the architectural and engineering features of the laboratory and its supporting mechanical systems which function to confine the spread of contamination.

sporicide - an agent that kills spores.

sterile - the absence of all life on or in an object. This is an absolute term; there is no such thing as "nearly sterile", "partially sterile" etc.

sterilize - 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.

supply air - air entering the biological safety cabinet through the work opening and/or through the supply opening to make up for the volume of air exhausted. In Class II cabinets this air passes through a supply HEPA filter before moving vertically over the work surface.

teratogen - a drug or other agent that causes abnormal development of a fetus.

thermo-anemometer - an instrument for measuring air velocity based on the removal of heat from a sensor as air passes by it.

total containment - (containment level IV) no escape of aerosol is permitted. This is the level of containment **ultraviolet (UV) radiation** - the short wavelengths of light beyond the violet end of the visible spectrum. A narrow range of the UV spectrum has antimicrobial activity but is limited by poor penetrating power (UV lamps often lose the antimicrobial portion of their spectrum even though no loss in intensity is visibly obvious).

vector - a carrier, usually an arthropod or insect that transmits the causative organisms of disease from infected to non-infected individuals.

ventilated hood - see horizontal laminar flow hood.

vertical laminar flow hood - a ventilated cubicle with solid sides and an open front, having a table-height work surface and minimum turbulence air entering from a horizontally mounted HEPA filter providing unidirectional airflow down onto the work surface.

viable - literally "capable of life". Generally refers to the ability of microbial cells to grow and multiply as evidenced by, for example, the formation of bacterial colonies on an agar medium or, as with viruses, to divert the host cells metabolism to begin replication of the parasite. Frequently, organisms may be viable under one set of culture conditions and not under another set, making it extremely important to define precisely the conditions used for determining viability.

virucide - an agent that inactivates, destroys or kills viruses.

virulence - the relative power and degree of pathogenicity possessed by organisms to produce disease.

virus - a term for a group of microbes which, with few exceptions, are capable of passing through fine filters that retain bacteria; they are incapable of growth or reproduction apart from living cells.