LABORATORY BIOSAFETY
TRAINING MANUAL

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1.0 INTRODUCTION

Biohazardous infectious materials fall under WHMIS Class D3 – Poisonous and Infectious Material - and are defined as organisms that have been shown to cause disease or are reasonably believed to cause disease in persons or animals, and the toxins of these organisms.

Such organisms are denoted by the WHMS symbol shown here

The safe handling of infections substances requires precautions beyond those found in a normal chemical laboratory and the purpose of this manual is to provide an introduction to the hazards of these materials and the general principles and requirements for working with them in a safe manner.

This manual constitutes the biosafety training required to be taken by all persons who work with or around biological materials, including, for example, microorganisms, cells or cell lines, tissue cultures, recombinant DNA, viruses, animals or animal blood, and human blood, body fluids or tissues.

What is Biosafety?

The term biosafety relates to the measures and procedures employed when handling biohazardous materials to avoid infecting oneself, other persons, or contaminating the environment. It consists of the use of:

- engineering controls,
- administrative controls,
- practices and procedures, and
- personal protective equipment.

What is a Biohazard?

A biohazard is any potential hazard to humans, animals or the environment caused by a biological organism or by material produced by such an organism. Such organisms include:

- Bacteria
- Viruses
- Fungi
- Parasites
- Transformed cell lines

- Blood and body fluids
- Animal and human tissue

Working safely with biohazardous materials requires identifying the risks associated with the particular organism and introducing procedures, practices, equipment and facilities to control the risks and reduce them to acceptable levels.

The research environment, by its very nature, is constantly changing and it is not possible for safety specialists to be aware of and monitor each and every use of these materials. It is essential for the researcher to identify the potential hazards associated with the work and to institute the necessary practices and procedures. In addition, the University requires compliance with the Canadian *Biosafety Standards and Guidelines* published by the Public Health Agency of Canada.

2.0 LABORATORY ASSOCIATED INFECTIONS

There is an extensive body of literature documenting laboratory-associated infections. The precise number of such infections is not known as there is no requirement for central reporting and collection of such data. The published material is based on surveys conducted beginning around 1941. One can say that such infections have occurred with regularity and have occasionally resulted in death.

As of 1999, over 5000 cases of laboratory-acquired infections and 190 deaths have been reported, giving an overall mortality rate of around 4 percent. These figures are believed to be a significant underestimate because of underreporting. Additionally, only about 20% of infections can be attributed to any know, single exposure event. Exposure to aerosols was considered to be a plausible, but unconfirmed source of infection for the more than 80% of the reported cases in which the infected person had “worked with the agent”.

The most frequently reported laboratory-acquired infections from a total of 3,921 reported events are shown in Table 2.1.

TABLE 2.1
REPORTED LABORATORY-ACQUIRED INFECTIONS

Infection	% of cases		infection	% of Cases
Brucellosis	10.8 %		Typhus	3.2 %
Q Fever	7.1 %		Psittacosis	3.0 %
Typhoid Fever	6.5 %		Coccidioidomycosis	2.4 %
Hepatitis	6.0 %		Streptococcal infections	2.0 %
Tularemia	5.7 %		Histoplasmosis	1.8 %
Tuberculosis	4.5 %		Leptospirosis	2.2 %
Dermatomycosis	4.1 %		Salmonellosis	1.2 %
Venezuelan equine encephalitis	3.6 %		Shigellosis	1.5 %

The routes of exposure for laboratory workers and the practices leading to them are:

Ingestion

- Mouth pipetting
- Splashes of infectious material into the mouth
- Contaminated articles of fingers placed in the mouth
- Consumption of food or drink in the workplace

Inoculation

- Needlestick injuries
- Cuts from sharp objects
- Animal and insect bites and scratches

Contamination of Skin and Mucous Membranes

- Spills or splashes into eyes, mouth, nose
- Spills or splashes on intact of nonintact skin
- Contaminated surfaces, equipment, articles

Inhalation

- Numerous procedures that produce aerosols

The most frequent exposures occur through inhalation of aerosols and laboratory accidents. The types of accidents associated with laboratory infections are shown in Table 2.2

TABLE 2.2
ACCIDENTS ASSOCIATED WITH LABORATORY INFECTIONS

Accident	% of Infections
Splashes and sprays	26.7 %
Needlesticks	25.2 %
Sharp objects	15.9 %
Animal or ectoparasite bite/scratch	13.5 %
Mouth pipetting	13.1 %
Other, unknown	5.5 %

Manny common laboratory procedures can generate aerosols which can cause infection if inhaled. Depending on their size, the droplets will settle out of the air quickly on surfaces or be carried around rooms and buildings on air currents. Many of the major outbreaks of laboratory-acquired infections, which cannot be traced to a particular accident or incident are presumed to arise from aerosols.

Types of laboratory activities which can give rise to aerosols are shown in Table 2.3.

TABLE 2.3
LABORATORY ACTIVITIES THAT GENERATE AEROSOLS

Laboratory Activity	Microbiological Practice
Inoculating-loop manipulation	Subculturing and streaking culture “cooling” a loop in culture media Flaming a loop
Pipetting	Mixing microbial suspensions Pipette spills on hard surfaces
Needle and syringe manipulation	Expelling air Withdrawing needle from stopper Injecting animals Spray created when needle separates from syringe
Others	Centrifugation Using blenders, shakers, sonicators, and mixing instruments Pouring or decanting fluids Opening culture containers Spillage of infectious material Lyophilization and filtration under vacuum Egg inoculation and harvesting

The most important factor in the likelihood of a laboratory-acquired infection is the pathogenicity of the organism being handled. The dose, or number of organisms required to cause disease can vary widely depending of the specific organism and the route of entry. Table 2.4 gives some examples of the infectious doses for a number of pathogens.

TABLE 2.4
INFECTIOUS DOSE FOR HUMANS OF SPECIFIC AGENTS

Agent/Disease	Route of Entry	Infectious Dose (1)
Anthrax	Inhalation	≥ 1,300
Q fever	Inhalation	10
Tuberculosis	Inhalation	< 10
Tularemia	Inhalation	10
Measles	Intranasal spray	0.2
Venezuelan equine encephalitis virus	Subcutaneous	1
Escherichia coli	Ingestion	10^8
Salmonella spp.	Ingestion	10^5
Vibrio cholerae	Ingestion	10^8

(1) The infectious dose is the number of microorganisms that can cause disease in 25 to 50% of volunteers.

The very low inhalation dose for certain agents illustrates why it is extremely important to prevent aerosol formation in the laboratory and also indicates why the direct cause of many laboratory infections may go undetected.

The biosafety policies and procedures in place at the University are designed to minimize the risks associated with working with biohazardous agents. However it must be stressed that the single most effective way of preventing exposure is through trained staff proper microbiological techniques.

3.0 REGULATORY REQUIREMENTS

There are a number of acts, regulations and guidelines, both provincial and federal, which apply to biosafety.

3.1 Ontario *Occupational Health and Safety Act*

The Ontario *Occupational Health and Safety Act* governs workplace health and safety and sets out the rights and duties of all workplace parties. Two regulations under that Act which relate specifically to biohazards are:

1. *Control of Exposure to Biological or Chemical Agents*
2. *Workplace Hazardous Materials Information System (WHMIS)*

The Biological and Chemical Agents regulation places the following duty on employers:

3. (1) Every employer shall take all measures reasonably necessary in the circumstances to protect workers from exposure to a hazardous biological or chemical agent because of the storage, handling, processing or use of such agent in the work place.
3. (2) The measures referred to in subsection (1) shall include the provision and use of engineering controls, work practices, hygiene facilities and practices, and personal protective equipment.

Under the WHMIS legislation, biohazardous agents are classified as a controlled substance, Class D3- Poisonous and Infectious Material. Employers are required to:

- Label biohazardous material
- Provide instruction and training to workers
- Provide material safety data sheets where available from a supplier

3.2 *Human Pathogens and Toxins Act* and Regulations

Human pathogens and toxins are governed under the Human Pathogens and Toxins Act (HPTA), and under the Human Pathogens and Toxins Regulations (HPTR). The HPTA establishes a safety and security regime to protect the health and safety of the public against the risks posed by human pathogens and toxins. A license is required from the Public Health Agency of Canada to produce, possess, store, handle, transfer, import or export, or dispose of a human pathogen or toxin. The Canadian Biosafety Standards and Guidelines published by the Public Health Agency of Canada apply to all facilities in Canada that are governed by the HPTA and HPTR.

3.3 Canadian Biosafety Standards and Guidelines

The Canadian Institute for Health Research (CHIR) and the Natural Science and Engineering Research Council (NSERC) require, as a condition of their grants, that the University adhere to the *Canadian Biosafety Standards and Guidelines* published by the Public Health Agency of Canada. A copy of these guidelines is available at http://canadianbiosafetystandards.collaboration.gc.ca/cbsg-nldcb/index-eng.php.

3.4 Other Acts and Regulations

There are a number of other acts and regulations which pertain to the import and export of human and animal pathogens, plants and the transportation of dangerous goods. These do not impact on the biosafety practices within laboratories and they are not discussed in detail here. The major ones are listed below with references as to where more information may be obtained.

3.4.1 Human Pathogens Importation Regulations

A permit is required from the Public Health Agency of Canada to import a human pathogen into Canada. A human pathogen is any microorganism or parasite that causes disease in humans. This includes zoonotics. Human pathogens may be contained in cultures, diagnostic specimens, or tissue.

More information and the permit application are found on the Public Health Agency of Canada website at
http://www.phac-aspc.gc.ca/ols-bsl/pathogen/index.html .

3.4.2 Health of Animals Act and Regulations

A permit is required from the Canadian Food Inspection Agency for imported animal pathogens and pathogens associated with reportable animal diseases. Further details and the permit application can be found on the Canadian Food Inspection website at http://www.inspection.gc.ca/english/anima/animae.shtml

3.4.3 Plant Protection Act and Regulations

The importation and release of “plants with novel traits” are regulated by the Canadian Food Inspection Agency, Plant Biosafety Office. For information see their website at http://www.inspection.gc.ca/english/plaveg/bio/pbobbve.shtml

3.4.4 Transport of Dangerous Goods Regulations

Transport Canada regulations define labeling, packaging and documentation requirements for shipping infections substances including diagnostic specimens within Canada. Biohazardous materials fall under Class 6, Infectious substances. See the regulations at http://www.tc.gc.ca/tdg/menu.htm.

3.5 UOIT Biosafety Program Policies and Procedures

The UOIT Biosafety Program and procedures have been designed to comply with the requirements of the Ontario *Occupational Health and Safety Act* and the *Canadian Biosafety Standards and Guidelines*. The program is administered through the Office of Research Services and the Biosafety Officer. The Biosafety Program is detailed in the UOIT Biosafety Manual, a copy of which can be found

at
https://shared.uoit.ca/shared/department/research/documents/BIOSAFETY_MANUAL_R2.1.pdf

3.5.1 Biosafety Certificates

A biosafety certificate is required for all research and teaching laboratory activities which involve the use or manipulation of potentially hazardous biological agents and materials containing such agents (e.g. viruses, bacteria, fungi, parasites, recombinant DNA, prions and other microorganisms/genetic systems and human and animal tissues, cells, blood and body fluids), and which are:

(1) supervised or conducted by employees or members of the University, or
(2) conducted on University premises, or in a building or location administered by or under the control of the University, and
(3) supported by funds provided by or through the University.

The major purpose of the Biosafety Certificate is to ensure that a risk assessment is performed for work with biological agents, that an appropriate level of containment is selected, and that appropriate security controls are in place.

All faculty members proposing to conduct research using hazardous biological agents must complete an application for a Biosafety Certificate and submit it to the Office of Research Services.

The application will be reviewed by the University Biosafety Committee who will issue the certificate and specify the conditions under which the research is to take place.

3.5.2 Biosafety Committee

The University Biosafety Committee is responsible for establishing and maintaining a system to ensure that all activities within the University involving hazardous biological agents are conducted in a safe manner and in conformity with generally accepted standards.

4.0 BIOHAZARD RISK ASSESSMENT

4.1 Risk Groups

Biological organisms are classified into four risk groups based on the particular characteristics of the organism, such as:

- Pathogenicity
- Infectious dose
- Mode of transmission
- Host range
- Availability of effective preventive measures
- Availability of effective treatment

These classifications presume typical use in a research laboratory or growth in small volumes for research, diagnostic and experimental purposes. For large scale operations, additional requirements may be specified.

4.1.1 Risk Group 1

Risk Group 1 agents are those that are unlikely to cause disease in healthy workers or animals. They pose a low risk to individuals and populations.

Examples of Risk Group 1 Agents
Bacillus subtilis *Naegleria gruberi* *Infectious canine hepatitis virus* *E. coli*

4.1.2 Risk Group 2

Risk Group 2 agents can cause human disease but, under normal circumstances, are 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 prevention measures are available and the risk of spread is limited. These agents pose a moderate individual risk and a low population risk.

Examples of Risk Group 2 Agents
Measles virus
Salmonellae
Toxoplasma spp.
Hepatitis B Virus

4.1.3 Risk Group 3

Risk Group 3 agents will usually cause serious human disease or result in serious economic consequences, but they do not ordinarily spread by casual contact from one individual to another, or they cause diseases that are treatable by antimicrobial or antiparasitic agents. These agents pose a high individual risk and a moderate population risk.

Examples of Risk Group 3 Agents
M. tuberculosis
St. Louis encephalitis virus
Coxiella burnetti

4.1.4 Risk Group 4

Risk Group 4 agents are those which usually produce 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. These agents pose a high individual and a high population risk.

Examples of Risk Group 4 Agents
Ebola Zaire
Sin Nombre virus
Rift Valley Fever

It is the responsibility of the researcher to identify the risk group of the organisms which with which he/she proposes to work. The Office of Laboratory Security of the Pubic Health Agency of Canada provides Material Safety Data Sheets for a number of organisms on its website at http://www.phac-aspc.gc.ca/msds-ftss/index.html. These provide valuable information on the hazards of the organism. Another useful reference is the publication of the US Department of Health and Human Services *Biosafety in Microbiological and Biomedical Laboratories. (Available at http://bmbl.od.nih.gov/)*

4.2 Cell Lines

Cell lines and cell cultures are commonly used in microbiological laboratories, and there have been instances of laboratory acquired infections reported as a result of the manipulation of primary cell cultures.

Although cell lines do not inherently pose a risk to individuals handling them in the laboratory, they have the potential to contain pathological organisms, either through contamination, transformation or recombination. Cell lines can be contaminated with bacterial, fungi, mycoplasma, viruses and prions.

An assessment must be made as to the level of hazard associated with a particular cell line. This assessment must accompany the application for a biosafety certificate and will determine the containment level and procedures appropriate to handling that cell line.

Guidance on conducting the risk assessment is provided in the Public Health Agency of Canada *Canadian Biosafety Standards and Guidelines.*

4.3 Laboratory Animals

Work with animals poses a variety of hazards including exposure to infectious agents (zoonotic diseases), animal bites and scratches. In addition there are numerous additional regulatory requirements such as those of the Canadian Council for Animal Care.

These are covered in a separate course designed for those who work with animals.

5.0 CONTROL OF BIOHAZARDS

5.1 Containment Levels

The basic principle for the control of biohazards is described by the word “containment”. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons and the outside environment to potentially hazardous agents.

Containment is achieved through a combination of:

- Engineering Controls (facility design)
- Work practices and techniques
- Personal Protective Equipment

Four containment levels are defined based on a combination of engineering controls, operational procedures and safety equipment. These containment levels are applicable to facilities such as research laboratories, diagnostic, clinical, teaching and production facilities that are working with laboratory scale quantities of biohazardous agents.

The four containment levels correspond approximately to the biohazard risk group. That is, normally a risk group 1 agent would be assigned a Containment Level of 1, a risk group 2 agent, a Containment Level of 2 and so on. The Health Canada Guidelines provide for variances from this practice based on a risk assessment of the work to be performed.

Depending on the procedure being performed either a lower or a higher level of containment may be appropriate remembering that containment includes both the physical facilities and the operational procedures. An example provided in the Health Canada guidelines is for procedures involving HIV. Primary diagnostic tests for HIV may be done in a containment level 2 physical laboratory with the use of containment level 3 operational protocols, but growing and manipulating cultures of HIV may require both level 3 physical facilities and operational protocols.

The selection of the appropriate containment level is therefore a part of the process of applying for and receiving a biosafety certificate.

Containment Level 1

Containment Level 1 is a basic level of containment requiring no special design features beyond those suitable for a well-designed and functional laboratory. Work may be performed on an open bench top and containment is achieved through the use of practices normally employed in a basic microbiological

laboratory. No special primary or secondary barriers are required other than a sink for handwashing.

Appropriate safe working practices are described in Section 6 – Work Practices and Procedures.

Containment Level 2

The primary hazards associated with organisms requiring Containment Level 2 are through the ingestion, inoculation and mucous membrane route. Agents requiring Level 2 Containment are not generally transmitted by airborne routes, but care must be taken to avoid the generation of aerosols or splashes. Aerosols can settle on bench tops and become an ingestion hazard through contamination of the hands.

With good microbiological techniques these organisms can be used safely on open benches, **provided the potential for producing splashes or aerosols is low**.

Primary containment devices such as biological safety cabinets and centrifuges with sealed rotors or safety cups are to be used for operations which have the potential to create aerosols or splashes. Personal protective equipment (i.e. gloves, laboratory coats, protective eyewear) should also be used as appropriate. Environmental contamination must be minimized by the use of handwashing sinks and decontamination facilities (e.g. autoclaves)

Containment Level 3

Agents requiring Containment Level 3 may be transmitted by the airborne route, often have a low infectious dose to produce effects and cause serious life-threatening disease.

Containment Level 3 emphasizes additional primary and secondary barriers to minimize the release of infectious organisms into the immediate laboratory and the environment. All laboratory manipulations must be carried out in a biological safety cabinet or other enclosed equipment.

Additional features to prevent transmission of organisms are appropriate respiratory protection, HEPA filtration of exhausted laboratory air and strictly controlled laboratory access.

Containment Level 3 facilities have special design requirements beyond those of a basic laboratory. There are no Level 3 facilities at the University.

Containment Level 4

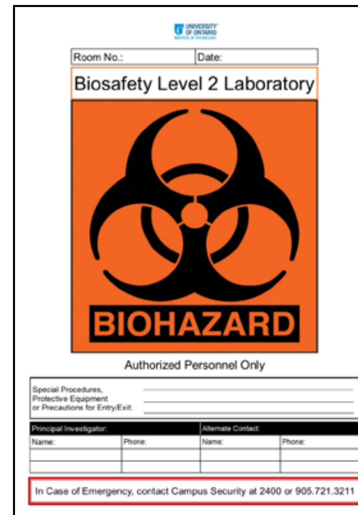
Level 4 is the maximum containment available and is used for agents which have the potential for aerosol transmission, often have a low infectious dose and produce very serious and often fatal disease for which there is generally no vaccine or treatment available.

This level of containment represents an isolated unit functionally, and often structurally, independent of other areas. Maximum containment is achieved by complete sealing of the facility perimeter, with confirmation by pressure decay testing; isolation of the researcher from the pathogen by his or her wearing of a positive pressure suit and decontamination of air and other effluents produced by the facility.

There is no Level 4 facility at the University.

5.2 Signs and Labels

All laboratories assigned a containment level of 2 or greater, must have a biohazard warning sign posted on the outside of all entry doors giving the containment level and the name of the contact person or laboratory supervisor.



All containers, equipment and storage units used with biological agents, as well as biohazardous wastes must have biohazard labels.

6.0 WORK PRACTICES AND PROCEDURES

The single most important factor in working safely with biohazardous materials is the use of good microbiological work practices.

6.1 Seven Basic Rules

The most common means of exposure can be essentially eliminated by following the seven basic rules of biosafety.

Seven Basic Rules of Biosafety

1. Do not mouth pipette.
2. Manipulate infectious fluids carefully to avoid spills and the production of aerosols and droplets.
3. Restrict the use of needles and syringes to those procedures for which there are no alternatives; use needles, syringes and other “sharps” carefully to avoid self-inoculation; and dispose of “sharps” in leak- and puncture-resistant containers.
4. Use protective laboratory coats and gloves.
5. Wash hands following all laboratory activities, following the removal of gloves, and immediately following contact with infectious materials.
6. Decontaminate work surfaces before and after use, and immediately after spills.
7. Do not eat, drink, store food, or smoke in the laboratory.

6.2 General Laboratory Safety Practices

The *Canadian Biosafety Standards and Guidelines* specify work practices which must be followed by all laboratories handing infectious agents. These are detailed in that publication for all four containment levels and in the University Biosafety Manual for levels 1 and 2. Common to all biohazard labs are the following:

- An up-to-date procedures manual,
- Training of staff in the hazards and necessary precautions,
- Eating, drinking, smoking storing of food or personal items in a lab are prohibited,
- Insertion and removal of contact lenses in a lab is prohibited; use of contact lenses only if other forms of corrective eyewear are not suitable,
- Oral pipetting prohibited,
- Long hair to be tied back or restrained,
- Access to laboratory limited to authorized personnel,

- Doors to laboratories must not be left open,
- Open wounds, cuts, scratches covered with waterproof dressings,
- Laboratories to be kept clean and tidy,
- Proper protective clothing to be worn (lab coats, eye protection, gloves),
- Protective lab clothing not to be worn in non-lab areas,
- Use of needles, syringes and other sharp objects strictly limited,
- Frequent hand washing,
- Clean and decontaminate work surfaces regularly,
- Decontaminate all materials leaving the laboratory,
- Regular efficacy monitoring of autoclaves with biological indicators,
- Proper disinfectants available at all times,
- Leak proof containers for transport of infectious material,
- Reporting of spills, accidents or exposure to infectious material, and
- A rodent and insect control program.

A Level 2 facility requires in addition to these:

- Use of biological safety cabinets for operations that may produce aerosols or involve high concentrations or volumes of infectious material,
- Appropriate signage posted outside the laboratory indicating the containment level,
- Restricted entry,
- Written emergency procedures.

6.3 Handwashing

Handwashing is an effective means of preventing infections if done frequently and properly.

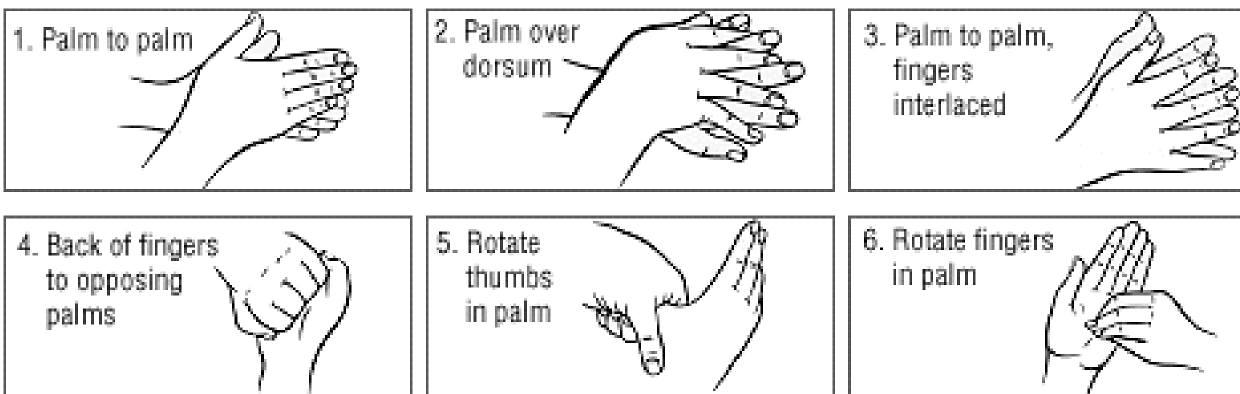
Handwashing should be done:

- Before starting any manipulations,
- Before leaving the laboratory,
- When hands are obviously soiled,
- Before and after any task in a biosafety cabinet,
- Every time gloves are removed,
- Before contact with face and mouth, and
- At the end of the day.

For a Level 1 laboratory a non-antiseptic soap may be uses; for a Level 2 laboratory antiseptic hand-washing solutions are required.

Proper Hand Washing Protocol

- Wet hands with tepid water,
- Dispense soap into cupped hand,
- Spread soap around hands and between fingers,
- Wash hands for at least 10 seconds,
- Rinse thoroughly under tepid water,
- Dry hands thoroughly with paper towels.



6.4 Personal Protective Equipment

Personal protective equipment is the last line of defense against exposure and is to only be worn in the laboratory. Protective equipment and clothing should not be worn outside of the laboratory.

Laboratory Coats

Laboratory coats should be long-sleeved and knee length. They should only be worn in the laboratory and not worn outside the laboratory.

Footwear

Closed-toed shoes only should be worn. Sandals should not be worn in the laboratory.

Gloves

For work with biological agents use latex, nitrile or vinyl gloves.

Latex gloves can give rise to allergic reactions in some people so watch for any allergic reaction on the hands. Non-powdered latex gloves are preferable to the powdered variety as there is evidence that the latex protein allergens from the glove fasten onto the powder. When the gloves are removed the power along

with the absorbed latex proteins becomes airborne where it can be inhaled or come into contact with body membranes. Studies have shown that in workplaces where only powder-free gloves are used, the levels of latex protein allergens are low or undetectable.

Eye and Face Protection

When working with liquids that can be splashed into the eyes, goggles should be worn which seal around the eyes. Safety glasses with side shields are designed for flying particles and do not offer protection against liquids.

Respiratory Protection

The use of respirators is a last resort and they should not be used as a normal means of protection. The use of a respirator requires special training and fit-testing to ensure a proper seal around the face.

7.0 HUMAN PATHOGENS

In general, the risk to laboratory personnel working with an agent that only infects and causes diseases in animals is less than the risk in working with tissues and cells from humans and other primates. If the human material contains a viable human pathogen, it will likely be a human pathogen with the potential to infect and cause disease in other humans.

Human blood and body fluids are a potential source of pathogenic microorganisms that may present a risk to workers who are exposed during the performance of their duties. The sources include:

- Blood
- Semen
- Vaginal secretions
- Other body fluids (cerebrospinal, amniotic, synovial)
- Tissue cultures
- Organ cultures
- Infected experimental animals

The pathogens of primary concern are:

- the human immunodeficiency virus (HIV),
- hepatitis B virus (HBV),
- Hepatitis-C virus (HVC).

and can also include any other sexually-transmitted or other disease pathogenic to humans.

Infection control techniques for dealing with potentially contaminated human body fluids were developed following the AIDS outbreak in the early 1980s. They were termed “universal precautions” but today are more generally referred to by the terms “standard precautions” and “additional precautions”.

7.1 Standard Precautions

Standard precautions are basically good hygiene habits such as hand washing, the use of gloves and other barriers, correct sharps handling and aseptic techniques.

Standard precautions apply to all human blood and body fluids, no matter what their infections state is know or suspected to be. The first rule is to **assume that all human blood and body fluids are infectious and treat them as such**.

Hepatitis B Virus (HBV)

Pathogenicity:

- Two forms – symptomatic and asymptomatic
- Non specific symptoms (anorexia, vague abdominal discomfort, nausea and vomiting, fever may be absent or mild, sometimes rash, often progressing to jaundice
- Severilty ranges from mild to fatal
- Long-term fatality rate 2-3% due to cancer or cirrhosis of the liver

Incubation Period:

- 24-180 days: average 60-90 days

Laboratory Acquired Infections:

- most frequently acquired laboratory infection
- incidence in some categories of lab workers up to 7 times greater than in general population
- primary exposure from parenteral innocuoation, droplet exposure of mucous membranes, contact exposure of broken skin

Survival outside Host:

- survives in dried blood for weeks,
- stable on environmental surfaces for at least 7 days at 25 C.

Immunization:

- available and recommended for health care and laboratory workers exposed to human blood and body fluids

Source: Public Health Agency of Canada, Material Safety Data Sheet for Hepatitis B virus.

Hepatitis C Virus (HBC)

Pathogenicity:

- Two forms – symptomatic and asymptomatic
- Non-specific symptoms (anorexia, vague abdominal discomfort, nausea and vomiting, progressing to jaundice)
- Severity ranges from mild (90% of cases) to fatal
- Chronic liver disease common in 50-80% of infections

Incubation Period:

- 2 weeks to 6 months; commonly 7-10 weeks

Laboratory Acquired Infections:

- medical personnel have slightly higher HBC antibody prevalence than general population
- risk of infection lower than HBV
- primary exposure from parenteral inoculation of blood and plasma products; other unknown factors

Survival outside Host:

- survives in dried blood for long periods (weeks)

Immunization:

- none available

Source: Public Health Agency of Canada, Material Safety Data Sheet for Hepatitis C virus.

Human Immunodeficiency Virus (HIV)

Pathogenicity:

- Non-specific symptoms (anorexia, lymphadenopathy, chronic diarrhea, weight loss, fever and fatigue, opportunistic infections and malignant diseases).

Incubation Period:

- 6 months to 7 years

Laboratory Acquired Infections:

- 5 reported laboratory acquired HIV infections (splashing of infected materials, puncture wounds, inapparent skin exposure)
- 18 reported cases in health care workers worldwide
- exposure from direct contact with skin an mucous membranes eye, nose and mouth; accidental parenteral inoculation; ingestion; hazard of aerosol exposures unknown

Survival outside Host:

- drying in environment causes rapid (within several hours) 90-99% reduction in HIV concentration

Immunization:

- none available

Source: Public Health Agency of Canada, Material Safety Data Sheet for Human Immuno deficiency virus.

7.1 Standard Precautions (continued)

Standard precautions are basically the good laboratory practices outlined in Section 6, but they are used outside laboratories in the health care setting. They can be briefly summarized as:

- use appropriate barrier precautions to prevent skin and mucous membrane exposure,

- use of gloves
- use of protective equipment to protect mouth, nose and eyes
- frequent hand washing
- precautions to prevent needlestick injuries
- proper management of wastes
- prohibition on mouth pipetting
- frequent decontamination of work surfaces and equipment

7.2 Additional Precautions

Additional precautions are used in addition to standard precautions where there is know to be infection and depend on the particular organism. Conditions which indicate the need for additional precautions are:

- prion diseases (e.g. Creutzfeldt-Jakob disease),
- diseases with airborne transmission (e.g. tuberculosis),
- diseases with droplet transmission (e.g. mumps, rubella, influenza, pertussis),
- transmission by direct or indirect contact with dried skin or contaminated surfaces.

8.0 BIOLOGICAL SAFETY CABINETS

8.1 Types of Biological Safety Cabinets

Biological safety cabinets are the most effective and most commonly used primary containment devices used in laboratories working with infectious agents. They are second only to safe work practices in preventing infections. In Level 2 and higher facilities they are used for procedures with the potential to generate infectious aerosols and for high concentrations or volumes of infections material. They operate to minimize contact with biological agents through the use of directional airflows.

There are three classes of cabinet – Class I, Class II and Class III. They have different characteristics and selection of the proper cabinet requires a careful evaluation of the activities to be carried out.

Any cabinet used should meet the National Sanitation Foundation (NSF) Standard No. 49-2002 (independent standard for the design, manufacture and testing of BSCs) and bear a NSF 49 seal.

<u>Class I Cabinets</u>

Class I Cabinets are negative pressure ventilated cabinets with an unrecirculated air flow away from the operator that is discharged through a HEPA (High Efficiency Particle Air) filter. The cabinet air can either be discharged into the laboratory through the filter or connected to a building ventilation system and discharged outside into the atmosphere.

Class I cabinets provide good operator protection, but do not protect the material inside the cabinet from contamination since the inward air flow from the laboratory is unfiltered.

The Class I Cabinet is designed for general microbiological research with low and moderate risk agents.

Class II Cabinets

Class II Cabinets are designed for personnel, sample and environmental protection. They are designed for work involving microorganisms in containment level 2,3, and 4 laboratories. Class II Cabinets are the types most commonly used in research laboratories.

Class II Cabinets are subdivided into four subclasses – A1, A2, B1, and B2 on the basis of their construction, airflow velocities and patterns and exhaust systems.

Some of their important characteristics and differences are summarized in Table 8.1.

TABLE 8.1
CHARACTERISTICS OF CLASS II BIOLOGICAL SAFETY CABINETS

Type A1	Type A2	Type B1	Type B2
Air is recirculated within the cabinet	Air is recirculated within the cabinet	Recirculates 30% of the air within the cabinet.	Does not recirculate air within the cabinet; 100% of cabinet air is exhausted.
Cabinet air may be exhausted back into the laboratory or to the ourdoors via the building exhaust system after passage through a HEPA filter.	Cabinet air may be exhausted back into the laboratory or to the ourdoors via the building exhaust system after passage through a HEPA filter.	Exhaust air is hard-ducted through a dedicated duct and exhausted to the atmosphere after passage through a HEPA filter.	Exhaust air is hard-ducted through a dedicated duct and exhausted to the atmosphere after passage through a HEPA filter.
May have positive pressure contaminate ducts and plenums.	Has ducts and plenums under negative pressure.	Contains negative pressure plenums.	Contains negative pressure plenums.
Maintains a minimum average face velocity of 0.38 m/s.	Maintains a minimum average face velocity of 0.5 m/s.	Maintains a minimum average face velocity of 0.5 m/s.	Maintains a minimum average face velocity of 0.5 m/s.
Not suitable for work with low levels of volatile toxic chemicals and volatile radionuclides	Is suitable for work with minute quantities of volatile toxic chemicals and trace amounts of radionuclides	Suitable for work with low levels of volatile toxic chemicals and trace amounts of radionuclides.	Suitable for work with volatile toxic chemicals and radionuclides.

Class III Cabinets

Class III Cabinets are designed for work with Level 4 pathogens and provide an alternative to the negative-pressure suit made for maximum containment laboratories. They protect both the worker and the product from contamination.

Class III Cabinets are totally enclosed and gas-tight with HEPA filtered supply and exhaust air. Work is performed with attached long-sleeved gloves. The cabinet is kept under negative pressure of at least 120 Pa and airflow is maintained by dedicated exterior exhaust system. The exhaust air is passed through two HEPA filters in series or through a single HEPA filter followed by incineration before discharge from the facility. Removal of materials from the cabinet is through either a dunk tank, a double door autoclave, or air-lock pass-through for decontamination.

8.2 Installation and Certification

Biological Safety Cabinets should be installed in accordance with the requirements outlined in the Canadian Standards Association Standard Z316.3-95 – *Biological containment cabinets (class I and II): installation and field testing.* They should be located away from high traffic areas, doors and air supply/exhaust grilles that may interrupt airflow patterns. Wherever possible a 30 cm clearance should be provided on each side of the cabinet to allow for maintenance access.

The correct operation of the biological safety cabinet must be verified before use and annually thereafter. The cabinet must also be retested after any repairs or relocation.

Certification of cabinets is carried out by an external company and is arranged annually through the University. A copy of the certification report must be kept by the user and the cabinet must bear a label indicating the date of the last certification and the name of the certifier.

Annual testing of biological safety cabinets used in Level 2 facilities is a requirement of the Biosafety Certificate issued by the University.

8.3 General Guidelines for Working in a Biosafety Cabinet

The general work guidelines provided here are from the *Laboratory Biosafety Guidelines.*

1. If the cabinet is equipped with UV lights for disinfection, turn the UV light off before working in the cabinet. UV radiation can pose a significant hazard to the skin and eyes. Turn on the fluorescent light in the cabinet.

2. Ensure the blower is turned on and allow it to run for 5-10 minutes to purge airborne contaminants from the work area before starting work.

3. Check air intake and exhaust grilles for obstructions.

4. Confirm the inward air flow by checking the flow monitor. Verify the flow by holding a tissue at the middle of the edge of the viewing panel and ensuring that it is drawn in.

5. Disinfect the interior surfaces with a suitable non-corrosive disinfectant.

6. Place essential items for the procedure in the cabinet. The working surface may be lined with absorbent paper with plastic backing if desired. Segregate “clean” items from “contaminated” items.

7. Don protective clothing and gloves as appropriate.

8. Perform operations as far to the rear of the cabinet as possible, particularly operations which could generate aerosols.

9. Avoid movement of materials or excessive movement of hands and arms through the from access opening during use. Avoid impeding the air flow into the cabinet or any movement which could cause turbulence and draw air out of the cabinet. When you do enter or exit the cabinet allow the air flow to stabilize before resuming work.

10. Keep discarded, contaminated materials to the rear of the cabinet; do not discard materials in containers outside the cabinet.

11. Do not work with open flames inside the cabinet.

12. If there is a spill during use, decontaminate the surface of all objects inside the cabinet, disinfect the working area while it is still in operation. Do not turn off the blower.

13. After completion of the work allow the cabinet to run for 5 minutes with no activity.

14. Close or cover open containers before removing them from the cabinet.

15. Surface disinfect objects in cabinet before removing them from the cabinet.

16. Remove contaminated gloves and dispose of them as appropriate. Wash hands.

17. Don clean gloves and place all potentially contaminated materials are placed in biohazard bags within the cabinet. This includes any absorbent paper used to line the work surface.

18. Using a suitable non-corrosive disinfectant (e.g. 70% ethanol) disinfect interior surfaces of the cabinet.

9.0 DECONTAMINATION

A basic principle of biosafety is that all contaminated materials must be decontaminated prior to disposal.

Decontamination includes both:

<u>Sterilization</u> – the complete destruction of all microorganisms, and

<u>Disinfection</u> – the reduction of the number of pathogenic organisms to the point where they pose no danger of disease.

Decontamination can be achieved through both physical and chemical means. The physical agents most commonly used are dry and wet heat. There are a variety of chemical disinfectants in use and the choice depends on the resistance of the microorganisms of concern.

9.1 Dry Heat

Dry heat (from ovens) is usually used to sterilize metal and glassware. It is the only suitable means of sterilizing oils and powers.

Objects are sterilized by dry heat when subjected to 170 C for 1 hour, to 160 C for 2 hours or 120 C for 16 hours.

An open flame is a form of dry heat used to sterilize inoculating loops and the mouths of culture tubes and to dry the insides of pipettes. When flaming objects in the laboratory, care must be taken to avoid the formation of floating ashes and aerosols. These can be a means of spreading infectious agents if the organisms in them are not killed by incineration.

9.2 Moist Heat/Autoclaving

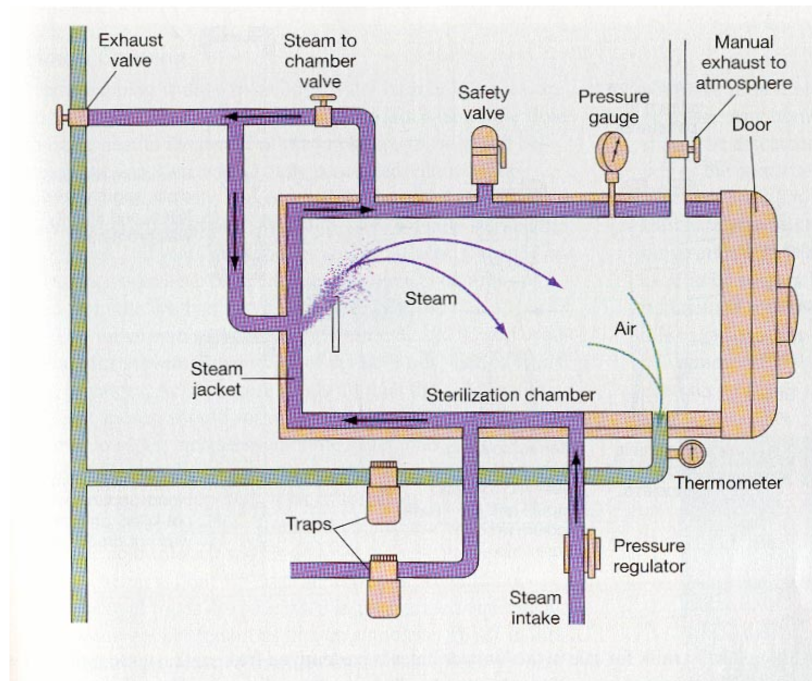
Steam autoclaving is the method of choice decontaminating cultures, laboratory glassware, pipettes, syringes, or other small items contaminated with infectious agents.

Material which cannot be autoclaved includes certain plastics, chemical or radioactive waste, animal carcasses, and large volumes of contaminated clothing.

The effectiveness of steam autoclaving depends on various factors which influence the temperature to which the material is subjected and the contact time. Attention must be paid to the packaging and size of containers, and their

distribution in the autoclave. Containers of waste must allow for steam penetration and be arranged in the autoclave to permit free circulation of steam.

Figure 9.1
A TYPICAL AUTOCLAVE



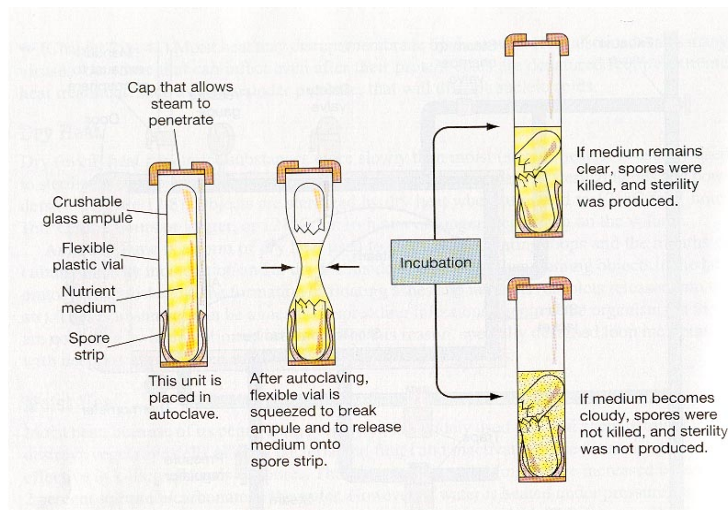
There are two major types of autoclaves – gravity displacement, where the incoming steam displaces air in the autoclave, and vacuum, where the autoclave is evacuated before steam is introduces. The former typically operates at 15 lb/in^2 pressure and 121 C, the latter at 27 lb/in^2 pressure and 132 C.

Operating parameters should be developed through the use of biological indicators with typical loads. Biological indicators should also be used on a regular basis for routine monitoring of the effectiveness of the sterilization process.

Biological Indicators

A biological indicator consists of a heat resistant endospore such as *Bacillus stearothermophilis*. These are available commercially in the form of a spore strip and an ampule of medium sealed in a soft plastic vial. The vial is placed in the centre of the material to be sterilized and is autoclaved. After the autoclave cycle is completed, the inner ampule is broken releasing the medium and the whole container is incubated. If the sterilization was effective, the spores will have been killed and no growth will occur in the medium.

Sometimes an indicator dye is added which will turn colour if microbial growth occurs due to the accumulation of acid by-products. This is faster than waiting for sufficient growth to turn the medium cloudy.



Autoclaves are pressure vessels operating at high pressures and temperatures. It is important to develop standard operating procedures and a legal requirement to have them regularly inspected and certified by trained personnel.

9.3 Chemical Disinfection

Chemical disinfectants are used for the decontamination of surfaces and equipment than cannot be autoclaved and for cleanup of spills of infectious material, and other items for which heat treatment is not feasible. Disinfectants are used on inanimate objects in contrast to antiseptics which are used on living tissue.

Selection of an appropriate disinfectant spends on the resistance of the microorganism of concern.The least resistant (or most susceptible to chemical disinfectants) are vegetative bacteria, fungi, and enveloped viruses;

mycobacteria and nonenveloped viruses are more resistant; and bacterial spores and protozoan cysts are generally the most resistant.

Disinfectants can be classified as high-level, intermediate-level and low level depending on their effectiveness.

High level disinfectants

High level disinfectants destroy vegetative bacteria, mycobacteria, fungi and enveloped (lipid) and nonenveloped (non lipid) viruses, but not necessarily bacterial spores. High level disinfectants must be capable of sterilization when contact time is extended (6 to 10 hours) but normal contact times are 10 to 30 minutes. Items must be thoroughly cleaned prior to high level disinfection.

Intermediate level disinfectants

Intermediate level disinfectants kill vegetative bacteria, most viruses and most fungi, but not resistant bacterial spores. They are commonly used to disinfect laboratory benches and as part of detergent germicides used for housekeeping purposes.

Low level disinfectants

Low level disinfectants kill most vegetative bacteria and some fungi as well as enveloped (lipid) viruses (e.g. hepatitis B, C, hantavirus and HIV). Low level disinfectants do not kill mycobcteria or bacterial spores. Low level disinfectants are typically used to clean environmental surfaces.

The selection of a disinfectant should be selected based on the function that it is expected to perform. Ideally a disinfectant should be broad spectrum (eliminating as wide a range of organisms as possible), nonirritating, nontoxic, noncorrosive and inexpensive.

Major factors which influence the effectiveness of a disinfectant are:

1. the type of contaminating microorganism.
2. the amount of contamination.
3. the amount of protein present; high protein based materials absorb and neutralize some chemical disinfectants,
4. the presence of organic matter and other compounds such as soaps which may neutralize some disinfectants,
5. the chemical nature and mode of action of the disinfectant,
6. the concentration and quantity of the disinfectant, and

7. the contact time and temperature; sufficient time and appropriate temperature must be allowed for action of the disinfectant and may depend on the degree of contamination and organic matter load.

Some high-level disinfectants are glutaraldehydes, chlorine, formaldehyde and hydrogen peroxide, Some intermediate level ones are ethyl and isopropyl alcohols, chlorine, phenol and iodophors. Some low level ones are hydrogen peroxide and quaternary ammonium compounds.

When selecting a disinfectant for any particular organism one should consult the relevant material safety data sheets(http://www.phac-aspc.gc.ca/msds-ftss/index.html#menu).

The two most common disinfectants used in laboratories are alcohol and chlorine.

Alcohols

Ethyl and isopropyl alcohol are excellent disinfectants that are commonly available and inexpensive. The optimal concentration range is 60 – 90% solutions with water. Methyl alcohol is not as effective a disinfectant and it should not be used alone as an antiseptic or disinfectant.

Ethyl and isopropyl alcohol are not high-level disinfectants as they do not inactivate bacterial endospores and some viruses. They are effective against Hepatitis-B, C and HIV.

Alcohols are flammable and evaporate rapidly, making extended contact time difficult unless the item to be decontaminated is immersed in the solution.

Chlorine

Hypochlorites are the most widely used of the chlorine disinfectants and are available in liquid (sodium hypochlorite) and solid (calcium hypochlorite and sodium dichloroisocyanurate) forms.

Chlorine solutions are high level disinfectants because they inactivate all bacteria, viruses, fungi, parasites and some spores. They are fast-acting, very effective against Hepatitis-B, C and HIV, and are inexpensive and readily available. Household bleach is a 4-6% aqueous solution of sodium hypochlorite (approximately 50,000 ppm free chlorine).

When potable (clean water is available, a 0.1% chlorine solution is satisfactory for high-level disinfection; if the chlorine is diluted with contaminated (unfiltered) water, a higher concentration (0.5%) should be used because most of the chlorine will be inactivated by the microscopic organic material in the water.

Chlorine disinfectants diluted in tap water can loose their potency on standing if left in an open container and have a limited shelf-life. After 30 days solutions stored in a polyethylene container can loose 40-50% of their concentration. Ideally solutions for surface decontamination should be made fresh.

Table 8.1 gives directions for preparing and using chlorine-based disinfectants. (taken from BC Centre for Disease Control, BCCDC Laboratory Services, Guide to Selection and use of Disinfectants).

TABLE 9.1
DIRECTIONS FOR PREPARING AND USING CHLORINE-BASED DISINFECTANTS

Product	Intended Use	Dilution	Available Chlorine
Household Bleach (5% sodium hypochlorite with 50,000 ppm)	Cleanup blood spills (contact time > 10 minutes)	1 part bleach to 9 parts water	0.5% 5000 ppm
	Surface disinfection (contact time > 5 minutes)	1 part bleach to 50 parts water	0.1% 1000 ppm
	Food surfaces (contact time > 2 minutes)	1 part bleach to 200 parts water	0.025% 200 ppm
	Instruments.surfaces contaminated with tissue infective for Creutzfeld-Jakob disease (contact time 1 hour) (Instruments require sterilization following disinfection)	1 part bleach to 1 part water or undiluted	2.5-5% 20,000 to 50,000 ppm
Sodium dichloroisocyanurate (NaDCC) powder with 60% available chlorine	Cleanup blood spills	Dissolve 8.5 g in 1 litre of water	0.85% or 5000 ppm
Chloramine-T powder with 25% available chlorine	Cleanup blood spills	Dissolve 20 g in 1 litre of water	2% or 5000 ppm

10.0 WASTE MANAGEMENT

Disposal of biohazardous waste requires well-defined procedures to prevent exposure to infectious agents. Detailed procedures are provided in the University Biosafety Manual.

Biohazardous waste can generally be categorized as being of three types:

- Non-anatomical (glassware, sharps, absorbent paper)
- Anatomical (tissues, organs, body parts, animal carcasses)
- Cytotoxic (chemotherapy waste, items contaminated with chemotherapy drugs)

Of these three categories, only the non-anatomical waste can be autoclaved, the other two types must be collected and incinerated.

The following general principles are applicable to the disposal of infectious biohazardous wastes.

1. “Sharp” waste must be handled carefully and segregated into separate containers. Great care must be taken to avoid puncture or needlestick injuries.
2. Non-anatomical wastes must be collected in the appropriate containers and sterilized or disinfected before disposal as non-hazardous waste.
3. Generally, steam autoclaving is the preferred method, however waste containing significant amounts of chemicals or radioactive material cannot be autoclaved and must be chemically disinfected.
4. Anatomical and cytotoxic waste must be disposed of through an external company which is licensed by the Ministry of Environment to dispose of this type of waste.

For detailed procedures consult the University Biosafety Manual or the laboratory specific manual.

11.0 EMERGENCY PROCEDURES

Each laboratory should develop and implement a plan to handle accidental spills of infectious material or releases of infectious microorganisms into the laboratory. The details of the plan will depend on the infectious agent, the magnitude of the spill and whether an aerosol is generated.

Minor spills can be handled immediately by cleaning up with a suitable disinfectant, while very large spills or aerosols may require shutdown of the ventilation system and decontamination of the entire room.

The basic steps to follow in any emergency spill are:

- prevent exposure of personnel
- apply first aid if needed
- contain the spill
- decontaminate/disinfect
- cleanup
- disposal
- report the spill to the laboratory supervisor
- provide followup medical care with any exposed personnel depending on the agent

11.1 Spills in General Laboratory Areas

- Immediately notify other persons in the lab of the spill and evacuate the area
- If the spill is small enough cover it with absorbent material
- Keep the area evacuated for at least 30 minutes to allow any generated aerosols to settle
- Assemble supplies and protective equipment
- Add disinfectant and allow sufficient time for the disinfectant to work before proceeding
- Pick up any broken glass or sharps material with forceps or tongs and place in a sharps container
- Place absorbent material in biohazardous waste and wipe down spill area again with disinfectant
- Wipe down adjacent areas beginning with the adjacent area and moving towards the spill area
- Package and autoclave all waste

11.2 Spills within a Biological Safety Cabinet

- Leave the ventilation running for at least 10-15 minutes to clear any aerosols
- Cover the spill with absorbent material
- Soak the spill area with an appropriate disinfectant and let stand a sufficient time for the disinfectant to work before proceeding
- Pick up any broken sharps with forceps or tongs and place in a sharps container
- Place absorbent material in a biohazardous waste bag
- Disinfect all materials in the cabinet and wipe down interior surfaces again with disinfectant
- Package and autoclave all waste

11.3 Spills within a Centrifuge

- Close the centrifuge lid and allow any aerosols to settle for at least one hour
- Ensure the centrifuge is turned off, disconnect the power and place a warning sign on the centrifuge
- Using forceps or tongs, remove any samples and/or intact or broken glassware into a container of disinfectant
- Disinfect centrifuge rotors, and buckets in an appropriate disinfectant; allow sufficient contact time for the disinfectant to work
- Thoroughly wipe down the inside of the centrifuge and all parts, including the lid; use paper towels soaked in disinfectant
- Rinse all parts with water
- Package and autoclave all waste

11.4 Emergency Medical Procedures

Emergency medical procedures apply when any person is exposed to blood or body fluids, infections or communicable disease or zoonotic agents. Exposure may be via a needlestick, cut or puncture wound, animal bite or scratch, mucous membrane contact or non-intact skin contact.

- Wash the exposed site immediately.
- If a needlestick, cut, puncture, animal bite or scratch, wash with soap and water after allowing the wound to bleed freely.
- If mucous membrane contact (eyes, nose, mouth), or non-intact skin contact (cuts, rash or dermatitis), flush with water and the nearest faucet or eye wash station.
- Immediately inform the laboratory supervisor.

- Seek prompt medical attention, giving the medical provider details on the agent.
- Complete the accident/incident report.

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