Global Health Development

EMPHNET
The Eastern Mediterranean Public Health Network

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TRAINING AND EDUCATIONAL MATERIALS ON BIOSAFETY AND BIOSECURITY

The Biological Risk Management Handbook

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This Handbook has been produced in collaboration with the Iraqi Ministry of Higher Education and Scientific Research and Ministry of Health

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Foreword

Global Health Development | Eastern Mediterranean Public Health Network (GHD | EMPHNET) has long recognized the importance and the critical need for biosafety and biosecurity in the EMR .In response, GHD | EMPHNET collaborated with Iraq Ministry of Higher Education (MOHE) and Ministry of Health (MOH), to develop this handbook to acknowledge and implement basic concepts in biological safety and to develop national codes of practice for the safe handling of pathogenic microorganisms in laboratories.

On behalf of GHD | EMPHNET, I would like to express my great pleasure to introduce the first Iraqi national Biorisk Management Handbook. The Handbook provides technical guidance to all personnel who work in biological laboratories and who actively handle or manage biological agents and toxins, as well as other valuable laboratory materials. The document describes many aspects of Biorisk Management including general Laboratory Design Features, Roles and Responsibilities within an Institution, Pathogen Hazards, Bio-Risk Assessment and Management, Laboratory Design: Containment Levels 1-4Laboratory, Laboratory Associated Infections (LAIs), Biosafety Cabinets (BSCs), Biohazardous or Infectious Waste and Regulated Medical Waste, Disinfection and Sterilization, Personal Protective Equipment (PPE), Good laboratory Practice (GLP), Spill Procedures, Laboratory Emergencies, Shipping of Infectious Substances, Occupational Health and Medical Surveillance, Bloodborne Pathogens Program and Exposure Control Plan, Bioethics and Dual Use Research of Concern and Biosecurity.

On this occasion, I would like to thank the Iraqi MOHE, MOH and representatives from health and academic institutions for their enormous efforts in developing and reviewing the materials especially with the difficult situation worldwide due to COVID-19 pandemic. My gratitude and appreciation are also extended to the consultants for their efforts in developing chapters of the handbook.

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Introduction

Infectious microorganisms remain a source of infection, and even mortality, among laboratory workers. There is a steady increase in both the number of laboratories handling pathogens and in the number of scientists wishing to import new or exotic strains for further study. The release of human and animal pathogens and toxins from laboratories or other containment zones may pose a risk to public health, animal health, or both. Consequently, the handling or storing of infectious material or toxins necessitates an awareness and application of biosafety and biosecurity practices among laboratory personnel and those who work with pathogens, toxins, or infected animals in containment zones. Increasing demands are also being placed on regulatory authorities to ensure that such pathogens are handled in a safe and secure manner.

Risks associated with infectious material or toxins can be minimized through the application of appropriate biosafety and biocontainment principles and practices. "It is good for you; it is good for the science and it is the law!"

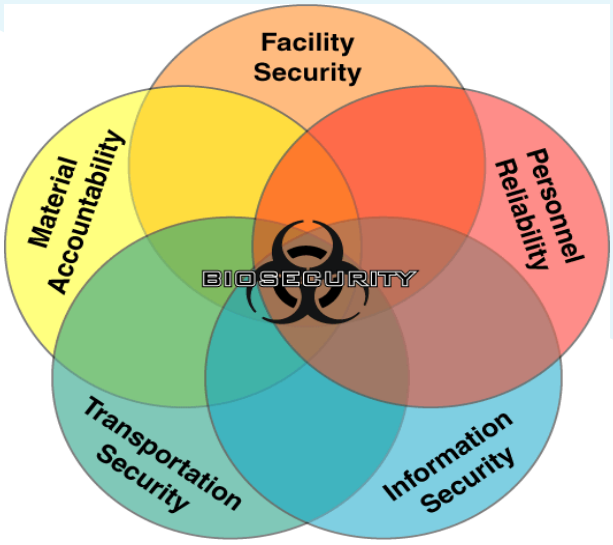
To be safe and compliant it is quite easy, it is all about diligence, knowing who is responsible, knowing your risk and practicing good laboratory practice. It is necessary to remain vigilant and alert to hazards that may be in the laboratory; complacency can lead to laboratory-associated infections.

Glossary

Laboratory Biosafety: containment principles, technologies, and practices implemented to prevent unintentional exposure to pathogens and toxins, or their unintentional release (Laboratory Biosafety Manual, Third edition, WHO, 2004).

Reference: Sandia National Laboratories Albuquerque, NM, USA

Laboratory Biosecurity: protection, control, and accountability for valuable biological materials within laboratories, in order to prevent their unauthorized access, loss, theft, misuse, diversion, or intentional release (Laboratory Biosecurity Guidance, WHO, 2006).



Reference: Sandia National Laboratories Albuquerque, NM, USA

Biologic Terrorism: Use of biologic agents or toxins (e.g., pathogenic organisms that affect humans, animals, or plants) for terrorist purposes.
Biohazard: A biological agent or a condition that constitutes a threat to humans, especially in biological work, research, or experimentation. The potential danger, risk, or harm from exposure to such an agent or condition.

- Viruses, bacteria, fungi, and parasites and their toxins.
- Blood and body fluids, as well as tissues from humans and animals.
- Transformed cell lines and certain types of nucleic acids.
- A potential hazard to humans, animals or the environment caused by a biological organism, or by material produced by such an organism

Risk: A measure of the potential loss of a specific biologic agent of concern, based on the probability of occurrence of an adversary event, effectiveness of protection, and consequence of loss.

Risk Assessment: An analysis of the probability and the consequences of loss, theft and potential misuse of pathogens and toxins.

Bioethics: The study of the ethical and moral implications of biological discoveries, biomedical advances, and their applications as in the fields of genetic engineering and drug research. Bioethics is one of the three components that contribute to a successful biorisk management culture.

Code of Conduct, Code of ethics & Code of Practice: Non-legislated guidelines that one or more organizations and individuals voluntarily agree to abide by, that set out the standard of conduct or behavior with respect to a particular activity.

Accountability: Accountability ensures that valuable biological materials (VBM, see definition below) are controlled and traced as intended, by formally associating the specified materials with the individuals who provide oversight and are held responsible for them.

Vulnerability: An exploitable capability, security weakness, or deficiency at a facility. Exploitable capabilities or weaknesses are those inherent in the design or layout of the biologic laboratory and its protection, or those existing because of the failure to meet or maintain prescribed security standards when evaluated against defined threats.

Vulnerability Assessment: A systematic evaluation process in which qualitative and quantitative techniques are applied to arrive at an effectiveness level for a security system to protect biologic laboratories and operations from specifically defined acts that can oppose or harm a person's interest.

Threat: The capability of an adversary, coupled with intentions, to undertake malevolent actions.

Threat Assessment: A judgment, based on available information, of the actual or potential threat of malevolent action.

Select Agent: The possession, use, and transfer of select agents and toxins that have the potential to pose a severe threat to public health and safety are regulated.

Genetically Modified Organisms (GMOs): Organisms whose genetic material has been altered using techniques generally known as "recombinant DNA technology". Recombinant DNA technology is the ability to combine DNA molecules from different sources into one molecule in a test tube. GMOs are often not reproducible in nature, and the term generally does not cover organisms whose genetic composition has been altered by conventional cross-breeding or by "mutagenesis" breeding, as these methods predate the discovery (1973) of recombinant DNA techniques.

Valuable Biological Materials (VBMs): Biological materials that require (according to their owners, users, custodians, caretakers or regulators) administrative oversight, control, accountability, and specific protective and monitoring measures in laboratories to protect their economic and historical (archival) value, and/or the population from their potential to cause harm. VBM may include pathogens and toxins, as well as non-pathogenic organisms, vaccine strains, foods, genetically modified organisms (GMOs), cell components, genetic elements, and extraterrestrial samples.

Biological Safety Officer or Biosafety Responsible Official: Individual responsible for developing and implementing biological hazard monitoring and controlling programs in the workplace. Serves as liaison with state and federal regulatory agencies, campus health services, public safety and provides assistance and emergency response for incidents involving biological and chemical hazardous materials and regulated medical waste.

Institutional Biosafety Committee: It develops institutional biosafety policies and codes of practice. It also reviews research protocols for work involving infectious agents, animal use, recombinant DNA, and genetically modified materials. Other functions of the committee may include risk assessments, formulation of new safety policies and arbitration in disputes over safety matters. The membership of the biosafety committee should reflect the diverse occupational areas of the organization as well as its scientific expertise.

Principal Investigator: The individual responsible for the conduct of the project. This responsibility includes the intellectual conduct of the project, fiscal accountability, administrative aspects, and the project's adherence to relevant policies and regulations.

Decontamination: Free of contamination, the destruction of microorganisms to a lower level such that it removes danger of infection to individuals.

Sterilization: The complete destruction of all viable microorganisms.

Disinfection: Use of agents (physical or chemical) to destroy harmful organisms on inanimate objects (not necessarily all organisms).

Biomedical Waste: Discarded biological material from teaching, clinical and research laboratories, and operations. Biomedical waste includes but is not limited to animal waste, biological laboratory waste, human anatomical waste, human blood and body fluid waste and sharps.

Containment: Describes safe methods for managing infectious agents in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other people, and the outside environment to potentially hazardous agents. The three elements of containment include laboratory practice and technique, safety equipment, and facility design.

Ignorance: not knowing how to do things or what to do.

Incompetence: not being able to do things.

Negligence: Conscious failure to do what should be done.

General Laboratory Design Features

1. Ample space must be provided for the safe conduct of laboratory work and for cleaningandmaintenance.
2. Walls, ceilings, and floors should be smooth, easy to clean, impermeable to liquids and resistant to the chemicals and disinfectants normally used in the laboratory. Floorsshouldbeslip-resistant.
3. Bench tops should be impervious to water and resistant to disinfectants, acids, alkalis,organic solvents, and moderate heat.
4. Illumination should be adequate for all activities. Undesirable reflections and glare should be avoided.
5. Laboratory furniture should be sturdy. Open spaces between and under benches, cabinets and equipment should be accessible for cleaning.
6. Storage space must be adequate to hold supplies for immediate use and thus prevent clutter on bench tops and in aisles. Additional long-term storage space, conveniently located outside the laboratory working areas, should also be provided.
7. Space and facilities should be provided for the safe handling and storage of solvents,
8. Facilities for eating and drinking and for rest should be provided outside the laboratory working areas.
9. Hand-washing basins, with running water if possible, should be provided in each laboratory room, preferably near the exit door.
10. Doors should have vision panels, appropriate fire ratings, and preferably be self-closing.
11. At Biosafety Level 2, an autoclave or other means of decontamination should be available in appropriate proximity to the laboratory.
12. Safety systems should cover fire, electrical emergencies, emergency showers and eyewash facilities.
13. First-aid areas or rooms suitably equipped and readily accessible should be available
14. In the planning of new facilities, consideration should be given to the provision of mechanical ventilation systems that provide an inward flow of air without recirculation. If there is no mechanical ventilation, windows should be able to be opened and should be fitted with arthropod-proof screens.

15. A dependable supply of good quality water is essential. There should be no cross connections between sources of laboratory and drinking-water supplies. An anti-Black flow device should be fitted to protect the public water system.
16. There should be a reliable and adequate electricity supply and emergency lighting to permit safe exit. A stand-by generator is desirable for the support of essential equipment, such as incubators, biological safety cabinets, freezers, etc., and for the ventilation of animal cages.
17. There should be a reliable and adequate supply of gas. Good maintenance of the installation is mandatory.
18. Laboratories and animal houses are occasionally the targets of vandals. Physical and fire security must be considered. Strong doors, screened windows and restricted issue of keys are compulsory. Other measures should be considered and applied, as appropriate, to augment security.
19. Finally, a culture of biosafety and biosecurity should be built within the Institution.

General Laboratory Safety Guidelines

People who work in scientific laboratories are exposed to various hazards. Most workplaces have hazards that are well recognized (those of ordinary fire, for example) with well-defined actions to control the situation.
Laboratories, however, involve a greater variety of possible hazards and some of these hazards need precautions not ordinarily encountered.

Electrical safety

The typical laboratory requires a large quantity of electrical power. This increases the likelihood of electrically related problems and hazards. One must address both the electrical shock hazard to the facility occupants and the fire hazard potential.
The major hazards associated with electricity are electrical shock and fire. Electrical shock occurs when the body becomes part of the electric circuit, either when an individual comes in contact with both wires of an electrical circuit, one wire of an energized circuit and the ground, or a metallic part that has become energized by contact with an electrical conductor.

The severity and effects of an electrical shock depend on a number of factors, such as the:

a. Pathway through the body
b. The amount of current
c. The length of time of the exposure
d. Whether the skin is wet or dry.

Water is a great conductor of plug electricity, allowing current to flow more easily in wet conditions and through wet skin. The effect of the shock may range from a slight tingle to severe burns to cardiac arrest.

In addition to the electrical shock hazards, sparks from electrical equipment can serve as an ignition source for flammable or explosive vapors or combustible materials.

The following general principles should be applied to electrical safety

- All electrical outlets must be earthed
- Where possible individual items of electrical equipment should be earthed
- Electrical loads should be regularly checked
- All electrical equipment must be evaluated regularly.
- Regular "on load" testing of generators should be performed.
- Electrical cords or other lines shall not be suspended unsupported across rooms or passageways.
- Do not route cords over metal objects such as emergency showers, overhead pipes or frames, metal racks, etc.
- Do not run cords through holes in walls, ceilings, doorways, or windows. Do not place under carpet, rugs, or heavy objects.
- Do not place cords on pathways or other areas where repeated abuse can cause deterioration of insulation.
- Multi-outlet plugs shall not be used unless they have a built-in circuit breaker. This causes overloading on electrical wiring, which will cause damage and possible overheating.
- Only qualified and trained people should repair or modify electrical or electronic equipment.
- If at any time, there is any concern regarding electrical equipment for electrical safety you must contact the Biosafety Officer or your laboratory manager/ supervisor. If you or anyone else has received an electric shock from any item of equipment, no matter how small, make sure that no one else touches it until it has been investigated further.

Never try to repair any electrical equipment or tamper with electrical equipment if you suspect that there is a problem.

Fire Safety

The fire triangles or combustion triangles are simple models for understanding the necessary ingredients for most fires. The triangle illustrates the three elements a fire needs to ignite: heat, fuel, and an oxidizing agent (usually oxygen). A fire naturally occurs when the elements are present and combined in the right mixture. A fire can be prevented or extinguished by removing any one of the elements in the fire triangle. For example, covering a fire with a fire blanket removes the oxygen part of the triangle and can extinguish a fire.

Figure1: The fire triangle

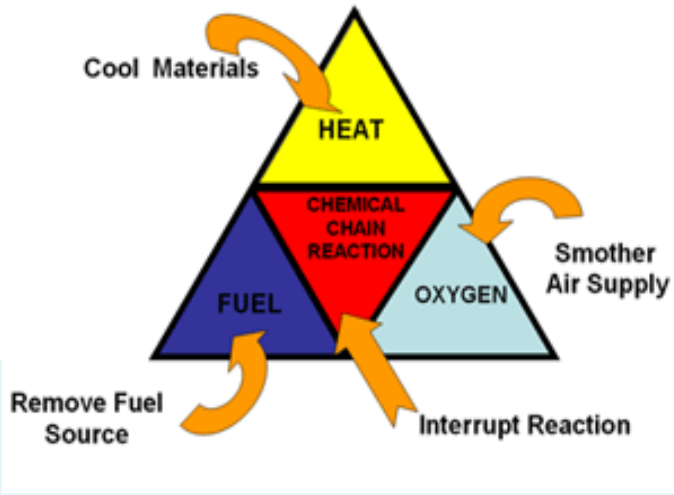
Fire classes

There are different types of fire. Before extinguishing a fire, determine what exactly is burning. All combustible substances are divided into groups (classes). These fire classes range from A (solids) to F (oils and fats). see figure below:

CLASSES OF FIRES	TYPES OF FIRES	SYMBOL
A	Wood, paper, fabric, plastic, and most kinds of trash.	
B	Flammable **liquids** (for example,gasoline).	
C	Burning **gases** (for example,natural gas).	
D	Combustible **metals*** such as magnesium, potassium, titanium, and zirconium. * Exception of the metals that burn in contact with air or water (for example, sodium).	
E	Fires involving potentially energized **electrical equipment**.	
F	Unsaturated **cooking oils** in well insulated cooking appliances located in commercial kitchens.	

“Fire Extinguishers”

Not all fires are the same. Different fuels create different fires and require different types of fire extinguishing agents. A potential fire can often be controlled before it really takes hold, if the right firefighting equipment is used.
Fire extinguishers put out fire by taking away one or more elements of the fire triangle / tetrahedron.



Extinguishers categories

Different types of fire extinguishers are designed to fight different classes of fire. It is important to know what types of fire you can attempt to extinguish with them. Each facility is required at the minimum.
Fire extinguishers are located in every lab or directly outside each lab. Fire extinguishers are serviced annually, and inspections are documented on the hang tags. Personnel who use the lab on a regular basis, or other specifically assigned personnel should conduct a visual inspection of each unit to confirm that they are present and that the pressure gauge is in the green, operational zone. There should be nothing placed in front of fire extinguishers so that they may be easily accessed. Facility personnel should be trained on how to use the extinguisher and the evacuation plan.
Below is an overview of each type of fire extinguisher including what fire classes they tackle.

Water fire extinguishers	AFFF Foam fire extinguishers	Carbon Dioxide (CO2) fire extinguishers
		
They are suitable for fighting **Class A** fires (combustible solid materials) . Remember water conducts electricity and SHOULD NOT be used on electrical equipment. They all have a red label. 	They are highly effective on **Class B** fires (flammable liquids) such as petrol. The foam works to cover and seal the surface of the flammable liquid. This stops the vapour from reaching the air, preventing re-ignition. They also work well on **Class A** fires. They all have a cream label 	It was originally designed for use of flammable liquids therefore is highly suited for **Class B** fires. It is also extremely effective on **electrical fires** as co2 is not a conductor. Carbon Dioxide smothers the fire cutting off the oxygen supply. Carbon Dioxide extinguishers leave behind no harmful residue. They all have a black label 

ABC Powder extinguisher	Wet Chemical fire extinguishers	Water Mist fire extinguishers
		
It is so named due to the fact it can be used on **Class A, Class B** and **Class C** fires, as well as **electrical**. It is available in a range of sizes and ideal for environments containing mixed fire risks. It is not recommended for use within small rooms due to the risk of inhalation and loss of vision. Note. There is a special dry powder extinguisher which is used specifically for Class D Fires. 	They are the most effective against Class F fires (cooking oils and fats) e.g fats, grease and oil. Therefore they are practical in a kitchen environment. The wet chemical rapidly extinguishes the flames, cools the burning oil and chemically reacts to form a soap-like solution, sealing the surface and preventing reignition. They can also be used on Class A fires. 	They tackle Class A, Class B and Class C fires, rated risks as well as Class F and Electrical fires. Water Mist is a new technology that works on the basis of cooling fire, suffocating it and then cooling the burning media to prevent re-ignition using microscopic particles of water. Water mists extinguishers are ideal for covering areas of a building where multiple fire risks can be found. 

How to Use a Fire Extinguisher

It is easy to remember how to use a fire extinguisher if you can remember the acronym PASS,

which stands for Pull, Aim, Squeeze, and Sweep.

i. Pull the pin-This will allow you to discharge the extinguisher.

ii. Aim at the base of the fire- If you aim at the flames (which is frequently the temptation), the extinguishing agent will fly right through and do no good. You want to hit the fuel at the base of the fire.

iii. Squeeze the top handle or lever- This depresses a button that releases the pressurized extinguishing agent in the extinguisher.

iv. Sweep from side to side- Until the fire is completely out. Start using the fire extinguisher from a safe distance, then move forward. Once the fire is out, keep an eye on the area in case it reignites

Fire evacuation plan

In case of fire alarm triggering, all personnel have to stop immediately their work, leave all their belongings behind and proceed calmly to the evacuation of the building to gather on the parking.

Assisting other personnel

In summary one shall use **"RACE"** which is an acronym for:

R=Rescue persons in danger (this can be done without putting your life in danger.

A=Activate or sound alarm

C=Confine the fire by closing doors and windows

E=Extinguish the fire with the nearest fire appropriate fire extinguisher

Never Fight A Fire If:

i. **You do not know what is burning.** If you do not know what is burning, you don't know what type of extinguisher to use. Even if you have an ABC extinguisher, there may be something in the fire, which is going to explode or produce highly toxic smoke.

ii. **The fire is spreading rapidly beyond the spot where it started.** The time to use an extinguisher is in the incipient, or beginning, stages of a fire. If the fire is already spreading quickly, it is best to simple evacuate the building, closing doors and windows behind you as you leave.

iii. **You do not have adequate or appropriate equipment.** If you do not have the correct type or large enough extinguisher, it is best not to try to fight the fire.

iv. **You might inhale toxic smoke**. If the fire is producing large amounts of smoke that you would have to breathe in order to fight it, it is best not to try. Any sort of combustion will produce some amount of carbon monoxide. These gases can be fatal in very small amounts.

v. **Always keep an exit at your back.**

Chemical safety

Workers in microbiological laboratories are not only exposed to pathogenic microorganisms, but also to chemical hazards. It is important that they have proper knowledge of the toxic effects of these chemicals, the routes of exposure and the hazards that may be associated with handling and storage. safety data sheets (SDS), which describe the hazards associated with the use of a given chemical, are available from chemical manufacturers and/or suppliers.

The SDS is provide an easy to understand, standardized document that informs the user of important information regarding the material. The SDS is a 16-section document containing details on properties, hazards, storage and transport, regulatory status, protective measures, and emergency procedures. These should be accessible in laboratories where these chemicals are used. All laboratories should have in place a chemical hygiene plan and "Hazard Communication.

The "Right-to-Know" law as prescribed in the OSHA, 2007 states that every employee has a right to know the properties and potential safety and health hazards of substances to which they may be exposed. The goals of Right to Know are to help reduce the risks involved in working with hazardous materials/chemical, to transmit vital information to employees about real and potential hazards of substances in the work place, to reduce the incidence and cost of illness and injury resulting from hazardous substances.

Routes of Exposure

Exposure to hazardous chemicals may occur by:

1. Inhalation: Chemicals may cause irritation, sensitization, allergic reactions, respiratory disease, or cancer.
2. Contact: Contact with skin may cause chemical burns, conjunctivitis of the eyes, or systemic poisoning.
3. Ingestion: Hazardous chemicals may be accidentally swallowed via mouth pipetting, or contamination of food or drinks.
4. Through broken skin: Hazardous chemicals may enter the body via cuts, abrasions or needle-sticks.

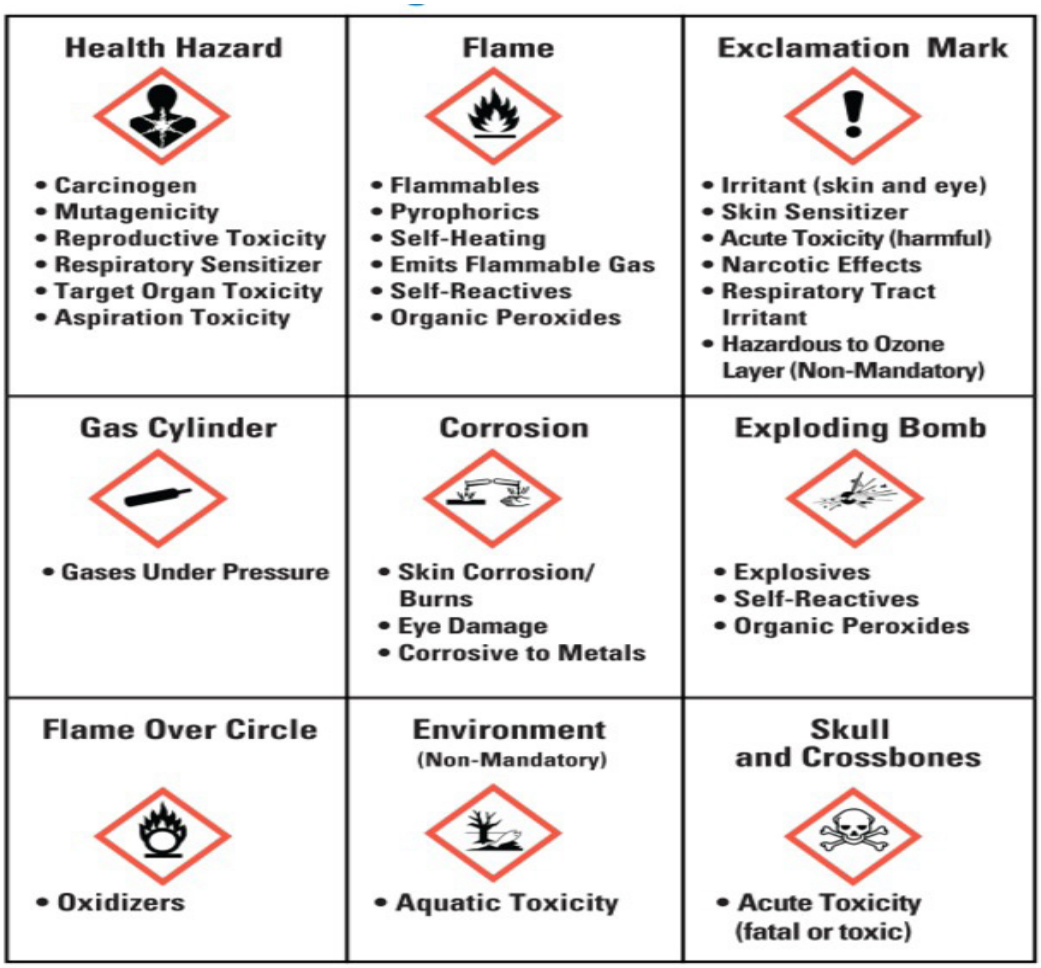
Chemical Segregation

As many as a quarter of all chemical incidents occur because of incorrect storage, leading to reactions between substances. These incidents are easily preventable if safety and chemical segregation rules are carefully followed. It is important to ensure that all members of the laboratory team have up-to-date training on the proper storage of chemicals and how to achieve safe chemical segregation. It is important to identify incompatible chemicals in the laboratory and design the chemical storage to suit the substances that work with.

Incompatible chemicals are substances that produce a reaction when they come into contact with each other. In an uncontrolled environment, a reaction can cause serious damage or even be fatal. Chemicals that react to produce heat, pressure, fire, explosion, or another type of violent reaction are deemed to be incompatible and should be stored carefully to prevent uncontrolled mixing. Basic hazard groups include:

1. Acids
2. Bases
3. Flammables
4. Oxidizers
5. Toxics
6. Peroxide forming chemicals
7. Pyrophoric forming substances
8. Water reactive chemicals
9. Explosives

Below the hazard standard pictograms



Below are some common hazard groups to segregate:

10. No acids with bases
11. No bases with acids
12. No acids or bases with flammables
13. No oxidizers near compressed flammable gases

- Incompatible chemicals must not be stored near each other. In an emergency situation of a fire, earthquake or a spill, incompatible chemicals could mix and react to cause toxic fume production or an explosion.

Handling and Transportation of Chemicals

Many laboratory accidents occur by carrying chemicals from one place to another or transferring them from one container to another. The chemicals used in a laboratory are often corrosive, toxic or flammable and any accident involving these has the potential for personal injury. Therefore, it is good practice to assume that all chemicals are potentially hazardous.

1. When large bottles of acids, solvents, or other liquids are transported within the laboratory without a cart, only one bottle should be carried at a time. The bottle should be carried with both hands, one on the neck of the bottle and the other underneath. Do not hook a finger through the glass ring on top of the bottle, allowing it to dangle while being transported. Never carry or attempt to pick up a bottle by the cap.
2. Large quantities of concentrated mineral acids, e.g., sulfuric, nitric and hydrochloric acids, shall be kept in specific storage rooms or cabinets for corrosive substances.

Storage of chemicals

Chemicals are major cause of hazards at health care settings. In this regard they shall be carefully separated and grouped before storage. Chemical information is outlined in the Material Safety Data Sheet (MSDS). The grouping and storage should be based on the major chemical classes.

- Only amounts of chemicals necessary for daily use should be stored in the laboratory.
- Bulk stocks should be kept in specially designated rooms or buildings.
- Chemical containers must be labeled with a minimum of chemical name, hazard warnings and target organs.
- Containers shall be dated when received and opened. Accompanying material safety data sheets must be kept.
- Store chemicals based on compatibility and not in alphabetical order.
- Do not store chemicals above the eye level.
- Store larger containers on lower shelves.
- Avoid storing chemicals in aisle ways.
- Store separately acids from bases.
- Use secondary catch basins for concentrated acids and bases.
- Keep corrosives away from organic chemicals and combustible materials.
- Store chemicals away from sources of heat (e.g., ovens or steam pipes) and direct sunlight.
- Store liquids inside chemically resistant secondary containers (such as trays or tubs) that are large enough to hold spills.
- Store volatile toxic and odorous chemicals in a way that prevents release of vapors (e.g., inside closed secondary containers, ventilated cabinets, paraffin sealing).
- Store flammables requiring refrigeration in laboratory-safe refrigerators.
- Never pipette by mouth. Always use a bulb to pipette.
- Do not store food in chemical storage refrigerators.
- Label chemical storage refrigerators with the following: No Food – Chemical Storage Only.
- Label reactive or unstable chemicals (e.g., ethers) with the date of receipt and the date opened.
- Develop a chemical inspection plan/procedure for every facility.
- Use chemicals on rotational basis (Old ones first before the new batches).

- Out-of-date chemicals shall be disposed of on a periodic basis to reduce overall hazard potential and minimize inventory tracking and updating.
- Chemical containers that have been emptied have to be cleaned off remaining residues by triple rinsing with water or other suitable solvent and air-dried before disposal.

Storage of compressed and liquefied gases

Compressed gas cylinders and liquefied gas containers should be securely fixed (e.g. chained) to the wall or a solid bench. It should be stored in bulk in an appropriate facility at some distance from the laboratory. This area should be locked and appropriately identified. Should not be placed near radiators, open flames other heat sources, sparking electrical equipment, or in direct sunlight. Small, single-use gas cylinders must not be incinerated.
The following risk control measures must be used when moving gas cylinders:

- only properly trained personnel are permitted to move gas cylinders.
- only purpose-built and serviceable trolleys are to be used for gas cylinder transportation.
- gas cylinder isolation valves are to be closed or isolated and not leaking prior to movement; and
- all associated distribution equipment is to be disconnected and removed before moving the cylinder.

Table 1 - Examples of incompatible combinations of some commonly used chemicals.

Chemical	Keep from contact with:
Acetic Acid	chromic acid, nitric acid, hydroxyl compounds, perchloric acid, peroxides, permanganate
Acetylene	chlorine, bromine, copper, fluorine, silver, mercury
Alkali Metals	water, chlorinated hydrocarbons, carbon dioxide, halogens
Ammonia, Anhydrous	mercury, chlorine, calcium hypochlorite, iodine, bromine, hydrofluoric acid

Chemical	Keep from contact with:
Ammonium Nitrate	acids, metal powders, flammable liquids, chlorates, nitrites, Sulphur, finely divided combustible materials
Aniline	nitric acid, hydrogen peroxide
Carbon, Activated	calcium hypochlorite, all oxidizing agents
Chlorates	ammonium salts, acids, metal powders, sulphur, finely divided combustible materials
Chromic Acid	acetic acid, naphthalene, camphor, glycerin, turpentine, alcohol, flammable liquids
Chlorine, Bromine	ammonia, acetylene, butadiene, butane, methane, propane (or other petroleum gases), hydrogen, sodium carbide, turpentine, benzene, finely divided metals
Flammable Liquids	ammonium nitrate, inorganic acids, hydrogen peroxide, sodium peroxide, halogens
Hydrogen Peroxide	copper, chromium, iron, most metals or their salts, alcohols, acetone, aniline, nitromethane, flammable liquids, oxidizing gases
Nitric Acid	acetic acid, aniline, chromic acid, hydrocyanic acid, hydrogen sulphide, flammable liquids, flammable gases
Sodium Peroxide	alcohol, glacial acetic acid, acetic anhydride, benzaldehyde, carbon disulphide, glycerin, ethylene glycol, ethyl acetate, methyl acetate, furfural
Sulphuric Acid	potassium chlorate, potassium perchlorate, potassium permanganate (or compounds with similar light metals, such as sodium, lithium, etc.)

Roles and Responsibilities within an Institution

Everyone entering or using a laboratory has responsibility for safety. These responsibilities ensure the safety of each lab and persons therein. It is important that all policies, procedures and guidelines are followed.

Biosafety Officer

Each institute or laboratory shall have a laboratory biosafety officer. The biosafety officer shall be appointed by (identified and recommended by the committee according to set criteria) the head of the laboratory. The officer must have appropriate technical background and be well versed in safety issues.

Wherever possible a biosafety officer should be appointed to ensure that biosafety policies and programmes are followed consistently throughout the laboratory. The biosafety officer executes these duties on behalf of the head of the institute or laboratory.

In small units, the biosafety officer may be a microbiologist or a member of the technical staff, who may perform these duties on a defined part-time basis. Whatever the degree of involvement in biosafety, should have a sound Laboratory biosafety background, be actively involved in the work of the laboratory, and have experience and training in the broader aspects of biosafety , should possess the professional competence necessary to suggest, review and approve specific activities that follow appropriate biocontainment and biosafety procedures. The biosafety officer should apply relevant national and international rules, regulations and guidelines, as well as assist the laboratory in developing standard operating procedures.

The biosafety officer should also be able to communicate effectively with administrative, technical and support personnel.

Biosafety Officer Activities

The activities of the biosafety officer should include the following:

1. Perform internal biorisk assessments and audits, these audits will also include comprehensive workplace surveys to ensure compliance with appropriate local and national safety and health policies and standards.
2. Review and ensure implementation of customized biorisk Standard Operational Procedures (SOPs) and ensure documentation of all biosafety procedures and activities.
3. Discussions of the safety policy with the appropriate/responsible persons.
4. Verification that all members of the staff have received appropriate instruction and that they are aware of all hazards, and that members of the medical, scientific and technical staff are competent to handle infectious materials.
5. Provision of continuing education in biosafety.
6. Ensuring appropriate decontamination following spills or other incidents involving infectious material(s).
7. Providing regular reports on safety status of the laboratory to the laboratory in-charge and archiving all documentation related to biosafety and biosecurity (for example, accident report forms and the accident record book).
8. Put in measures to ensure all materials are decontaminated and that infectious wastes are safely disposed of after treatment.
9. Ensuring the disinfection of any apparatus requiring repair or servicing before it is handled by non-laboratory personnel.
10. Establishment of procedures for recording the receipt, movements, and disposal of pathogenic material and for notification by any research worker or laboratory staff of the introduction of infectious materials that is new to the laboratory.
11. Advising the director or laboratory in charge of the presence of any agents that should be notified to the appropriate local or national authorities and regulatory bodies.
12. Coordination with medical staff regarding possible laboratory-acquired infections.
13. Reviewing the safety aspects of all plans, protocols, and operating procedures for clinical diagnostic work prior to the implementation of these procedures or studies.
14. Develop a system (plan) to deal with any emergencies (as per the risk assessment) that arise in the laboratory.
15. Carry out periodic inspections of containment facilities.
16. Assist team leaders in assessing training needs for those working with biological agents.

Biosafety Committee

A biosafety committee should be constituted to develop institutional biosafety policies and codes of practice. The biosafety committee should also review research protocols for work involving infectious agents, animal use, recombinant DNA, and genetically modified materials. Other functions of the committee may include risk assessments, formulation of new safety policies and arbitration in disputes over safety matters.

The institutional biosafety committee is comprised of no fewer than five members with at least two members who are not directly affiliated with the institute and represent the interests of the surrounding community. the committee include members with expertise in biosafety and physical containment, recombinant DNA technology, animal or plant research and have available consultants with knowledge of institute policies, applicable laws, standards of professional conduct, etc. Collectively members of the committee shall have the expertise and capability to evaluate the risks associated with research involving recombinant and synthetic nucleic acid molecules as well as research with biological organisms and materials.

Biosafety Committee responsibilities include:

1) Review of all research that is conducted at or sponsored by the institute. This review will include:
    a. Independent assessment of the risks associated with the research and verification of containment levels to ensure that all risk assessments are in place as required.
    b. Assessment of facilities, equipment, procedures, practices, training and all other elements associated with the research to:
        i. Ensure facilities are suitable for the work.
        ii. Ensure personnel are trained.
        iii. Monitor working standards would enable the monitoring of work with, for example, human pathogens, clinical material and cell and tissue culture.
2) Notification of the Principal Investigator of the committee's actions.
3) Periodic review of recombinant DNA and biological research.
4) Adopt emergency plans for accidental spills, personnel contamination, loss of containment and research related illnesses.
5) Keep a record of meetings, providing sufficient detail to serve as a record of major points of discussion and the committee's rationale for particular decisions.
6) Reports any significant problems.

Biosafety Committee Membership

The membership of the biosafety committee should reflect the diverse occupational areas of the organization as well as its scientific expertise. The composition of a basic biosafety committee may include:

1) Biosafety officer(s)
2) Scientists
3) Medical personnel
4) Veterinarian(s) (if work with animals is conducted)
5) Representatives of technical staff
6) Representatives of laboratory management.

The biosafety committee should seek advice from different departmental and specialist safety officers (e.g. with expertise in radiation protection, industrial safety, fire prevention, etc.) and may at times require assistance from independent experts in various associated fields, local authorities and national regulatory bodies. Community members may also be helpful if there is a particularly contentious or sensitive protocol under discussion.

Laboratory Managers

Technical or Laboratory Managers are required to ensure that:

- Effective strategies, systems and procedures are developed, implemented and monitored to ensure that work and learning in laboratories is undertaken strictly in accordance with these Guidelines.
- The Technical Staff they supervise receive the appropriate information, instruction, laboratory safety induction and training to carry out their work in accordance with these Guidelines.
- Risk assessments are conducted, documented and maintained for all hazardous substances and dangerous goods under their control.
- The Technical Staff they supervise are fully conversant with these Guidelines and understand their role in monitoring compliance.
- A failure on the part of any person to comply with these Guidelines is reported.
- Work relating to the (re)design, modification, repair and/or upkeep of a laboratory or facilities is undertaken in a manner that does not compromise the safety of person(s) or property or contravene these Guidelines.

- Appropriate emergency management plans are developed, implemented, and regularly tested.
- Staff and students are trained in what action(s) they must take should an emergency arise within a laboratory.
- Students have received the appropriate laboratory safety induction prior to commencing laboratory work.
- Staff and students have access to and wear the appropriate personal protective equipment whilst in a laboratory.
- Students are formally advised that they are required to take personal responsibility for ensuring their own safety and the safety of others.
- Effective protocols are developed, implemented, and monitored for the handling, storage, transport and disposal of hazardous equipment, materials, substances and wastes.
- All incidents, hazards and 'near miss' incidents are reported using Incident, Accident Report Form and a copy sent to the Head of Work Unit.

Laboratory Staff

Laboratory staff members share the responsibility towards safety in the laboratory by:

1. Adhering to Good Laboratory Practice (GLP).
2. Reporting and recording all accidents and biohazardous exposures, work related illnesses to the appointed biosafety officer and/or the supervisor.
3. Following all work protocols and operating procedures applicable to their activities.
4. Informing the supervisor and/or biosafety officer of any personal conditions such as an illness, use of medication, pregnancy, or reduced immunity which could make their work more hazardous to themselves or others.
5. Understand the risks of the project, procedures, and activities he or she is undertaking.
6. Take appropriate safety measures to protect themselves, co-workers, and the environment.
7. Use personal protective equipment as prescribed at all time when on duty.
8. Undergo all relevant training requirements for the allocated duties and responsibilities.

Laboratory Biosecurity Manual

Laboratory Biosecurity manual provides general guidelines, procedures, and requirements for the prevention of accidents and exposure to chemicals and is considered the minimum for the safe operation of a laboratory at the institutes.

WHO biorisk management Laboratory biosecurity guidance published in 2006 has already provided guidance to laboratory workers on how to perform laboratory work safely, to laboratory managers on how to set up a managerial approach to biosafety and to regulatory authorities, to help them consider necessary aspects for the development of adequate national biosafety regulations.

In the absence of national regulatory guidance, laboratory managers are encouraged to consider adopting a biorisk management approach adapted to their particular situation and developing guiding principles to be implemented in response to the specific needs of their facilities. This approach underlines the need to recognize and address the ongoing responsibility of institutions to ensure the expectation for a safe and secure laboratory environment.

Training

The institutions should ensure that requirements and procedures for biorisk-related training of personnel are identified, established, and maintained. All staff should be trained to foster correct attitudes and understanding of safe working practices including personal hygiene, appropriate use of personal protective equipment (PPE) with good microbiological techniques, safe use of equipment, recognition of hazards, risks and consequences before commencement of practical laboratory work.

Continuing education and training to maintain staff awareness of the safety implication of changing technology and improvements in safety practices should be undertaken and documented. Training should be offered regularly and taken recurrently. It should represent an opportunity for employees to refresh their memories and to learn about new

developments and advances in different areas. Personal training records shall be kept. Laboratory biosecurity training should be provided to all those working at a facility, including maintenance and cleaning personnel, and to external first responders and responsible staff involved in ensuring the security of the laboratory facility.

Training should provide for protection, assurance, and continuity of operations. Procedures describing the security roles, responsibilities, and authority of personnel in the event of emergencies or security breaches should also be provided during training, as well as details of security risks judged not significant enough to warrant protection measures.

Training should also provide guidance on the implementation of codes of conduct and should help laboratory workers understand and discuss ethical issues. Training should also include the development of communication skills among partners, the improvement of productive collaboration, and the endorsement of confidentiality or of communication of pertinent information to and from employees and other relevant parties.

Training is also important in providing occasions for discussions and bonding among staff members, and in strengthening of the team spirit among members of an institution. Training should include raising personnel awareness of biorisk issues including the relevance of human factors in biorisk management.

Laboratory Biosecurity Plan

For labs dealing with infectious substances, a biosecurity plan is just as important as a strong safety program. Biosecurity plans are designed to prevent the misuse, intentional release or theft of infectious agents. Such plans depend on what type of laboratory you manage; before creating a biosecurity program, the local environment, the kind of diagnostics and research, and the nature of the facility need to be considered. Law enforcement agencies should also be sure to include if necessary, in the creation of the plan, along with maintenance workers, lab staff, security staff, safety officers, principal investigators, administrators, and

scientific directors. Here are the main areas that should think about when drawing up the biosecurity plan:

i. Physical Protection. This involves the protection of the actual building housing the lab, including things such as locks on doors, perimeter security and practices limiting entry into the most sensitive areas of the lab such as areas where infectious agents are stored and animal testing areas.

ii. Personnel Suitability/Reliability. It is important to make sure staff undergo criminal background checks if they will be handling infectious agents. This section also involves ID badges for staff, limiting access to visitors, biosecurity training for staff, and anything else dealing with those who work in and visit the lab.

iii. Pathogen Accountability. These procedures deal with the inventory requirements for transfers within and outside the laboratory, inactivation, and disposal of cultures after they have been used, tracking of internal possession and proper labeling of substances.

iv. Biosecurity Incident and Emergency Response. This part of biosecurity plan involves how security issues are reported and investigated in the facility. Security issues can include everything from unauthorized entry to missing infectious agents. how the staff will deal with natural disasters should also consider, unintentional events such as releasing pathogens by mistake, and intentional events such as bomb threats. The proper training should be provided to all staff these plans will affect.

Conclusion

Biosecurity can be defined as the exclusion, eradication, and effective management of biorisks. Biorisk management of biological hazards can be broadly divided into:

i. actions that take place before the biological hazard has materialized (preventive measures);
ii. actions that take place during an outbreak (eradication); and
iii. actions aimed at reducing the consequences of the presence of the hazard.

The biorisk management approach is composed of a biosafety, a laboratory biosecurity, and an ethical component. It offers laboratory facilities a programme that should help them to account for and protect their valuable scientific assets. The main components of Biorisk management programmes are:

- Identify valuable biological materials (VBM) that require protection.
- Establish clear guidance, roles, responsibilities, and authorities for those who work with or have access to valuable biological materials (VBM) and to the facilities that contain them.
- Promote a culture of awareness, shared sense of responsibility, ethics, and respect of codes of conduct within the international life science community.
- Develop policies that do not hinder the efficient sharing of reference materials and scientific data, clinical and epidemiological specimens, and related information.
- Develop policies that do not impede the conduct of legitimate research.
- Strengthen collaboration between the scientific, technical and security sectors.
- Provide appropriate training to employees of laboratory facilities.
- Strengthen emergency response.

Pathogen Hazards

The principal hazardous characteristics of an agent are: its capability to infect and cause disease in a susceptible human or animal host, its virulence as measured by the severity of disease, and the availability of preventive measures and effective treatments for the disease. Biological agents include:

- All microorganisms and their toxins
- Viruses and sub-viral particles (including prions)
- Recombinant products (plant, animal, microbial)
- Parasites (microscopic, as well as macroscopic)
- Cultured human and animal cells and the potentially infectious agents that these cells may contain
- Clinical specimens (human or animal blood, body fluids, cells, tissues, bone)
- Whole animals and tissues from experimentally infected animals
- Allergens (such as molds, microbial spores, and animal allergens)

a- Risk Groups

Classification of organisms according to risk group has traditionally been used to categorize the relative hazards of infective organisms. The factors used to determine which risk group an organism falls into is based upon the characteristics of the organism, such as

i. Pathogenicity
ii. Infectious dose
iii. Mode of transmission
iv. Host range
v. Availability of effective preventive measures
vi. Availability of effective treatment.

The World Health Organization (WHO) has recommended an agent risk group classification for laboratory use that describes four general risk groups based on these principal characteristics and the route of transmission of the natural disease. The four groups address the risk to both the laboratory worker and the community. The NIH Guidelines established a comparable classification and assigned human etiological agents into four risk groups based on hazard. The descriptions of the WHO and NIH risk group classifications are presented in Table 1.

Table 1: Classification of Infectious Microorganisms by Risk Group

Risk group	World Health Organization Laboratory Biosafety Manual 3rd Edition 2004	NIH Guidelines for Research involving Recombinant DNA Molecules 2002
Risk Group1 Example: laboratory strains of non-pathogenic E. coli, S. cerevisiae, Lactobacillus, B. subtilis	(low individual and community risk) This is any biological agent that is unlikely to cause disease in healthy workers or animals	Agents not associated with disease in healthy adult humans.
Risk Group 2 Examples: Streptococcus, Herpes virus, pathogenic E. coli, Campylobacter spp	(moderate individual risk, low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.	Agents associated with human disease that is rarely serious and for which preventive or therapeutic interventions are often available.
Risk Group 3 Examples: B. anthracis, hantavirus, yellow fever	(high individual risk, low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.	Agents associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).
Risk Group 4 Examples: Ebola, Hendra and Nipah viruses.	(high individual risk, high community risk) This is any pathogen that usually produces very serious human disease and may be readily transmitted from one individual to another, directly or indirectly, effective treatment and prevention measures are not usually available.	Agents likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).

b- Resources for assigning risk group/biosafety level

The assignment of an agent to a biosafety level for laboratory work must be based on a risk assessment. Such an assessment will take the risk group as well as other factors into consideration in establishing the appropriate biosafety level. For example, an agent that is assigned to Risk Group 2 may generally require Biosafety Level 2 facilities, equipment, practices, and procedures for safe conduct of work. However, if particular experiments require the generation of high-concentration aerosols, then Biosafety Level 3 may be more appropriate to provide the necessary degree of safety, since it ensures superior containment of aerosols in the laboratory workplace. The biosafety level assigned for the specific work to be done is therefore driven by professional judgement based on a risk assessment. the assignment of a biosafety level takes into consideration the organism (pathogenic agent) used, the facilities available, and the equipment practices and procedures required to conduct work safely in the laboratory.

Helpful resources:

WHO Biological Safety Manual

The NIH Guidelines for Research Involving Recombinant or Synthetic DNA

The Biosafety in Microbiological and Biomedical Laboratories (BMBL)

c- Relation of risk groups to biosafety levels, practices, and equipment

Four biosafety levels (BSLs) represent combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed and the documented or suspected routes of transmission of the infectious agents, as well as for the laboratory function or activity. The recommended biosafety level for an organism represents the conditions under which the agent can be ordinarily handled safely.

As a general rule for selecting the appropriate laboratory containment, the biosafety safety level (BSL) selected should correspond with the highest risk group (RG) category of the organisms involved. For example, work with vaccinia virus which is a Risk Group 2 (RG2) agent, should be conducted at BSL-2 or higher and Mycobacterium tuberculosis which is a Risk Group 3 (RG3) should be conducted at BSL-3.

Table 2 **relates but does not "**equate" risk groups to the biosafety level of laboratories designed to work with organisms in each risk group.

Table 2. **Relation of risk groups to biosafety levels, practices, and equipment**

Risk group	Biosafety level	Laboratory Type	Laboratory practices	Safety Equipment
1	Basic-Biosafety Level 1	Basic teaching, research	Good microbiological techniques (GMT)	None; open bench work
2	Basic-Biosafety Level 2	Primary health services, diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus biological safety cabinet (BSC) for potential aerosols
3	Containment-Biosafety Level 3	Special diagnostic services, research	Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment-Biosafety Level 4	Dangerous pathogen units	Level3 plus airlock entry, exit showers, special waste disposal	Class III BSC or positive pressure suits with class II BSCs, double-ended autoclaved (through the wall), filtered air

d- Human blood, blood products, body fluids, tissues, and cells

Infection is the factor that has been seriously concerned when handling blood and products of human origin as HIV, Hepatitis B and Hepatitis C are examples of the infectious materials from such samples. Other agents such as fungi, bacteria, or viral agents may also present causing infections. Representative infections include tuberculosis and brucellosis.
As such, all blood, blood products, body fluids, as well as other blood-related materials should be treated as if they bear the blood-related pathogens. Biosafety Level 2 containments should be reached, and universal precautions should be taken to prevent any contaminations or spread of disease. an employer must implement an exposure control plan for the worksite with details on employee protection measures. The plan must also describe how an employer will use engineering and work practice controls, personal protective clothing and equipment, employee training, medical surveillance, hepatitis B vaccinations, and other provisions as required by OSHA's Bloodborne Pathogens Standard. Engineering controls are the primary means of eliminating or minimizing employee exposure and include the use of safer medical devices, such as needleless devices, shielded needle devices, and plastic capillary tubes.

e- Recombinant DNA

Genetic material, either natural or synthetic, can be combined to construct novel rDNA. rDNA technologies, are widely used in modern-day research and industry and have many applications, including the production of transgenic animals, the cloning of microbial toxin genes, drug resistance genes, or other genes in expression vectors, as well as the production of full-length infectious viral clones. While there are numerous beneficial uses for rDNA technology, there is also the risk that this technology can be used to create new pathogenic organisms or to increase the pathogenicity of existing organisms, whether intentionally or not.

Genetically Modified Organisms (GMOs)

GMOs are organisms (i.e., plants, animals, or microorganisms) that are created through the alteration of genetic materials in a way that does not occur naturally through mating or natural recombination. The best-known method for creating GMOs is through the application of rDNA technologies. A GMO can be as simple as a point-mutated bacteria strain (e.g., E. coli DH5-Alpha) or rDNA cloned into a viral host (e.g., vaccinia virus vaccines) to overexpress a specific gene for further study. More complex GMOs include transgenic and knock-out animals (e.g., severe combined immunodeficiency mice) whose genome has been altered by the insertion, deletion, or alteration of DNA segments. Experiments involving the construction or use of GMOs should be conducted after performing a biosafety risk assessment. The pathogenic properties and any potential hazards associated with such organisms may be novel and not well-characterized. The properties of the donor organism, the nature of the DNA sequences that will be transferred, the properties of the recipient organism, and the properties of the environment should be evaluated. These factors should help determine the biosafety level that is required for the safe handling of the resulting GMO and identify the biological and physical containment systems that should be used. As for pathogens, four risk classes have been determined for GMOs:

Risk class 1	GMO activities holding no or a negligible risk	Activities for which level 1 containment is appropriate to protect human health as well as the environment
Risk class 2	GMO activities holding a low risk	Activities for which level 2 containment is appropriate to protect human health as well as the environment
Risk class 3	GMO activities holding a moderate risk	Activities for which level 3 containment is appropriate to protect human health as well as the environment
Risk class 4	GMO activities holding a high risk	Activities for which level 4 containment is appropriate to protect human health as well as the environment

Biosafety considerations for Biological Expression systems

Biological expression systems consist of vectors and host cells. A number of criteria must be satisfied to make them effective and safe to use.

Plasmid pUC18.

- Frequently used as a cloning vector in combination with Escherichia coli K12 cells, the pUC18 plasmid has been entirely sequenced.

- All genes required for expression in other bacteria have been deleted from its precursor plasmid pBR322. E. coli K12 is a non-pathogenic strain that cannot permanently colonize the gut of healthy humans and animals.

Routine genetic engineering experiments can safely be performed in E. coli K12/pUC18 at Biosafety Level 1, provided the inserted foreign DNA expression products do not require higher biosafety levels.

Biosafety considerations for expression vectors

Higher biosafety levels may be required when:

1. The expression of DNA sequences derived from pathogenic organisms may increase the virulence of the GMO
2. Inserted DNA sequences are not well characterized, e.g. during preparation of genomic DNA libraries from pathogenic microorganisms

3. Gene products have potential pharmacological activity
4. Gene products code for toxins.

Viral Vectors

Viral vectors are vehicles used to deliver genetic material into host cells for subsequent gene expression. These systems have been used for both research and gene therapy applications. Viral vector systems used for recombinant gene transfer are usually based on viruses present in the human population such as adenoviruses, herpes viruses, and retroviruses. Genetic modifications are typically made to these vectors to improve gene delivery efficiency and to enhance their safety.

Retroviral vector systems, including lentiviral vectors derived from HIV-1, are competent

gene transfer vehicles that are widely used for their stable integration into the chromosomes of non-dividing and dividing cells, and for their long-term transgene expression.
Adenovirus vectors are used for the transfer of genes to other cells. Such vectors lack certain virus replication genes and are propagated in cell lines that complement the defect. Stocks of such vectors may be contaminated with replication-competent viruses, generated by rare spontaneous recombination events in the propagating cell lines, or may derive from insufficient purification.
These vectors should be handled at the same biosafety level as the parent adenovirus from which they are derived.

Transgenic and "knock-out" animals

- Animals carrying foreign genetic material (transgenic animals) should be handled in containment levels appropriate to the characteristics of the products of the foreign genes.
- Animals with targeted deletions of specific genes ("knock-out" animals) do not generally present particular biological hazards.
- Examples of transgenic animals include animals expressing receptors for viruses normally unable to infect that species.
- If such animals escaped from the laboratory and transmitted the transgene to the wild animal population, an animal reservoir for that virus could theoretically be generated.
- This possibility has been discussed for poliovirus and is particularly relevant in the context of poliomyelitis eradication. Transgenic mice expressing the human poliovirus receptor generated in different laboratories were susceptible to poliovirus infection by various inoculation routes and the resulting disease was clinically and histopathologically similar to human poliomyelitis.
- However, the mouse model differs from humans in that alimentary tract replication of orally administered poliovirus is either inefficient or does not occur.
- It is therefore very unlikely that escape of such transgenic mice to the wild would result in the establishment of a new animal reservoir for poliovirus.
- Nevertheless, this example indicates that, for each new line of transgenic

animal, detailed studies should be conducted to determine the routes by which the animals can be infected, the inoculum size required for infection, and the extent of virus shedding by the infected animals.

In addition, all measures should be taken to assure strict containment of receptor transgenic mice

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Transgenic plants

- Transgenic plants expressing genes that confer tolerance to herbicides or resistance to insects are currently a matter of considerable controversy in many parts of the world.
- The discussions focus on the food-safety of such plants, and on the long-term ecological consequences of their cultivation.
- Transgenic plants expressing genes of animal or human origin are used to develop medicinal and nutritional products.

A risk assessment should determine the appropriate biosafety level to produce these plants.

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Risk assessments for work with GMOs should consider the characteristics of donor and recipient/host organisms.

Examples of characteristics for consideration include the following:

- Risk assessments for work with GMOs should consider the characteristics of donor.
- Recipient/host organisms.
- Examples of characteristics for consideration include the following.

Hazards arising directly from the inserted gene (donor organism)

Assessment is necessary in situations where the product of the inserted gene has known biologically or pharmacologically active properties that may give rise to harm, for example:

1. Toxins
2. Cytokines
3. Hormones
4. Gene expression regulators
5. Virulence factors or enhancers
6. Oncogenic gene sequences
7. Antibiotic resistance
8. Allergens.

The consideration of such cases should include an estimation of the level of expression required to achieve biological or pharmacological activity.

Hazards associated with the recipient/host

1. Susceptibility of the host
2. Pathogenicity of the host strain, including virulence, infectivity, and toxin production
3. Modification of the host range

4. Recipient immune status
5. Consequences of exposure

Hazards arising from the alteration of existing pathogenic traits

Modification of normal genes may alter pathogenicity. In an attempt to identify these potential hazards, the following points may be considered

1. Is there an increase in infectivity or pathogenicity?
2. Could any disabling mutation within the recipient be overcome as a result of the insertion of the foreign gene?
3. Does the foreign gene encode a pathogenicity determinant from another organism?
4. If the foreign DNA does include a pathogenicity determinant, is it foreseeable that this gene could contribute to the pathogenicity of the GMO?
5. Is treatment available?
6. Will the susceptibility of the GMO to antibiotics or other forms of therapy be affected as a consequence of the genetic modification?
7. Is eradication of the GMO achievable?

Further considerations

- The use of whole animals or plants for experimental purposes also requires careful consideration.
- Investigators must comply with the regulations, restrictions, and requirements for the conduct of work with GMOs in host countries and institutions.
- Countries may have national authorities that establish guidelines for work with GMOs and may help scientists classify their work at the appropriate biosafety level.
- In some cases, classification may differ between countries, or countries may decide to classify work at a lower or higher level when new information on a particular vector/ host system becomes available.
- Risk assessment is a dynamic process that takes into account new developments and the progress of science.

f- Cultured cells and tissue

Cell lines and cell cultures are commonly used in diagnostic, research, and industrial laboratories. Many cell lines do not inherently pose a risk to the individuals manipulating them in the laboratory; however, they have the potential to contain pathogenic organisms such as bacteria (e.g., mycoplasmas), fungi, viruses, or prions. This can occur either naturally through contamination by adventitious organisms (e.g., mycoplasma and moulds), or experimentally (e.g., transduction, transfection, or infection).

Cell lines available from commercial sources are generally very well-characterized and the presence of infectious contaminants is documented. Some commercially available and established cell lines and cell cultures may contain parts of human or animal pathogens as a result of a previous infection (e.g., a latent virus or presence of a helper virus) or as the result of genetic engineering to include a pathogen's genetic information that retains pathogenicity.

Freshly prepared cell lines from a primary culture may have a higher risk of contamination, especially if the cell line was obtained from a source known to be or suspected of being infected with a pathogen. There have been documented Laboratory Acquired Infections (LAIs) associated with the manipulation of primary cell cultures. Bacterial and fungal contamination in cell lines can be readily identified; however, viruses are not as easily identified and can pose a significant hazard. Growth conditions (e.g., pH, temperature, medium supplements) may cause altered expression of oncogenes, expression of latent viruses, interactions between recombinant genomic segments, or altered expression of cell surface proteins.

The presence of biologically active mycoplasma products, the stability of mycoplasma antigens, and the fact that a number of mycoplasmas are zoonotic pathogens may make them an additional hazard to consider when working with cell lines.

Workers who handle or manipulate human or animal cells and tissues are at risk for possible exposure to potentially infectious latent and adventitious agents that may be present in those cells and tissues. This risk is well understood and illustrated by the reactivation of herpes viruses from latency, the inadvertent transmission of disease to organ recipients, and the persistence of human immunodeficiency virus (HIV), HBV, and hepatitis C virus (HCV) within infected individuals in the U.S. population. There also is evidence of accidental

transplantation of human tumor cells to healthy recipients which indicates that these cells are potentially hazardous to laboratory workers who handle them. In addition, human and animal cell lines that are not well characterized or are
obtained from secondary sources may introduce an infectious hazard to the laboratory. For example, the handling of nude mice inoculated with a tumor cell line unknowingly infected with lymphocytic choriomeningitis virus resulted in multiple LAIs. The potential for human cell lines to harbor a blood borne pathogen led the Occupational Health and Safety Administration (OSHA) to interpret that the occupational exposure to blood borne pathogens final rule would include primary human cell lines and explants.
Close attention to the maintenance of a high standard of general aseptic technique in the tissue culture laboratory will remain the primary protective action that prevents laboratory acquired infection. It is clear that many other developments, such as newly defined growth media and new virus screening techniques, will Source materials contribute to ensuring the safety of raw materials used in cell culture. However, the researcher working with animal cells should always bear in mind that it is attention to the quality of reagents, work practices and thorough documentation which will provide high-quality results and a safe working environment.

Bio-Risk Assessment and Management

Laboratory Bio-Risk Assessment

One of the most helpful tools available for performing a bio-risk assessment is the listing of risk groups for biological agents. However, simple reference to the risk grouping for a particular agent is insufficient in the conduct of a risk assessment. Other factors that should be considered, as appropriate, include:

1. Pathogenicity of the agent and infectious dose
2. Potential outcome of exposure
3. Natural route of infection
1. 4. Other routes of infection, resulting from laboratory manipulations (parenteral, airborne, ingestion)
4. Stability of the agent in the environment
5. Concentration of the agent and volume of concentrated material to be manipulated
6. Presence of a suitable host (human or animal)
7. Information available from animal studies and reports of laboratory-acquired infections or clinical reports
8. Laboratory activity planned (sonication, aerosolization, centrifugation, etc.)
9. Any genetic manipulation of the organism that may extend the host range of the agent or alter the agent's sensitivity to known, effective treatment regimens
10. Local availability of effective prophylaxis or therapeutic interventions.

On the basis of the information ascertained during the bio-risk assessment, a biosafety level can be assigned to the planned work, appropriate personal protective equipment selected, and standard operating procedures (SOPs) incorporating other safety interventions developed to ensure the safest possible conduct of the work.

➢ Specimens for which there is limited information

In these cases, it is prudent to take a cautious approach to specimen manipulation.

- Standard precautions should always be followed, and barrier protections applied (gloves, gowns, eye protection), whenever samples are obtained from patients.
- Basic containment – BSL 2 practices and procedures should be the minimum requirement for handling specimens.

- Transport of specimens should follow national, international or both rules and regulations.

Some information may be available to assist in determining the risk of handling these specimens:

Medical data on the patient

- Epidemiological data (morbidity and mortality data, suspected route of transmission, other outbreak investigation data)
- Information on the geographical origin of the specimen.

➢ Material Safety Data Sheet (MSDS)

Material Safety Data Sheets (MSDS's) / Safety Data Sheets (SDS's) are required as part of an Institutions Safety Program to meet regulatory standards.

Safety Data Sheets information must be accessible for employees during all shifts, including laboratory personnel. Access to MSDS's / SDS's can mean access to paper copies or electronic access via the Internet.

- An MSDS is a document that relays vital information about certain chemicals and biological agents.
- Each component of the MSDS is broken down into sections.
- Each section goes into detail about what precautions to take and the characteristics of the substance.
- This is for both chemicals and biological agents

Laboratory Bio-Risk Management-Standard CWA 15793:2011

Part I: Philosophy of Bio-Risk Management

- Overview & Objectives

In recent years, there has been a push to improve biosafety and biosecurity practices in laboratories globally. Much of the practices being promoted were developed in the USA, where the first biosafety conference was held in 1955 to allow professional biologists working with deadly pathogens to share good biosafety practices. Since those days, there have

been incredible engineering advances, both in buildings and in equipment, to provide engineering approaches to biosafety through secondary and tertiary containment.

However, in much of the world, public health officials have to deal with deadly endemic pathogens but do not have the budgets to build, equip, operate and maintain expensive laboratories that use all these engineering approaches.
Some Middle East and North Africa (MENA) countries have highly excellent biological laboratories, but the capacity building in biosafety vary among the countries of the region. Many MENA countries

and for many laboratories, guidance or specific requirements for the appropriate handling and storage of Valuable Biological Materials (VBM) do not yet exist. Simple practices and techniques predominate in some labs and the perceptions of risk vary among the regions. A lack of funding and other resources is likely to be the primary factor that prevents laboratories from applying appropriate biosafety measures; a lack of funds, in some countries of the region, prohibits laboratories from purchasing the necessary equipment and hiring qualified staff. A lack of awareness or education is also a significant hurdle; many scientists are guided by their employers or employee training, but biosafety training is minimally conducted in all MENA countries. Furthermore, every country in the region looks to its government to help shape its biosafety practices in the laboratory; yet, for many countries, no national regulations or guidance exists.

Regardless of the engineering level of a laboratory, developing human practices - based on management systems approaches - is essential to operating safely and responsibly any biological facility dealing with pathogens. Indeed, sometimes, such as in field epidemiology,

human practices may be the only biosafety option available.

To respond to this need to develop a performance-based biorisk management system standard, in February 2008 the European Committee for Standardization (CEN) developed the CEN Workshop Agreement (CWA) 15973. There are now efforts underway internationally to turn this agreement into an International Standards Organization (ISO) standard.

The objective of this section is to enable those operating biological laboratories – even if they have little prior knowledge of formal biosafety and biosecurity concepts - to develop a biorisk management system for their facility that will be in compliance with the CWA 15973 (and thus the new ISO standard once it is finalized).

➢ Performance Standards

The standard requires organizations to `demonstrate that appropriate and validated risk reduction procedures have been established and implemented'. To do this, the standard envisages that organizations shall be able to:

- "Establish and maintain a biorisk management system to control or minimize risk to acceptable levels in relation to employees, the community and others as well as the environment which could be directly or indirectly exposed to biological agents or toxins.
- Provide assurances that the requirements are in place and implemented effectively.
- Seek and achieve certification or verification of the biorisk management systems by an independent third party; and
- Provide a framework that can be used as the basis for training and raising awareness of laboratory biosafety and laboratory biosecurity guidelines and best practices within the scientific community".

➢ Elements of the Plan

CWA 15973 states the following about the requirements for a qualifying biorisk management policy:

"The organization's top management shall develop, authorize and sign a policy concerning

the management of laboratory biorisk (laboratory biosafety and laboratory biosecurity). It shall clearly state the overall biorisk management objectives and a commitment to improving biorisk management performance.

"The policy shall be appropriate to the nature and scale of the risk associated with the facility and associated activities and commit to:

a. Protecting staff, contractors, visitors, community and environment from biological agents and toxins that are stored or handled within the facility.
b. Reducing the risk of unintentional release of, or exposure to biological agents or toxins.
c. Reducing the risk to an acceptable level of unauthorized intentional release of hazardous biological materials, including the need to conduct risk assessments and implement the required control measures.
d. Complying with all legal requirements applicable to the biological agents and toxins that will be handled or possessed, and with the requirements of this standard.
e. Ensuring that the need for effective biorisk management shall take precedence over all non 'health and safety' operational requirements.
f. Effectively informing all employees and relevant third parties and communicating individual obligations with regard to biorisk to those groups.
g. Continually improving biorisk management performance."

Bearing in mind that the policy should be appropriate to the specific hazards and risks resulting from the activities and facilities in question, a biorisk management system should draw from the following elements as necessary:

1) General Concepts

a) Laboratory Biosafety and Biosecurity
b) Management: Institutional Roles
c) Concepts of Risk
d) Biological Risk
e) The Human Factor
f) Bioethics

2) Safe Laboratory Design and Operations

a) Risk Group and Biosafety Measures
b) Microbiological Risk Assessment in the Laboratory
c) Biological Safety Cabinets
d) Airflow
e) Laboratory Design and Facilities
f) Animal handling and care
g) Vectors and pests
h) Standard Operating Procedures

3) Waste Management and Clean Up

a) Infectious Waste Management
b) Waste Disposal Procedures
c) Autoclave Operations
d) Disinfection
e) Heat Treatments

4) Personnel Safety

a) Personnel Protective Equipment
b) Work practice
c) Aerosol hazards
d) Post-work Safeguards and Procedures
e) Specimen collection
f) Safety for Support Staff
g) Biosafety and Recombinant DNA in Laboratories
h) Biosafety Aspects of Transmissible Spongiform Encephalopathy Agents
i) Laboratory Animal Handling Practices

5) Accidents and Emergencies

a) Spills
b) Sticks
c) Responses to injuries
d) Emergency Procedures:
   - Fire
   - Medical emergency in the lab
   - Shut down procedures for emergency evacuation
   - Lock in/down procedures in case of attack/breach
   - Major breach/failure of containment
e) Electrical Power Failure in a Lab
f) Handling and Storage of Chemicals

6) Shipping Infectious Substances
7) Occupational Health

a) Medical surveillance program:
   i) baseline medicals resulting in an assessment of whether the person is fit for the intended duties
   ii) vaccines and prophylaxes
   iii) regular health monitoring
   iv) procedures to follow in case of potential exposure to pathogens
b) Laboratory-associated infections
c) Cases of laboratory safety incidents
d) Risk of blood borne Pathogens

8) Reference Documents

➢ Conformity and Compliance

The standard requires that the organization's biorisk management system identifies and fulfils all its legal obligations. This means that it must have the ability to access, comprehend and ensure compliance with, inter alia, all relevant regulations and legislation:

- International
- Supranational (eg EU)
- National/Federal
- Regional/State
- Provincial
- City
- Local

➢ Continual Review and Improvement

A key element of the standard is that it requires the organization to continually measure and review performance with a view to continual improvement of the biorisk management system. In this regard, the standard notes:

"… key factors in establishing and implementing a successful biorisk management system include … focus on continual improvement:

- Making continual improvement an objective for every individual in the organization.
- Using period assessment against establish risk-criteria to identify areas for potential improvement.
- Continually improving the effectiveness and efficiency of processes.
- Promoting prevention activities.
- Providing personnel in the organization with appropriate education and training including the methods and tools of continual improvement.
- Establishing measures and goals for improvement.
- Recognizing improvement.

And requires:

"The organization should regard this as a recurring, step-by-step activity. When opportunities for improvement are identified and justified, the organization needs to decide how they are to be implemented based on the available resources. The justification should be founded on an analysis of the potential gains in terms of improved control of risk. Improvements may typically address issues like:

- Training and awareness programmes.
- Internal communications.
- Effectiveness of reviews.
- Preventative actions.
- Effectiveness of follow-up activities.
- Documented procedures and instructions.

Part II: Creating the Plan

➢ Who should create the plan?

CWA 15973 states that:

"The organization shall ensure that a risk assessment system is established, implemented

and maintained in accordance with this standard and that the performance of the risk management system is reported to senior management for review and as a basis for improvement.

"The organization shall identify resource requirements and provide adequate resources, including the assignment of trained personnel for management, performance of work, and verification activities, including internal review".

While safety is the responsibility of everyone who works in and enters the laboratories, and all line managers responsible for them, the work of creating the plan needs clearly defined tasks and responsible persons. In larger organisations, these would typically involve:

1. The Board of Directors: Reviewing biosafety and biosecurity performance and continual improvement efforts should be a standing item on the Board's agenda.
2. The Laboratory/Facility Director: The director of a facility ordinarily has ultimate responsibility for ensuring that the management systems are adequate to the task – including budgeting and resource availability and aligning professional rewards and punishments with biosafety and biosecurity performance
3. An Institutional Biosafety Committee should be staffed with persons who, taken together, have the entire breadth of expertise and experience to make competent recommendations and decisions concerning risk management and risk avoidance (i.e., deciding not to proceed with activities for which there are insufficient risk management capabilities). Their task is to undertake the technical work to assess risks associated with proposed activities and to decide whether to proceed and, if so, with what risk mitigation measures.
4. A Biosafety Officer(s) or Infection Control Officers (in a medical setting) generally have responsibility to serve on the IBC and to be the chief monitor of compliance with the plan and performance against the defined targets.
5. Line managers (production managers in a production facility, scientific management in a research facility, medical director in a health facility) will have responsibility for knowing the plan as it addresses their own work and that of their staff, ensuring that all staff are aware of the plan as it affects them and that staff have all necessary training to enable them to comply with the plan, and for the compliance with the plan by all staff.

6. An Occupational Health Officer, usually a medical doctor or an occupational health nurse, will provide input on:
    a. Vaccines and prophylaxis available for personnel working with pathogens or in areas where pathogens are handled.
    b. Medical surveillance programmes to help quickly identify any acquired infections.
    c. Education of staff on the human symptoms of any pathogens present in the facility.
    d. Recommended first aid and medical treatment in case of accidents or infections; and
    e. Liaison with any outside medical providers for facility staff to ensure they are aware of the potential of acquired infections.
7. The Facility Maintenance Manager(s) responsible for maintaining equipment and building systems required for safe operation of the facility, will be responsible for:
    a. Risks arising from the facility and the equipment
    b. Coordinating building, systems, and equipment maintenance in a safe manner
    c. Liaising with any contractors to ensure they are aware of special risks and are suitably trained, etc…

    This function may be split between multiple persons (eg biosafety cabinet engineer, building
    engineer) but any division of responsibility should be clearly delineated.
8. The Security Manager will be responsible for liaising with all to address such issues as controlled access to facilities, secure storage of pathogens, and general facility security.
9. The Animal Handling Manager, if animals are used at the facility, shall be responsible for providing input on safety issues pertaining to animal care and use. This person will advise others on zoonotic diseases and liaise with veterinarian support.

The roles and responsibilities for biosafety and biosecurity for these key players should be defined and documented, as it should be for anyone in the organization with responsibility for any one or more of the following:

- Initiate action to prevent or mitigate adverse impacts of risk
- Amend risk control actions until the level of risk becomes acceptable

- Identify and record problems with risk management
- Initiate, recommend or provide solutions

- Communicate and consult internally and externally as appropriate.

Everyone in the facility should be made aware of these persons' responsibilities and authority to perform the listed functions.

➢ When should the plan be created?

CWA 15973 states that:

"The organization shall ensure the approach to risk assessment is defined with respect to its

scope, nature and timing so that it is proactive rather than reactive.

"The following should trigger either a new risk assessment or review of an existing on:

1. Commencement of new work or changes to the programme or work, including the introduction of new biological agents or alterations to workflow or volume
2. New construction/modifications to laboratories, plant and equipment or its operation
3. Introduction of altered and unplanned staffing arrangements (including contractors, visitors and other non-core personnel)
4. Significant alterations to Standard Operating Procedures (SOPs) or working practices (e.g. disinfection/waste management methodologies, PPE provision/ usage, entry/exit protocols, etc…
5. When unexpected events that may have relevance for the management of biorisk are observed
6. When actual or potential non-conformity with internal/external rules and regulations is identified (e.g. introduction of new legislation or major accident exposure)
7. When considering emergency response and contingency planning requirements
8. As part of the existing management system review process (e.g. annually, or at other appropriate and predetermined frequency)"

What this means is that the biosafety and biosecurity plan should be in place before an activity commences and should be reassessed and modified as necessary as new information becomes available. Furthermore, if a project comprises multiple stages, then there should be a plan review and adaptation before each subsequent stage commences.

➢ What is the Process for Creating a Plan?

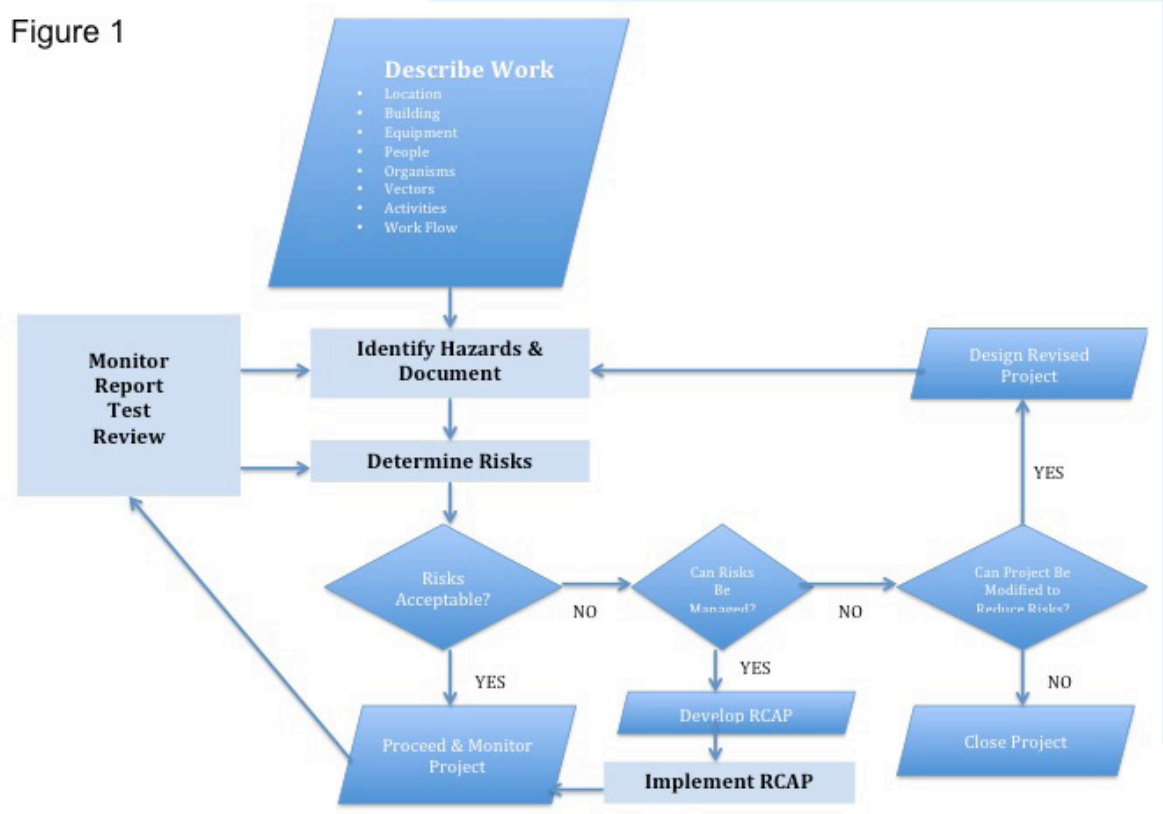
CWA 15973 identifies the following steps in creating a plan (see Figure (1) below for graphical representation of the process):

1. Identify and document the hazards associated with proposed work:
2. Assessment and recording of biorisks
3. Management plans and budgets for implementing the plan
4. Review of the plan to ensure its compliance with local legal and regulatory requirements
5. Establishment of objects and targets against which to measure performance of the plan
6. Development of monitoring controls to record the effectiveness of the plan

Step 1: Identifying and Documenting Hazards

CWA 15973 states that:

Figure 1



Flow Chart for Implementing a Risk Control Plan following CWA15973

"The hazards associated with proposed work shall be identified and documented."

What is a hazard?

A hazard is anything that has the potential for causing harm to humans, animals, plants and the environment, regardless of the probability of it happening. A hazard might be:

- A physical situation (eg a fire or an explosion)
- An activity (eg pipetting or using a centrifuge)
- Or a material (eg a biological agent or toxin, dangerous chemicals, asphyxiating gases)

How should the hazard be assessed?

The hazard should be assessed in the local context of the laboratory. For example, if a pathogen is spread by a vector that cannot survive at the laboratory's location, it may not be considered a hazard to the surrounding community, whereas the same pathogen at a location where the vectors can survive would be.

Who identifies the hazards?

It is recommended that everyone who works in the laboratory be involved in this process, and additionally that organizational experts on safety and risk management be involved (the latter if appropriate) together with the other experts identified at the beginning of this chapter (the biosafety officer, occupational health, facility manager, security manager, animal handlers).

What information should be considered in identifying the hazards?

Hazard identification should include information from:

1. Group experience and knowledge
2. External or specialized expertise not found in the facility (e.g. experts on vaccines and prophylaxis for, or treatment of, the human diseases caused by pathogens handled)
3. Results of previous assessments
4. Surveys of previous accidents/reviews of previous incidents
5. Hazardous materials data
6. Information on hazardous organisms
7. Guidelines and codes of practice
8. Facility drawings

9. SOPs, manuals, etc…
10. Process maps

How should hazards be recorded?

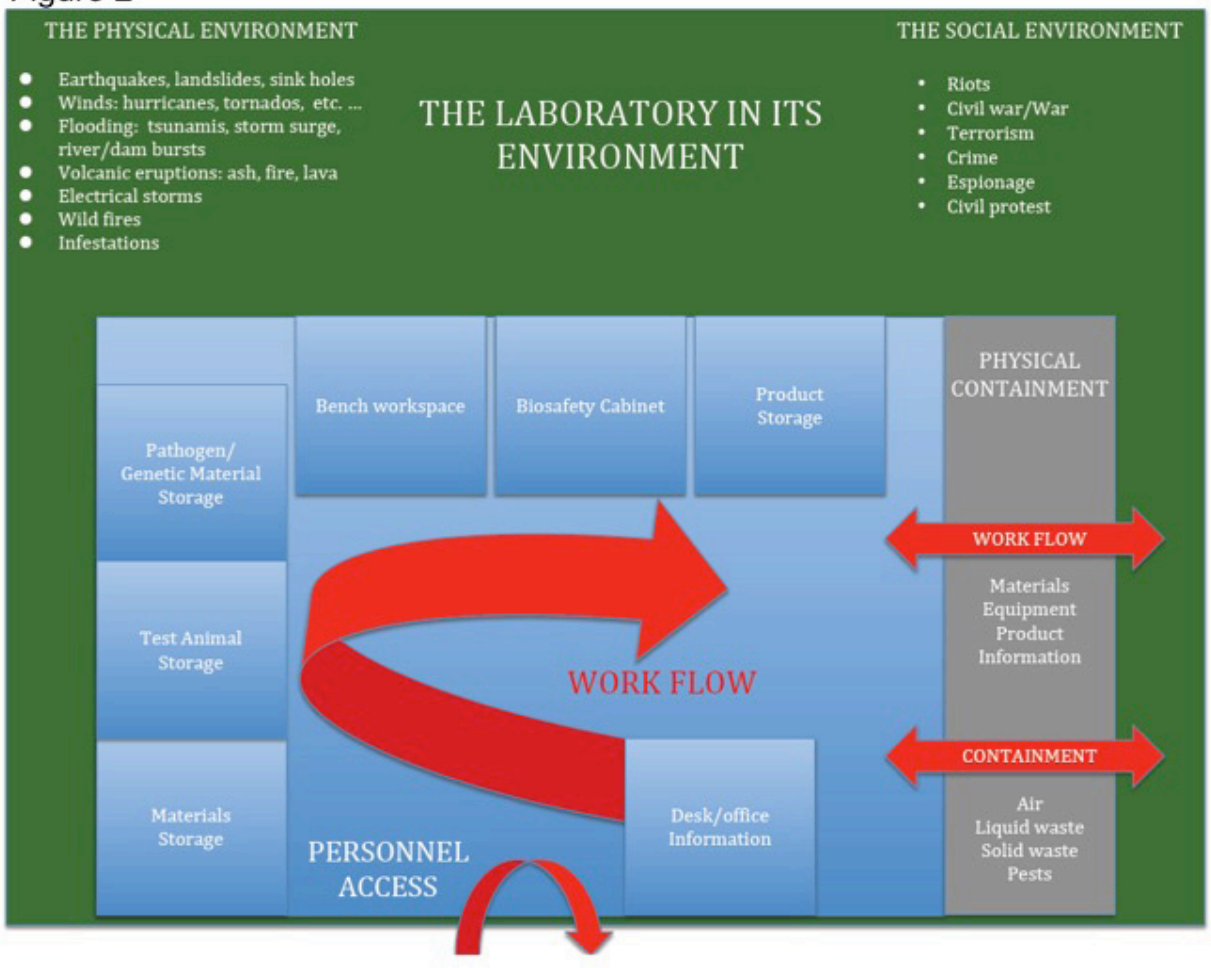
Hazards should be recorded in a manner that someone not involved in the original exercise can:

- Understand completely each hazard,
- Locate where it is present and
- Review the process and evaluate the list of hazards

Figure (2) gives a schematic of the types of hazards a typical facility might be exposed to:

i. Location and the physical and social environment
ii. Building(s) – shell, systems (HVAC, water, drains, electrical, gas)
iii. Equipment – biosafety cabinets, centrifuges, autoclaves, cell separators, etc…
iv. Personnel:
    1. Access
    2. Reliability
v. Pathogens/organisms/genetic material/vectors
vi. Other hazardous materials
vii. Animals/plants (for veterinary or botanical labs)
viii. Activities/techniques
ix. Methods and Work Practices, including workflow
x. Information

Figure 2



Step 2: Risk Assessment

CWA 15973 states that:

> "The organization shall ensure that suitable methodologies for assessing and recording risks are identified, implemented and maintained."

Risks should be categorized to identify those that need to be eliminated or controlled, with descriptions of likelihood and consequences. For each risk, a risk profile (i.e. acceptable level of that risk) should be defined and used in the assessment.

Assessments may be qualitative, semi-quantitative, or quantitative. The method should be suitable to the situation.

For biological agents and toxins, the inherent risk should be considered, using risk grouping descriptions, material safety data sheets etc…

Once risks have been assessed and a control proposed to avoid, eliminate or mitigate the risk, the risk of the proposed work practice including the control measures should be reviewed and assessed to ensure that risk is indeed lowered below the risk profile to acceptable levels. If it is not, alternative, or additional control measures should be identified until the resultant work practice with control measures is deemed acceptable.

Step 3: Risk Management Plan

CWA 15973 states that:

> "The organization shall ensure that suitable methodologies for the allocation of actions resulting from risk assessments, including timelines, responsible persons and associated reporting and approval mechanisms are identified, implemented and maintained."

The risk management approach should include a control plan to include:

1. Who is responsible and accountable for implementation of each element of the plan?
2. What resources are to be utilized (people, hours, budget, training, etc)
3. Timetable for implementation, with timely reviews to check milestones/rate limiting steps are implemented on time
4. Details of the mechanism and frequency of controlling for compliance with the plan

Risk management strategies should include the hierarchies of control to avoid, eliminate, mitigate, or manage risks. These are, where possible while achieving the objectives required:

- The elimination of hazardous work
- Substitution of hazardous work with a process using an alternative less hazardous organism or activity
- Isolation of the hazard to a confine area or time

- The use of engineering controls, such as biosafety cabinets or controlled access, to limit potential for exposure to the hazard
- Administrative controls, such as SOPs, to ensure that only qualified and trained staff perform hazardous activities and then in prescribed ways with all appropriate precautions and safety measures in place

- The reliance on Good Microbial Technique and Personal Protective Equipment. In order to

- limit the potential for contamination in the first instance, and to protect against infection should contamination occur.

Step 4: Conformity and Compliance

CWA 15973 states that:

> "The organization shall ensure that all relevant requirements are identified and fulfilled within the biorisk management system. Legal requirements include national/federal, regional/state, provincial, city and local regulatory requirements with which the organization shall comply."

The organization should adopt measure to identify legal and other requirements for the facility in relation to the biological agents and toxins that will be held and used, but also other regulations including:

1. Worker protection and rights
2. Environmental impact
3. General health and safety (eg fire and electrical)

In addition to those already existing, the organization should monitor new and upcoming requirements. This information should be kept up to date and the requirements incorporated into the biorisk management system of the facility.

Step 5: Metrics: Objectives, Targets and Programme

CWA 15973 states that:

> "The organization shall establish, implement and maintain documented biorisk control objectives and targets for an effective control of biorisk at relevant functions and levels in the organisation.

"Management shall establish the controls and put in place documented procedures for monitoring the effectiveness of the controls being applied to reduce or eliminate the hazards identified in the risk assessment process."

The controls can be monitored by:

- Regular audits
- Corrective action reporting processes where problems have been identified
- Investigation of incidents and accidents and improving controls and their implementation
- Ensuring that adequate resources, including training, are provided to maintain the effectiveness of the controls.

Step 6: Evaluation: Assessing the Effectiveness of the Plan

The plan should include formal mechanisms for regular periodic evaluation of the effectiveness of the plan and, where appropriate, identifying changes to the plan to improve effectiveness.

➢ Writing the Plan: Elements of the Plan

A biorisk management plan should follow an outline similar to the one below.

i. Philosophy and approach
ii. Organizational structures and key personnel.
    a. The roles, responsibilities and authorities of:
        i. Top Management/Board of Directors
        ii. Senior Management/Director
        iii. The Biorisk Management Committee
        iv. The Biorisk Management Advisor/Biosafety Officer
        v. Senior Scientific/Production/Medical Personnel
        vi. Occupational Health
        vii. Facility Management
        viii. Security
        ix. Animal Handling

b. Plans to ensure that personnel are aware of and trained to be able to work in compliance with the plan. This should cover:
    i. Recruitment criteria
    ii. Controls for internal promotions and work reassignments
    iii. Descriptions of competences required to perform assigned duties safely
    iv. Training requirements, curricula, and programmes as appropriate
    v. Continuity and succession planning, to ensure that procedures are followed in the absence of key personnel, and that required competences are present whenever hazardous work is performed.
    vi. Consultation and communication to ensure staff are aware of all relevant elements of the plan

iii. Standard Operating Procedures (SOPs) shall be developed as appropriate to cover:
    1. General Safety:
        a. General laboratory safety
        b. Fire safety
        c. Electrical safety
        d. Radiation safety
        e. Chemical safety
        f. Use of gasses
        g. Hot and cold work
        h. Equipment under pressure
        i. Laboratory animal care and use
        j. General housekeeping, eg storage and tidiness
    2. Maintenance of accurate inventories of pathogens and toxins
        a. Identification and logging of pathogens and toxins
        b. Restricted access to pathogens and toxins
        c. Effective physical security measures (locks, alarms, access controls, etc…)
        d. Sample identification
        e. Storage of pathogens and toxins according to risk levels, ie segregated storage
        f. Determination of what materials should be controlled
        g. Determination of what information needs to be kept

3. Change management to ensure that all changes associated with the design, operation and maintenance of the facility are properly documented and subject to a new hazard identification and risk assessment
4. Good Microbial Technique
    a. Animal handling
    b. Centrifugation
    c. Needles and Sharps
    d. Correct use of vacuum pumps
    e. Culture, purification, and storage techniques
    f. Minimization and containment of aerosols
    g. Pipetting
    h. Sonication and other forms of cell/tissue disruption
    i. Use of biosafety cabinets
    j. Use of disinfectants:
        i. Spill control
        ii. Routine decontamination
        iii. Hand washing
        iv. Showering
5. Inactivation of Biological Agents and Toxins
    a. Personnel
    b. Clothing and PPE
    c. Glassware
    d. Equipment
    e. Cultures and associated materials
    f. Spill clean-up materials and equipment
    g. Possibly infectious organisms, toxins and contaminated materials
    h. Paper and plastic waste
    i. Needles, syringes and sharps
    j. Wastewater, including from sinks and showers
    k. Air
    l. Filters and air handling systems

m. Discarded equipment
n. Animals exposed to pathogens or toxins
o. Animal carcasses and bedding
p. Facilities

6. Waste Management
    a. Minimizing the creation of waste
    b. Documentation of waste creation, processing and disposal
    c. Short-term storage of waste pending processing or disposal
    d. Segregation of waste per its processing requirements
    e. Packaging and handling of waste
7. Clothing and Personal Protective Equipment (PPE)
    a. Criteria for PPE selection
    b. Identification and outfitting of those requiring PPE
    c. Training in criteria for PPE selection, SOPs and competences
    d. Routine maintenance and checking of PPE
    e. Replacement schedule for PPE and inventory management, including for surge needs
    f. Documentation of and training in the hazards associated with working in PPE
    g. Cleaning of PPE and validation of decontamination
8. Occupational Health
    a. Baseline medical and assessment of medical fitness for assigned work
    b. Vaccines and prophylaxis
    c. Information on human symptoms for handled pathogens and toxins
    d. Routine medical monitoring and surveillance
    e. Procedures in case of potential exposure
    f. First aid SOPs
    g. Wallet cards
    h. Contact lists for medical care specialists
9. Personnel Issues
    a. Personnel Reliability
    b. Contractors, visitors, and suppliers
    c. Exclusion of persons as necessary

10. Infrastructure and Operational Management
    a. Planning, design, and verification of facilities
    b. Commissioning and decommissioning
    c. Maintenance, Control, Calibrations, Certification and Validation of Plant and Equipment
    d. Physical Security
    e. Information Security
    f. Personal Security
11. Emergency Response and Contingency Plans
    a. Acquired, or potential, infection
    b. Other emergency evacuation of personnel from high containment area
    c. Natural disasters
    d. Fire
    e. Flood
    f. Breach of Security
    g. Explosion
    h. Theft of pathogens or toxins
    i. Unexpected virulence of organism
    j. Facility or equipment failure
    k. Failure of disinfection
    l. Loss of power and water
    m. Major spillage or aerosol release
    n. Environmental release
    o. Terrorism, sabotage or vandalism
    p. Adverse media attention

iv. Controls:
    1. Documentation of work programme, planning and capacity
    2. Performance measurement
    3. Checks and corrective action

v. Reporting and Record Keeping

vi. Inventory Monitoring and Control

vii. Accident Investigations/Post-Mortems

viii. Inspection Audit
ix. Plan Review

SOPs play a large part in the plan, and so it is important that those creating the plan know how to write effective SOPs. Appendix (1) contains information from a variety of sources on how to do this.

Part II: Implementing the Plan

- Staff appointments and responsibilities

General

It is important that personnel are aware of their responsibilities and approved procedures for performing their duties, and that they are trained to be able to work in compliance with these.

For each activity that represents a biohazard, management should define competency levels that cover the appropriate levels of education, training and experience required to perform the activity safely and in accordance with the biorisk management plan.

Personnel performing such tasks should be assessed to ensure that they meet all relevant competency levels. Personnel records should record such verified competencies.

Thus, administrative measures should be established to:

a) Ensure that all relevant competency needs are defined
b) Define how successful completion of training can be demonstrated/tested
c) Define what constitutes a demonstration of ability to perform tasks under supervision
d) Define what constitutes a demonstration of ability to perform tasks unsupervised
e) The restrictions to be placed on personnel what have not demonstrated competence in order to ensure that they do not perform tasks for which they are not authorized
f) Maintain accurate and current records.

New Appointments

Recruitment for any position that involves working on activities or in areas covered by the biorisk management plan should assess whether the candidate has the appropriate experience, qualifications, aptitude, physical and mental health, and competence to do the defined work.

Background checks and reference checking should be used to verify a candidate's claims to competency.

When personnel are assigned new responsibilities within the organization, particularly if they are being cleared to work with higher risk pathogens, management should verify their competence to conduct their new duties.

All new personnel working in hazardous environments, whether freshly recruited or reassigned from within the organization, should work under the close supervision of experienced personnel until they have demonstrated requisite competence to work safely.

Staffing Levels

For any activity requiring supervision or the availability of support services, measures should be established to ensure that these activities are not undertaken in the absence of such supervision or support.

The organization should identify roles and individuals to ensure that the integrity of the biorisk management plan is not compromised through short- or long-term absences.

Succession planning should ensure that no individual holds critical technical, management, or scientific knowledge regarding the safe operation of the facility that is not available to others in the event of their unavailability or departure.

➢ Communications

It is important for the organization, upon completion and upon each revision of its biorisk management plan, to communicate relevant biorisk information to its personnel and other relevant parties, such as contractors and health care providers.

The communication of this vital information should be documented, eg. by having employees and contractors sign copies of the literature informing them of risks and procedures.

In addition, key information should be posted publicly in relevant areas of the facility, reminding personnel, for example, of the presence of hazards, of correct procedures or of the symptoms of infection.

Organisations should consider holding regular team meetings and briefings, in addition to formal training sessions to ensure effectiveness and comprehensiveness of this communication and consultation process.

The communications and consultation measures should be extended, as appropriate, to include:

a) Local, national, and international governmental organisations
b) Relevant regulatory agencies
c) Certifiers and commissioners
d) Emergency services and healthcare providers
e) Contractors and suppliers (including cleaners, maintenance providers, security personnel)
f) Local community representatives

In addition, organisations should establish a mechanism to identify existing or emerging technologies or other relevant information relating to the containment of pathogens and toxins handled or stored, vaccines, prophylaxis and treatments. This information should be shared with other relevant staff.

Communication methods to be considered include:

a) E-mails
b) Signage
c) Newsletters
d) Documents
e) Team briefings (including webinars or video conferencing)
f) Availability and maintenance of reference libraries
g) Bulletin boards

➢ Personnel Training

Training requirements and procedures must be identified, established, reviewed, and maintained for any position requiring biorisk-related training. This training should include raising staff awareness of all biorisk issues, the human symptoms resulting from infections by the pathogens handled by the organization, the occupational health available to them, and the human factors in biorisk management.

Thus, organisations should:

a) Define biosafety training needs
b) Establish procedures to ensure that personnel receive all the training required for their position
c) Define how they will determine the effectiveness of the training (and hence the trainee's competence relating to that training)
d) Define frequency and types of refresher training required by personnel
e) Establish measures to ensure that persons do not perform tasks they are not trained and cleared to perform
f) Maintain current and accurate records of staff training and training results

➢ Control Structures and Practice`

The organization should identify those operations and activities that are associated with possible biological risk and where control measure shall be applied. It shall pan these activities, including maintenance, to ensure that they are carried out under specified conditions, following all appropriate SOPs as defined and detailed in the biorisk management plan.

General Safety

The organization shall ensure that a formal process is in place to identify and manage risk associated with general safety. It shall adopt a preventative and proactive approach to managing sources of risk, both to protect workers from direct hazards associated with their work and to address the implications for biorisk in the event of an accident or incident resulting from such sources. Measures should be identified and implement to detect, mitigate, and respond to emergencies, taking into consideration potential implications for pathogens and toxin control in such measures.

Inventory Control

The organization shall ensure that an accurate and up-to-date inventory of pathogens and toxins is established and maintained. Complete and current back-up copies shall be maintained and stored at a physically separate location so that, should there be catastrophic damage to the facility, those records can be retrieved.

All transfers of pathogens and toxins from storage to other locations within the facility and vice versa, or into and out of the facility, shall be logged and recorded, and be performed in line with current SOPs under the risk management plan.

The inventory control process should be based on risk and include:

- Identifying all pathogens and toxins held, including cultures and other sources (e.g. infected tissues, diagnostic samples, animals)
- Restricting access to pathogens and toxins to authorized personnel and limiting authorization to those who have a demonstrable legitimate need
- Implementing effective physical security measures (alarms, locks, access controls…)
- Developing and maintaining a reliable sample identification system, including labelling
- Segregating and storing pathogens and toxins according to risk
- Determining what materials should be controlled and what level of information should be kept in the inventory for those materials

Inventory information should include:

- The name(s) and contact information for the individual(s) responsible for the material and details of other personnel with access to the materials or immediate area based on the level of risk.
- Restricted access to the detailed inventory records to those individuals whose work requires access to that information
- Legible and robust identification numbers and other relevant identifiers
- Records of quantities/volumes of pathogens and toxins at an appropriate level and based on risk
- Records of materials consumed, destroyed, or removed from the facility where appropriate

Controls should be established so that all the necessary checks and documented assurances are received to ensure that requests for pathogens and toxins originate from legitimate facilities and individuals. Material may only be brought into the facility or sent elsewhere if authorized by those responsible for the facility. For materials deemed high risk, more stringent controls including shipment tracking and verification of receipt are important considerations.

➢ Metrics and Corrective Actions

It is important to measure and evaluate the effectiveness of the biorisk management plan so that continual improvements can be made as appropriate to safety performance. In order to do this, the organization must:

a. Assess appropriate and practical ways of measuring performance against the objectives of the biorisk management plan.
b. Systematically collect data accordingly by recording these measurements.
c. Evaluate overall performance by analysing this data; and
d. Based upon the evaluation, make improvements to the plan and/or the metrics used to assess performance.

There should be at least an annual management review of the biorisk management plan, and whenever serious questions about the effectiveness or comprehensiveness or the plan are raised, or whenever changes in the work programme call for it.

In conducting reviews, data from performance monitoring above should be supplemented with data from other sources, including audits and analysis from other sources, including external sources.

The organization shall put in place control mechanisms in order to identify situations that do not conform to the requirements and prescriptions of the biorisk management plan and to ensure that they do not result in undesirable consequences, and maintain records of the non-conformity and any subsequent action taken.

These controls shall also identify who is responsible for dealing with instances of non-conformity and their authority to eliminate the causes on non-conformity through corrective actions. The process should define how:

a. Non-conformity is identified
b. Causes of non-conformity are identified
c. The need for corrective action is evaluated to ensure no reoccurrence
d. The corrective action is determined, and determined to be sufficient
e. Corrective action is implemented and by whom
f. Corrective actions should be recorded
g. The effectiveness of the corrective action is reviewed and evaluated.

The organization shall also take preventative action to identify and eliminate causes of potential non-conformity in order to prevent their occurrence. The procedure established should define how:

a. The potential for non-conformity is determined and causes
b. The need for preventative action is evaluated
c. The preventative action is determined, and determined to be sufficient
d. Preventative actions should be recorded
e. The effectiveness of the preventative action is reviewed and evaluated.

➢ Reporting and Record Keeping

Records, logs, documents, and data should be established and organized to provide evidence of compliance with the biorisk management plan. At least one current copy of the records should be kept off-site as a backup in case of catastrophic failure at the facility.

These records should include, but not necessarily limited to:

a. Risk assessments, SOPs, and safety manuals
b. Job hazard analyses and charts of authority
c. Design records and commissioning/test plans, records, and associated data
d. Audit and inspection checklists
e. Laboratory biosafety manuals and risk assessments, authorizations, and other security documents
f. Training records
g. Personnel competency levels relating to biohazardous activities and access to materials or restricted access areas of the facility
h. Containment equipment certifications and maintenance logs

Depending on the nature of the work performed at a facility, consideration might be given to limiting access to these documents based on a need to know, business continuity considerations also being taken into account.

Procedures should be established for defining the controls needed for:

a. The identification, storage, protection, retrieval, retention time and disposal of records
b. The approval of documents prior to issue or public release in order to ensure that sensitive information such as location of pathogens within the facility is not inadvertently released
c. The review, update, and re-approval of documents and for the change control and revision process.

➢ Inventory Monitoring

Stocktaking of inventories of pathogens and toxins should be conducted at regular, predetermined intervals based on risk (the higher the risk, the more frequent the review) to ensure that all items are accounted.

When inventories are checked, management should also review whether inventory of each item is still required and, if so, how the inventory can be minimized in order to reduce risk. Proactive measures should be taken to eliminate, substitute or minimize volumes and quantities of pathogens and toxins used, and the number of manipulations of them conducted.

The organization must establish procedures to investigate unaccounted for pathogens and toxins, appropriate to the level of risk release of those agents would engender.

➢ Accident Investigations/Post-Mortems

The organization shall establish and maintain documented procedures to define, record, analyse and learn from accidents, incidents and near-misses involving pathogens and toxins.

This starts with establishing a procedure to define what constitutes an accident, incident or near miss and to ensure that all relevant personnel know these definitions.

For example:

a. An accident could include a spill or a stick resulting an exposure to the agent by one or more persons in the facility, or release of a pathogen
b. An incident might include the malfunctioning of equipment vital to safe operations or failure on the part of a person to follow SOPs related to controlling biorisks
c. A near miss might include dropping a diagnostic sample tube without it breaking

Examining incidents and near misses in addition to accidents is important, as they are indications of when the risk management system nearly failed and so could be good indicators of where protections or procedures need to be improved.

Accident and incident investigation process should:

a. Identify the person responsible for maintaining the accident/incident reporting system
b. Define what constitutes an accident, incident or near miss and what triggers recording, reporting and investigation
c. Encourage reporting of accidents, incidents and near misses, with emphasis on learning from the situation rather than apportioning blame
d. Be designed to interview people as soon after the incident as possible to ensure fullest and most accurate recording of the facts pertaining to the situation
e. Identify root causes
f. Result in improvements to prevent recurrence
g. Specify what documentation is required to support the system
h. Identify reports to be generated, their frequency and distribution
i. Ensure analysis of trends in accidents, incidents and near misses
j. Identify where outside authorities (e.g. law enforcement, health, environment, or agriculture officials) may need to be brought into an investigation.

➢ Inspection and Audit

Inspections and audits shall be conducted with a frequency and level appropriate to the risk associated with the facility. These inspections and audits shall assess whether the facility is operating in accordance with the biorisk management plan, and whether the plan is meeting the anticipated standards of risk control and assured safety. Any issues of noncompliance or non-conformity with the plan shall be flagged and recommendations for corrective actions made, with the person responsible to taking them clearly identified.

Management shall ensure that any non-conformity or noncompliance issues identified in an inspection or audit are corrected without undue delay. Follow up inspections shall be scheduled to ensure that these corrective measures have been taken within the specified time.

➢ Plan Review

The biorisk management plan shall be reviewed by top management at planed intervals to ensure its continued suitability, adequacy, and effectiveness.

Reviews shall include assessments of:

a. Opportunities for improvements
b. Need for changes to the system, procedures, policies, and objectives.

Records of the review process shall be maintained.

Inputs for the management review might include, as appropriate:

a. Results of audits and inspections
b. Compliance with SOPs and work instructions
c. Status of risk assessment activities
d. Status of preventative and corrective activities
e. Implementation of follow-up actions from previous management reviews
f. Changes that could affect the system
g. Recommendations for improvement
h. Results of accident/incident investigations.

The review should result in decisions and recommendations for actions aimed at:

- Improvement of the effectiveness of the biorisk management system
- Improved risk assessments
- Amended requirements associated with these improvements
- Changes to budgets relating to changed resource needs.

Laboratory Design: Containment Levels 1-4

Containment is used to describe safe methods for managing infectious agents in the laboratory. Primary containment is the protection of the personnel and the immediate laboratory environment from exposure to the agents. This is achieved through good laboratory techniques and the use of safety equipment. Secondary containment is the protection of the environment external to the laboratory. It is achieved through the combination of facility design and operational practices. The three elements of containment include laboratory practice and technique, safety equipment, and facility designs.
Work is assigned a biosafety level based on the pathogens and operations to be performed, the documented or suspected routes of transmission for the agent, and the laboratory functions and activities. The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

a- Containment Level 1 (BSL1)

Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required but may be used as determined by appropriate risk assessment.

i. Laboratory Design and Physical Requirements

- Be separated from public areas by a door which should be kept closed when biohazards are in use.
- Have surfaces that can be readily cleaned and resistant to any disinfectants or other chemicals in use. Carpets and rugs in laboratories are not appropriate
- Have fly screens on any windows which can be opened
- Provide hooks for lab coats separate from personal clothing
- Have hand-washing stations, ideally near the exit.

· Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.

a. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

b. Chairs used in laboratory work must be covered with a non-porous material that can be easily cleaned and decontaminated with appropriate disinfectant.

· Where indicated by the chemical hazards in the laboratory, must have Emergency eyewash facilities and emergency shower equipment

· Have all appropriate door signage (e.g. biohazard sign, containment level, contact information, type of biohazardous material in use and any entry requirements such as PPE.

ii. Operational Practices

- Standard Microbiological Practices

1. The laboratory supervisor must enforce the institutional policies that control access to the laboratory.
2. Persons must wash their hands after working with potentially hazardous materials and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas. Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.

4. Mouth pipetting is prohibited; mechanical pipetting devices must be used.
5. Policies for the safe handling of sharps, such as needles, scalpels, pipettes, and broken glassware must be developed and implemented. Whenever practical, laboratory supervisors should adopt improved engineering and work practice controls that reduce risk of sharps injuries. Precautions, including those listed below, must always be taken with sharp items. These include:
   a. Careful management of needles and other sharps are of primary importance. Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.
   b. Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.
   c. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.
   d. Broken glassware must not be handled directly. Instead, it must be removed using a brush and dustpan, tongs, or forceps. Plastic ware should be substituted for glassware whenever possible.
6. Perform all procedures to minimize the creation of splashes and/or aerosols.
7. Decontaminate work surfaces after completion of work and after any spill or splash of potentially infectious material with appropriate disinfectant.
8. Decontaminate all cultures, stocks, and other potentially infectious materials before disposal using an effective method. Depending on where the decontamination will be performed, the following methods should be used prior to transport.
   a. Materials to be decontaminated outside of the immediate laboratory must be placed in a durable, leak proof container and secured for transport.
   b. Materials to be removed from the facility for decontamination must be packed in accordance with applicable local, state, and federal regulations.
9. A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present. The sign may include the name of the agent(s) in use, and the name and phone number of the laboratory supervisor or other responsible personnel. Agent information should be posted in accordance with the institutional policy.
10. The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.

iii. Safety Equipment

1. Special containment devices or equipment, such as BSCs, are not generally required.
2. Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination of personal clothing.
3. Wear protective eyewear when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials. Persons who wear contact lenses in laboratories should also wear eye protection.
4. Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Wash hands prior to leaving the laboratory. In addition, BSL-1 workers should:

a. Change gloves when contaminated, glove integrity is compromised, or when otherwise necessary.

b. Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

c. Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste. Hand washing protocols must be rigorously followed.

iv. Training Requirements

laboratory personnel must receive annual updates or additional training when procedural or policy changes occur. An effective safety programme begins with the laboratory managers, who should ensure that safe laboratory practices and procedures are integrated into the basic training of employees. Training in safety measures should be an integral part of new employees' introduction to the laboratory. Employees should be introduced to the code of practice and to local guidelines, including the safety or operations manual. Measures to assure that employees have read and understood the guidelines, such as signature pages, should be adopted. Laboratory supervisors play the key role in training their immediate staff in good laboratory techniques. The biosafety officer can assist in training and with the development of training aids and

documentation. Staff training should always include information on safe methods for highly hazardous procedures that are commonly encountered by all laboratory personnel and which involve:

1. Inhalation risks (i.e. aerosol production) when using loops, streaking agar plates, pipetting, making smears, opening cultures, taking blood/serum samples, centrifuging, etc.
2. Ingestion risks when handling specimens, smears, and cultures
3. Risks of percutaneous exposures when using syringes and needles
4. Bites and scratches when handling animals
5. Handling of blood and other potentially hazardous pathological materials
6. Decontamination and disposal of infectious material.

Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection. Individuals having these conditions should be encouraged to self-identify to the institution's healthcare provider for appropriate counseling and guidance.

b. Containment Level 2 (BSL2)

Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that:
1) laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures.
2) access to the laboratory is restricted when work is being conducted.
3) all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

i. Laboratory Design and Physical Requirements

Level 2 labs must:

- Meet all the facility requirements described above for Level 1 laboratories

· Doors must be closed at all times with access limited to authorized personnel only, and doors must be locked when the lab is not occupied.
· Have non-absorptive work surfaces that are scratch, stain, chemical, moisture, and heat resistant.
· Biological safety cabinets (BSCs) must be installed so that fluctuations of the room air supply and exhaust do not interfere with proper operations. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions.
·Vacuum lines should be protected with liquid disinfectant traps.
·An eyewash station must be readily available.
·There are no specific requirements for ventilation systems. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.
· HEPA filtered exhaust air from a Class II BSC can be safely recirculation back into the laboratory environment if the cabinet is tested and certified at least annually and operated according to manufacturer's recommendations. BSCs can also be connected to the laboratory exhaust system by either a thimble (canopy) connection or directly exhausted to the outside through a hard connection. Provisions to assure proper safety cabinet performance and air system operation must be verified.
· An acceptable means of waste treatment or disposal must be provided.
· are recommended and are often required, depending on the risk assessment.

ii. Operational Practices

- Standard Microbiological Practices
- Special Practices

1. All persons entering the laboratory must be advised of the potential hazards and meet specific entry/exit requirements.
2. Laboratory personnel must be provided medical surveillance, as appropriate, and offered available immunizations for agents handled or potentially present in the laboratory.
3. Each institution should consider the need for collection and storage of serum samples from at-risk personnel.
4. A laboratory-specific biosafety manual must be prepared and adopted as policy. The biosafety manual must be available and accessible.
5. The laboratory supervisor must ensure that laboratory personnel demonstrate proficiency in standard and special microbiological practices before working with BSL-2 agents.
6. Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport within a facility.
7. Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.

a. Spills involving infectious materials must be contained, decontaminated, and cleaned up by staff properly trained and equipped to work with infectious material.
b. Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.

8. Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety manual. All such incidents must be reported to the laboratory supervisor. Medical evaluation, surveillance, and treatment should be provided, and appropriate records maintained.
9. Animal and plants not associated with the work being performed must not be permitted in the laboratory.
10. All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.

c. Containment Level 2 Enhanced & Containment Level 2+

This biosafety level is also referred to as "Biosafety Level 2 Enhanced". Work conducted at BSL2+ usually involves work with biological agents that would normally be conducted at BSL2; however, the work may involve certain conditions which would necessitate an increased amount of precautions. One example might involve the use of oncogenes in lentiviral vectors. The potential for increased risk in these experiments should be considered carefully and might be further mitigated by including additional protective measures (e.g., the use of wrap-back disposable gowns and wearing double gloves, respiratory protection when handling material outside a biosafety cabinet, and restricting access to rooms when experiments are conducted). Other examples might include research involving higher volumes or concentrations of culture and/or procedures deliberately generating aerosols. Level 2+ labs must (in addition to level 2 requirements):

- have directional air flow into the lab.
- all work with biohazardous materials done in a biological safety cabinet (BSC)
- centrifuge rotors must have aerosol-resistant lids that are opened only inside the BSC.
- Lab specific training that is documented with a quiz is required for entry into Level 2+ labs.
- Lab personnel are responsible for all routine maintenance of a 2+ lab, including washing the floor.

· Because custodial assignments change frequently, it is usually not feasible for custodians to be trained to enter 2+ labs.
· Regular waste and glass waste should be surface decontaminated and put outside of door for custodians to pick up.
· Prior to annual floor stripping and sealing, lab personnel must surface decontaminate all readily accessible surfaces in the lab including the floor.

d. Containment Level 3 (BSL3)

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents and must be supervised by scientists competent in handling infectious agents and associated procedures.
All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices.
Workers in the laboratory where protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls. Protective clothing is not worn outside of the laboratory. Reusable clothing is decontaminated before being laundered. Clothing is changed when contaminated.

i. Laboratory Design and Physical Requirements

A BSL-3 laboratory has special engineering and design features.

1. The laboratory must be separated from the areas that are open to unrestricted traffic flow within the building. Additional separation may be achieved by placing the laboratory at the blind end of a corridor, or constructing a partition and door or access through an anteroom (e.g. a double-door entry or basic laboratory – Biosafety Level 2), describing a specific area designed to maintain the pressure differential between the laboratory and its adjacent space. The anteroom should have facilities for separating clean and dirty clothing and a shower may also be necessary.
2. Anteroom doors may be self-closing and interlocking so that only one door is open at a time. A break-through panel may be provided for emergency exit use.

3. Surfaces of walls, floors and ceilings should be water-resistant and easy to clean. Openings through these surfaces (e.g. for service pipes) should be sealed to facilitate decontamination of the room(s).
4. The laboratory room must be sealable for decontamination. Air-ducting systems must be constructed to permit gaseous decontamination.
5. Windows must be closed, sealed and break resistant.
6. A hand-washing station with hands-free controls should be provided near each exit door.
7. There must be a controlled ventilation system that maintains a directional airflow into the laboratory room. A visual monitoring device with or without alarm(s) should be installed so that staff can at all times ensure that proper directional airflow into the laboratory room is maintained.
8. The building ventilation system must be so constructed that air from the containment laboratory – Biosafety Level 3 is not recirculated to other areas within the building. Air may be high-efficiency particulate air (HEPA) filtered, reconditioned, and recirculated within that laboratory. When exhaust air from the laboratory (other than from biological safety cabinets) is discharged to the outside of the building, it must be dispersed away from occupied buildings and air intakes. Depending on the agents in use, this air may be discharged through HEPA filters. A heating, ventilation, and air-conditioning (HVAC) control system may be installed to prevent sustained positive pressurization of the laboratory. Consideration should be given to the installation of audible or clearly visible alarms to notify personnel of HVAC system failure.
9. All HEPA filters must be installed in a manner that permits gaseous decontamination and testing.
10. Biological safety cabinets should be sited away from walking areas and out of crosscurrents from doors and ventilation systems.
11. The exhaust air from Class I or Class II biological safety.

which will have been passed through HEPA filters, must be discharged in such a way as to avoid interference with the air balance of the cabinet or the building exhaust system.

12. An autoclave for the decontamination of contaminated waste material should be available in the containment laboratory. If infectious waste has to be removed from the containment laboratory for decontamination and disposal, it must be transported in sealed, unbreakable and leakproof containers according to national or international regulations, as appropriate.

13. Backflow-precaution devices must be fitted to the water supply. Vacuum lines should be protected with liquid disinfectant traps and HEPA filters, or their equivalent. Alternative vacuum pumps should also be properly protected with traps and filters.
14. The containment laboratory – Biosafety Level 3 facility design and operational procedures should be documented.

ii. Biosafety Level 3+ (BSL3+): This biosafety level is also referred to as "Biosafety Level 3 Enhanced". Work conducted at BSL3+ usually involves work with biological agents that would normally be conducted at BSL3; however, the work may involve certain conditions which would necessitate an increased amount of precautions. Examples would include using higher than normal volumes of culture, higher concentrations of culture, and/or production of aerosols. Also, some organisms require work at this containment (e.g., High Pathogenic Avian Influenza, 1918 Pandemic Influenza). The increased amount of precautions includes elements which would normally be used for work at biosafety level 4, which would typically mean the requirement to shower prior to entering the lab and showering prior to leaving the facility.

e. Containment Level 4 (BSL4):

Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is

frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory.

There are two models for BSL-4 laboratories:

1. A Cabinet Laboratory—Manipulation of agents must be performed in a Class III BSC. Passage through a minimum of two doors prior to entering the rooms containing the Class III biological safety cabinet(s) (cabinet
room) is required. In this laboratory configuration the Class III biological safety cabinet provides the primary containment. A personnel shower with inner and outer changing rooms is necessary. Supplies and materials that are not brought into the cabinet room through the changing area are introduced through a double-door autoclave or fumigation

chamber. Once the outer door is securely closed, staff inside the laboratory can open the inner door to retrieve the materials. The doors of the autoclave or fumigation chamber are interlocked in such a way that the outer door cannot open unless the autoclave has been operated through a sterilization cycle or the fumigation chamber has been decontaminated.

2. A Suit Laboratory_ A protective suit laboratory with self-contained breathing apparatus differs significantly in design and facility requirements from a Biosafety Level 4 laboratory with Class III biological safety cabinets. The rooms in the protective suit laboratory are arranged so as to direct personnel through the changing and decontamination areas prior to entering areas where infectious materials are manipulated. A suit decontamination shower must be provided and used by personnel leaving the containment laboratory area. A separate personnel shower with inner and outer changing rooms is also provided. Personnel who enter the suit area are required to don a one-piece, positively pressurized, HEPA-filtered, supplied-air suit. Air to the suit must be provided by a system that has a 100% redundant capability with an independent source of air, for use in the event of an emergency. Entry into the suit laboratory is through an airlock fitted with airtight doors. An appropriate warning system for personnel working in the suit laboratory must be provided for use in the event of mechanical system or air failure

The maximum containment laboratory – Biosafety Level 4 must be located in a separate building or in a clearly delineated zone within a secure building (Controlled access). Entry and exit of personnel and supplies must be through an airlock or pass-through system. On entering, personnel must put on a complete change of clothing; before leaving, they should shower before putting on their street clothing.

Negative pressure must be maintained in the facility. Both supply and exhaust air must be HEPA-filtered (Controlled air system).
All effluents from the suit area, decontamination chamber, decontamination shower, or Class III biological safety cabinet must be decontaminated before final discharge. Heat treatment is the preferred method. Effluents may also require correction to a neutral pH prior to discharge. Water from the personnel shower and toilet may be discharged directly to the sanitary sewer without treatment.

A double-door, pass-through autoclave must be available in the laboratory area. Other methods of decontamination must be available for equipment and items that cannot withstand steam sterilization.
Airlock entry ports for specimens, materials and animals must be provided. Emergency power and dedicated power supply line(s) must be provided. Containment drain(s) must be installed.

Table 1: Summary of Recommended Biosafety Levels for Infectious Agents

BSL	Agents	Practices	Safety Equipment	Laboratory Facilities
1	Not known to consistently cause disease in healthy adults	Standard Microbiological Practices	PPEs: laboratory coats; gloves; eye protection as needed	Open bench top sink required
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: Limited access Biohazard warning signs "Sharps" precautions Biosafety manual defining any needed waste decontamination or medical surveillance policies	Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials, PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: Controlled access Decontamination of all waste Decontamination of lab clothing before laundering Baseline serum	Class I or II BCSs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: Physical separation from access corridors Self-closing, double-door access Exhausted air not recirculated Negative airflow into laboratory
4	Dangerous/exotic agents which pose high risk of life-threatening disease, aerosol-transmitted lab infections; or related agents with unknown risk of transmission	BSL-3 practices plus: Clothing change before entering Shower on exit All material decontaminated on exit from facility	All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air- supplied, positive pressure personnel suit	BSL-3 plus: Separate building or isolated zone Dedicated supply and exhaust, vacuum, and decon systems Other requirements outlined in the text

f. How to choose the appropriate containment level for my research?

Microorganisms and clinical materials are assigned to one of four Biological Safety Levels (BSLs). Each BSL consists of combinations of safety equipment, facility design features, and laboratory practices and techniques that will reduce the risk of laboratory-acquired infections and prevent release of the agent to the environment. The recommended Biosafety level(s) for some organisms can be found in Section VII (Agent Summary Statements) of the BMBL. These BSLs represent the conditions under which the agent ordinarily can be handled safely.

Generally, work with known agents should be conducted at the biosafety level recommended in the BMBL. When the BMBL does not contain agent-specific guidance for a particular organism, the risk assessments for this agent using the best available information must be performed to determined appropriate biosafety level for the research. The risk group of the agent should be one factor considered in association with mode of transmission, procedural protocols, experience of staff, and other factors in determining the BSL in which the work will be conducted.

The laboratory director is specifically and primarily responsible for assessing the risks and applying the appropriate biosafety levels. The institution's Biological Safety Officer (BSO) and Institutional biosafety committees (IBC) can be of great assistance in performing and reviewing the required risk assessment. The IBC may specify practices that are more (or less) stringent than the BMBL or other guidance documents when specific information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered.

h. Conclusion

Biological Safety Levels (BSL) are a series of protections relegated to autoclave-related activities that take place in particular biological labs. They are individual safeguards designed to protect laboratory personnel, as well as the surrounding environment and community.

These levels, which are ranked from one to four, are selected based on the agents or organisms that are being researched or worked on in any given laboratory setting. For example, a basic lab setting specializing in the research of nonlethal agents that pose a minimal potential threat to lab workers and the environment are generally considered BSL-

1the lowest biosafety lab level. A specialized research laboratory that deals with potentially deadly infectious agents like Ebola would be designated as BSL-4—the highest and most stringent level.

Laboratory Associated Infections (LAIs)

Laboratory-acquired infections (LAIs) are defined as all infections acquired through laboratory or laboratory-related activities regardless of whether they are symptomatic or asymptomatic in nature. It is sometimes difficult to determine if a worker's infectious disease is caused by a microorganism that is present in the laboratory only or also in the community. LAIs are of public health concern, as an infected worker may present a risk of transmission to his colleagues, relatives, family members or other citizens.

The Occurrence of LAIs

LAIs can occur in biological facilities such as microbiological or animal facilities during research and investigations. The determination of the source of infection in a laboratory worker can be difficult because the etiological agent is sometimes present in the laboratory and outside the workplace in the population as well.

In addition, LAIs are an important issue in regard to public health, because an infected worker could be a transmission risk for other people. LAIs still occur, although comprehensive reports on LAIs are few, and based on internal reports of the infection laboratory or by official investigation.

Laboratory-acquired infections due to a wide variety of bacteria, viruses, fungi, and parasites have been described. Although the precise risk of infection after an exposure remains poorly defined, surveys of laboratory-acquired infections suggest that Brucella species, Shigella species, Salmonella species, Mycobacterium tuberculosis, and Neisseria meningitidis are the most common causes. Infections due to the bloodborne pathogens (hepatitis B virus, hepatitis C virus, and human immunodeficiency virus) remain the most common reported viral infections, whereas the dimorphic fungi are responsible for the greatest number of fungal infections. The largest survey of infections was reported in 1976 by Pike, who found that 4079 laboratory-acquired infections were due to 159 agents, although 10 agents accounted for >50% of the cases. At least 173 deaths have resulted from laboratory-acquired infection. At the time of Pike's survey, most LAIs (59%) occurred in research laboratories, compared with 17% in diagnostic laboratories. The highest mortality rate (7.8%) was associated with psittacosis. At that time, approximately 70% of LAIs resulted from work with the infectious agents (21%) or animals (17%), exposure to aerosols (13%), and accidents (18%). Less frequent sources of infection included clinical specimens (7%),

autopsies (2%), and contaminated glassware (1%). Most causes of LAI were unknown (82%), and in only 18% of the reported cases could the cause be attributed to accidents, associated with the use of sharps such as needles (25%), injuries by glass (16%), splashes or spills (27%), mouth pipetting (13%), and bites by laboratory animals (14%). Many of the LAIs of unknown origin were likely caused by exposure to an infectious aerosol.

Surveys of diagnostic laboratory workers in the United Kingdom conducted since 1971 have reported that tuberculosis and enteric infections (especially shigellosis) were the most common laboratory-acquired infections. A follow-up survey of UK laboratories from 1994–1995 reported that gastrointestinal infections predominated, particularly shigellosis. Similar results were obtained from a survey of clinical microbiology laboratories in Utah from the period 1978–1992, with shigellosis reported to be the most common laboratory-acquired infection. These results suggest a shift in the pattern of laboratory-acquired infections, with enteric infections predominating. However, no denominator data have been provided that would help determine the actual risk or incidence of infection for laboratory workers. A survey of symptomatic and asymptomatic LAIs has been conducted by Harding and Byers, who reviewed 270 publications from 1979 to 2004, a period during which much has been done to improve laboratory safety while the work load in laboratories increased. A decrease in the number of LAIs would therefore be expected; however, knowledge on the total population at risk and the total number of infections would be needed. Harding and Byers) found a total of 1,448 cases and 36 deaths, 6 of which were aborted fetuses. The infections occurred in clinical, research, teaching, public health, and production facility laboratories, with clinical and research laboratories accounting for approximately 76%.

In a 2002–2004 survey of clinical laboratory directors who participate in ClinMicroNet, an online forum sponsored by the American Society of Microbiology, 33% of laboratories reported the occurrence of at least 1 laboratory-associated infection. The 3 most common laboratory-acquired infections were shigellosis, brucellosis, and salmonellosis. In contrast, the highest incidences of infection were associated with Brucella species (641 cases per 100,000 laboratory technologists, compared with 0.08 cases per 100,000 persons in the general population) and Neisseria meningitidis (25.3 cases per 100,000 laboratory technologists, compared with 0.62 cases per 100,000 persons in the general population). Although LAIs caused by pathogenic bacteria have been described as the most common, LAIs caused by viruses have arisen nowadays

Causes and Factors involved in LAIs

Most LAIs are caused by microorganisms that are very pathogenic or that need a very low infectious dose, including arboviruses, Venezuelan equine encephalitis virus, hantavirus, HBV, HCV, Brucella spp., Coxiella burnetii, Francisella tularensis, Mycobacterium tuberculosis, Salmonella spp., Shigella spp., Chlamydia psittaci, Blastomyces dermatitidis, Coccidioides immitis, Cryptosporidium spp., and organisms causing typhus, streptococcal infections, histoplasmosis, leptospirosis, tularemia, coccidiomycosis, and dermatomycosis .

The most common route of exposure and accidental inoculation: Inhalation (aerosols), percutaneous inoculation (needle and syringe, cuts or abrasions from contaminated items and animal bites), contact between mucous membranes and contaminated material (hands or surfaces), ingestion (aspiration through a pipette, smoking or eating) . Indeed, aerosols have been responsible for major outbreaks of LAIs caused by Brucella spp., Coxiella burnetti (Q fever), Chlamydia psittaci (psittacosis), and M. tuberculosis.

Important factors to consider when assessing the risks for staff working in microbiology laboratory are the following:

- Mode of transmission
- Infectious doses for human
- Persistence or viability of infectious agents in the environment

The main hazards for inoculation include:

- parenteral inoculation,
- inhalation of infectious aerosols, and less commonly,
- accidental oral ingestion and direct contact with mucous membranes or (broken) skin.

Other Potential Hazards and Aerosolization: bloodborne pathogens, careless culturing, autoclaves , allergic reactions , ether and other chemicals , eating in the laboratory , Pipetting , sonication, electroporation , popping tube caps , vortexing , flow cytometry , Centrifugation and Innoculating loops .

Possible causes of LAIs are noncompliance with biosafety measures (e.g. inadequate decontamination or poor hygiene) , ignorance of biological characteristics (e.g. unknown transmission routes and sporulation) and bio- incidence due to human errors (e.g. splashes,

aerosols , needle sticks or cut with sharp and animal scratches and bites)or technical failure (e.g. equipment or infrastructures failure).

Some Causes of LAI's:

1. Training
- Failure to emphasize symptoms in training
- Non-compliance with training
2. Failure to use PPE
3. Biosafety cabinet failure
4. Lab staff are human/human errors/or inadequate training
- Failure to use eyewash after getting serum droplets in eye
- After handling culture cells, put fingers in mouth
5. Exposures due to cross-contamination of stock

Most LAIs in clinical laboratories were probably due to the absence of biosafety containment equipment in a number of these clinical laboratories, or the fact that during the early stages of culture identification, personnel are working with unknowns and may not be using adequate containment procedures. A small proportion of the LAIs resulted from actual accidents as reported aerially. Most were acquired by simply working in the laboratory or by exposure to infected animals. Characteristics of persons who have few accidents include adherence to safety regulations, a respect for infectious agents, "defensive" work habits, and the ability to recognize a potentially hazardous situation. In contrast, persons involved in laboratory accidents tend to have low opinions of safety programs, to take excessive risks, to work too fast, and to be less aware of the infectious risks of the agents they are handling. Also, men and younger employees (17 to 24 years old) are involved in more accidents than women and older employees (45 to 64 years old). While many reports emphasize the importance of personal protection, there are indications that extensive personal protection by use of double gloves, face masks, and protective clothing is not the sole solution, since such measures can reduce the dexterity of the laboratory worker, leading to increased accidents.

Prevention of LAIs

To Prevent LAI occurrences:

- Develop biosafety guidelines for work with pathogens.
- Use non-pathogenic or attenuated bacterial strains.
- Personnel must be aware of potential hazards and trained and proficient in the practices and techniques.
- Watch for symptoms, call health care provider if you or a family member have any of these symptoms.
- Lab coats only leave lab to be cleaned by the institution.
- No food, drinks or personal items like car keys, cell phones and mp3 players used while in the lab.
- Provide students with dedicated writing utensils, paper, and other supplies at each laboratory station.
- Require students and employees to wash their hands before leaving the laboratory.
- Aerosol Control: To protect against aerosols when using BL2 materials:
    i. Centrifuging: Use aerosol-proof rotors or safety caps with O-rings
    ii. Sonicating/homogenizing/using a blender: Use inside a Biosafety Cabinet.
    iii. Vortexing: Keep tubes capped.
    iv. Lyophilizing: Use HEPA filter for exhaust.
    v. Avoid flicking caps open to minimize aerosols.

It is important that each laboratory plan and implement their own pathogen-specific, preventative strategies to improve biosafety and biosecurity. This includes development and application of protocols specific for occupational health and safety incorporating

accident reporting and 'close call' incidents, and pre/post-exposure serological surveys. When working with pathogens, a risk-based approach should be applied for all biosafety programs focusing on pathogen-based factors. The factors to be considered are routes of

infection, infectious dose, quantity and concentration of the agent to determine the most appropriate risk mitigation strategies, such as administrative and engineering controls, and personal protective equipment. Furthermore, annual health checks and vaccinations, post-exposure prophylaxis including reporting and monitoring for post-vaccination adverse events and symptom monitoring, are recommended.

Specific Laboratory-Acquired Infections

- Laboratory-Acquired Brucellosis

Brucellosis has been recognized as one of the most significant causes of LAIs. Reports have shown that many of the infections are acquired through workers being unaware of contaminated/polluted microbial cultures from clinical cases. From 1979 to 2015, brucellosis has been reported as triggering 378 LAIs, and in 80% of the Brucella-associated LAIs, Brucella melitensis was found to be the major causative agent. In their study, Traxler group. revealed that amongst the 167 potential Brucella-exposed employees, 71 developed LAI brucellosis. Improper use of the biological safety cabinets, as well as lack of Brucella spp. (belonging to risk group Level 3) recognition isolated by laboratory staff are the important causes of Brucellosis outbreaks. On the other hand, the onset of laboratory-acquired brucellosis is not always related to the occupational accident but can occur due to direct contact, contaminated skin, needle stick injuries, and splashing in the conjunctivae, or mucous membranes. There are some reported cases of brucellosis infections that occurred following eating or drinking near a culture-processing workbench, and improper individual preventive measures while dealing with the contagious material. Lack of proper awareness for Brucella spp. pathogenicity and insufficient related to handling biohazard materials could also be a causative source to new infections.

- Laboratory-Acquired Tuberculosis

Initial inspections of laboratory-acquired tuberculosis documented the prevalence of

pathogenic Mycobacterium tuberculosis three to nine times higher amongst laboratory employees compared with the general population. Nonetheless, laboratory-associated tuberculosis is extremely challenging to recognize owing to the wide-environmental dissemination of these microorganisms and chronicity of the infection. The extreme

menace of LAI for laboratory staffs handling M. tuberculosis is related to the aerosol's generation. Also, the literature survey revealed some M. tuberculosis cases occurred due to inadequate isolation techniques and high capacities of specimens handled. It is important to handle mycobacteria in class II or III BSC to avoid their associated possible LAI. Recently, Wurtz et al. surveyed laboratory-acquired infections around the world in BSL-3 and BSL-4 laboratories. Out of 23 laboratories, only four reported around fifteen LAIs cases caused by four different pathogenic cultures. These have been classified as BSL-3 bacteria and belong to the species (1) Mycobacterium tuberculosis (ten cases), (2) Coxiella burnetii (two cases), and (3) Brucella melitensis (two cases), while other reported cased were caused by a BSL-2 virus. The laboratory personnel should undergo an annual Mantoux purified protein derivative skin test or an interferon-γ release assay to demonstrate conversion. The persons with positive test results should be further investigated for active tuberculosis by chest radiography.

- Other Bacterial-Associated LAIs

In addition to Brucellosis mentioned above and M. tuberculosis, several other bacterial strains have also been reported to cause LAIs though with lower frequencies. Amid these bacterial agents, Francisella tularensis is a zoonotic infection and usually occurs not only as a glandular ulcer form but also as pneumonia. There are some reported cases of F. tularensis mediated LAIs in the literature, which are more frequently linked to the bacterial cultures rather than on infected animals or clinical material. Considering the adverse consequences of antibiotic-based therapy treatment, appropriate vaccination along with accurate biosafety measures have been recommended as the most valuable tool to control these infections. Microorganisms such as Salmonella or Shigella belonging to Enterobacteriaceae have also been recognized to cause LAIs . Moreover, reports have shown that several other pathogenic agents like Escherichia coli, Clostridium difficile, or Klebsiella spp. may be classified as potential LAIs.

- Viral-Associated LAIs

In recent years, virus research is associated with widespread applications in biotechnological sectors, such as viral diseases, the development of novel vaccines, or GMOs. Despite scarce research investigation concerning virus associated LAIs, pathogenic infections with the human immunodeficiency hemorrhagic virus, West Nile Virus, Dengue, or Marburg virus have

been reported in the literature. Viral agents transmitted through blood and body fluids are responsible for most of the LAIs amongst healthcare employees in diagnostic laboratories. Despite that, the viral hemorrhagic fevers provoke the greatest fear, these viruses are rare causes of laboratory infection. Among the common blood-associated viruses, hepatitis B virus (HBV) is the leading cause of LAIs, and among all health care workers, the incidence of HBV infection in the United States is approximated to be 3.5–4.6 infections per 1000 workers . Encouragingly, among the laboratory staffs, there were no reported cases of HBV infection in the two most recent inspections of LAIs in the UK. These findings emphasized the implementation of universal precautions while handling blood specimens, developments in needleless devices, and the appropriate vaccination.

Because hepatitis B is a vaccine-preventable disease, all laboratory workers should be offered the hepatitis B vaccine without charge. Nonimmunized laboratory workers who have

percutaneous, ocular, or mucous membrane exposure to contaminated blood should receive PEP with hepatitis B immunoglobulin and vaccine.

During 2005–2006, there were 802 confirmed cases of hepatitis C (HCV) reported to the Centers for Disease Control and Prevention, with five occupational exposures to blood. However, very few data were found on the occurrence of HCV among laboratory employees with only one case in the US and UK.

Human immunodeficiency virus (HIV) infection related to contaminated blood or body fluids exposure consequences the paramount apprehension. From 1981 to 1992, HIV reports revealed a total of 32 healthcare workers in the US with occupationally acquired HIV infection. Among these, 25% of health care staffs were noted to be laboratory workers. Therefore, correct biosecurity and biosafety procedures, immune control approaches, education and training, and specialized laboratory facilities should be adopted to reduce the potential risk of LAIs or viral-associated diseases.

- Parasites Associated LAIs

Parasite-associated LAIs are uncommon in the diagnostic microbiology laboratories. Among the parasitic infections, LAIs caused by Leishmaniasis, fascioliasis, malaria, toxoplasmosis, trypanosomiasis, or schistosomiasis have been found to be the most adverse forms. Nearly, 313 cases of LAIs, with a variety of blood and intestinal protozoa, have been reported. Many of these cases occurred in reference and research laboratories. Among laboratory

staffs and healthcare employees, a total of 52 malaria cases have been reported, with 34 cases reviewed by Herwaldt. Out of these, 10, 9, and 15 cases were caused by Plasmodium cynomolgi, P. vivax and P. falciparum, respectively. The direct contact or exposure to parasites in the laboratory presumably increases the potential risk for acquiring parasitic infections. Several causes including needlestick injuries, barehanded work in the open field are the common means associated with parasitic LAIs. Since parasitic diseases are commonly characterized by a prolonged asymptomatic period, laboratory employees working with parasites are advised to be tested intermittently. Additionally, exceptional attention must be taken for childbearing women due to the hereditary transmission of some protozoan parasites.

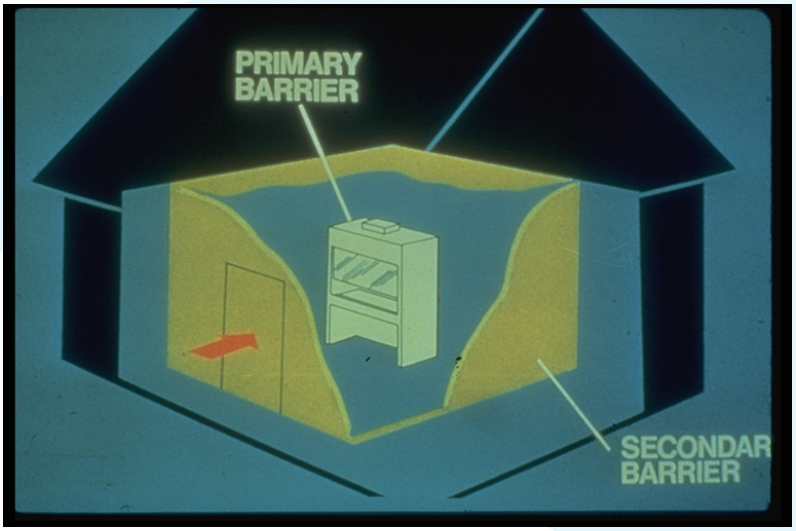
- Fungal-Associated LAIs

The dimorphic fungi, Blastomyces dermatitidis, Coccidioides immitis, and Histoplasma caspsulatum are the primary causative agents for most of the fungal-associated LAIs in the US. Although cutaneous infections are reported due to accidental inoculation, the majority of the LAIs occurred because of inhaling infectious conidia that led to pulmonary infection. The risk of fungal infection is probably lower in the mycology laboratories, because specimen handling is carried out in laminar-flow biological safety cabinets (BSCs), and culture plates are also sealed to avoid accidental opening. Nevertheless, infection risk is likely to increase on the aerobic culture bench because B. dermatitidis and C. immitis colonies can grow on conventional culture media within 2–3 days. Therefore, clinicians suspecting dimorphic fungal-associated infections should immediately alert the microbiology laboratory.

Biosafety Cabinets (BSCs)

Biological Safety Cabinets (BSCs) are among the most effective primary containment devices used in laboratories working with infectious agents. They act as primary barriers, when appropriate practices and procedures are followed, to prevent the escape of biological aerosols into the laboratory environment. This is an important function, because most laboratory techniques are known to produce inadvertent aerosols that can be readily inhaled by the laboratory worker.

They should be used in conjunction with good laboratory techniques, primary containment equipment and proper containment facility design. They replace work on open bench using personal protection equipment (PPE) as primary barrier. A BSC is used for work with Risk Group 2 agents that may generate infectious aerosols: opening tubes, inoculating loop, pipetting, opening sealed centrifuge tubes, mixing, and homogenizing, needles and syringes, pouring infectious materials.



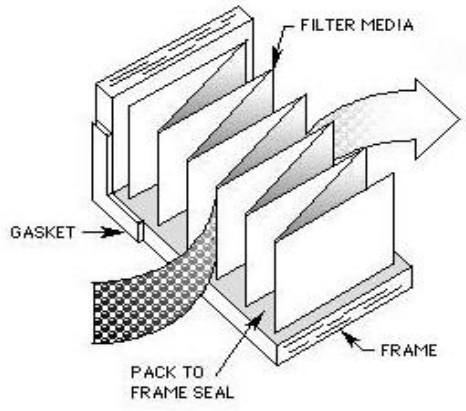
Three kinds of biological safety cabinets, designated as Class I, II and III, have been developed to meet varying research and clinical needs.

Most BSCs use high efficiency particulate air (HEPA) filters in the exhaust and supply systems. BSCs should be located away from doors, drafts, convection currents, diffusers, and high traffic areas. Proper maintenance of BSCs cannot be over emphasized. Biosafety Officers (BSOs) should understand that an active cabinet is a primary containment device

A BSC must be routinely inspected and tested by training personnel, following strict protocols, to verify that it is working properly. This process is referred to as certification of the cabinet and should be performed annually. Horizontal and vertical outflow cabinets ("clean-air workstations") are not biological safety cabinets and should not be used as such. They are used to protect a process, rather than the operator.

- The High Efficiency Particulate Air (HEPA) Filter

Control of airborne particulate materials became possible with the development of filters, which efficiently removed microscopic contaminants from the air and ensure that only microbe-free exhaust air is discharged from the cabinet. The HEPA filter was developed to create dust-free work environments. HEPA filters remove the most penetrating particle size (MPPS) of 0.3 µm with an efficiency of at least 99.97%. Bacteria, spores and viruses are removed from the air by these filters. HEPA filters do not filter out gasses and vapors.



- The Three Biological Safety Cabinet Classes (see figures at the end)

Class I

A Class I BSC provides only protection to the operator and the environment.
There is little to no protection to the product being manipulated. The HEPA filter in a Class I biological safety cabinet filters the air as it exits the cabinet. The air is drawn in through the opening in front access opening. The air flow into the cabinet is the "dirty room" air and flows across the work surface. The personal protection of a Class I cabinet is provided by the air flow into the cabinet and away from the operator. A Class I BSC is suitable for work with low risk materials.

Class II

A Class II BSC must provide protection to the operator, the environment and the product being used. Class II BSCs are further divided up into two categories, types A and B.
The types are then further divided. The knowledge of what the different types of Class II BSCs will aid in the selection of the proper cabinet for the work being performed.
Type A cabinets have a 70%/30% split in the amount of air recirculated within the cabinet and what is exhausted. In this case 70% of the air entering the cabinet would be recirculated and 30% would be exhausted through the HEPA system. Class II B1 has a 60%/40% split and the Class II B2 has a 100% exhaust with no recirculation.
Chemicals and radiologic should be manipulated in a fume hood. BSCs have limitations when manipulating chemicals and radiologic. Also, BSCs are not spark-proof. A build-up of vapours may cause an explosive atmosphere to accumulate inside the BSC. Fumes or vapours may pose a health hazard to the individuals in the laboratory. Again, for the use of chemical and radiologic a fume hood is the best practice.

Class III

Class III BSCs provide the highest level of protection of all. The Class III cabinets are a fully contained cabinet. To access the inside of the cabinet, there are full-length glove ports attached to the cabinet. The use of HEPA filters is employed for the air entering and the air exiting the cabinet.
The exit HEPA must be a double HEPA or can be a single and be associated with an incineration system.

- Selection of a Biological Safety Cabinet

A BSC should be selected primarily in accordance with the type of protection needed: product protection; personnel protection against Risk Group 1-4 microorganisms; personnel protection against exposure to radionuclides and volatile toxic chemicals; or a combination of these. Whether new or used, proper maintenance of BSCs will go a long way in assuring effective use of the containment device. Use of a certified contractor is recommended. Because of the size and weight of BSCs, there are specific considerations that apply to moving BSCs in order to assure that this is achieved safely and without spreading contaminants.

Selection of a Biological Safety Cabinet by Type of Protection Needed (WHO Biosafety Manual)

Type of protection	BSC selection
Personnel protection, microorganisms in risk group 1-3	Class I, Class II, Class III
Personnel protection, microorganisms in risk group 4, glove-box laboratory	Class III
Personnel protection, microorganisms in risk group 4, suit laboratory	Class I, Class II
Product protection	Class II, Class III only if laminar flow included
Volatile radioactive/chemical protection, minute amounts	Class IIB1, Class IIA2 vented to the outside
Volatile radioactive /chemical protection	Class I, Class IIB2, Class III

- Proper Use of a Biological Safety Cabinet

Start-Up Considerations

- The cabinet should be certified yearly to ensure proper function.
- Ensure that the sash is at the appropriate height.
- Adjust stool height so that underarms are level with the bottom of the sash.
- Check the pressure gauges to ensure that readings are within the acceptable range.
- If present, test the airflow alarm and ensure it is switched to the "on" position.
- Confirm inward airflow by holding a tissue at the middle of the edge of the sash to ensure that it is drawn in.
- Disinfect the interior surfaces with a disinfectant effective against the infectious material and toxins in use in the laboratory. If a corrosive disinfectant must be used, the surface should be rinsed with water after disinfection.
- Assemble all materials required for manipulation and load into the BSC.
- Care should be taken not to overcrowd or block the front or rear grilles to ensure that the appropriate airflow patterns are not compromised.

- When there is significant potential for splatter or splashes to occur during manipulations of infectious material or toxins, the work area should be lined with a plastic-backed absorbent pad.
- Place aerosol generating equipment (e.g., mixers, vortex) towards the back of the BSC, without blocking the rear grille.
- After loading material in the BSC, allow sufficient time for the airflow to stabilize before initiating work.

Working in the BSC

- Perform operations as far to the rear of the work area as possible. Ensure that elbows and arms do not rest on the grille or work surface.
- Avoid excessive movement of hands and arms through the front opening. Such movements disrupt the air curtain at the front of the BSC, which can allow contaminants to enter or escape the BSC. Arms should enter/exit the BSC slowly and perpendicular to the front opening.
- Keep a bottle of an appropriate disinfectant in the BSC while work is performed to avoid having to move hands outside of the BSC.
- Segregate non-contaminated ("clean") items from contaminated ("dirty") items.
- Work should always flow from "clean" to "dirty" areas.
- Material should be discarded in a waste container located towards the rear of the cabinet workspace. Do not discard materials in containers outside of the cabinet.
- Decontaminate the surface of all objects in the BSC in the event of a spill.
- The work area should be decontaminated while the BSC is still in operation.
- Open flames in the BSC create turbulence, disrupt airflow patterns, and can damage the HEPA filter. Consequently, sustained open flames in BSCs are prohibited, and on-demand open flames are to be avoided. Non-flame alternatives (e.g., microincinerators, or sterile disposable inoculation loops) should be used whenever possible. However, on-demand open flames (e.g., touch-plate microburners) may be used as the duration of time for which the flame is produced can be controlled and limited. Natural gas and propane should not be used in a BSC.
- Work in a BSC should only be conducted by one person at a time.
- Equipment creating air movement (e.g., vacuum pumps, centrifuges) may affect the integrity of the airflow and should not be used within the BSC.

- Windows that open should be kept closed when the BSC is in use.

Completion of Work in the BSC

- Upon completion of work, allow sufficient time for the air in the BSC to pass through the filter before disrupting the air curtain by removing hands or unloading material from the BSC.
- Close or cover all containers.
- Decontaminate the surface of items before removing them from the BSC.
- Disinfect the interior surfaces of the BSC, including sides, back, and interior of the glass, with a disinfectant effective against the agents in use.
- If a corrosive disinfectant is used, the surface should be rinsed with water after disinfection to avoid corrosion of the stainless-steel surfaces.
- Routinely remove the work surface and disinfect the tray beneath it.
- Routinely wipe the surface of the lights within the BSC with ethanol.

NB: Ultraviolet Light Considerations

UV lamps are not required or recommended in BSCs. The use of UV irradiation germicidal lamps is strongly discouraged due to their limited effectiveness at disinfecting the inside of BSCs. Personnel wishing to use UV irradiation in BSCs should receive training on the safe work practices required and the hazards of UV radiation beforehand.

Biohazardous or Infectious Waste & Regulated Medical Waste

Biohazardous waste defines as infectious or physically dangerous medical or biological waste that because of its characteristics may cause, or significantly contribute to, an increase in mortality or an increase in serious irreversible or incapacitating reversible illness; or pose a substantial present potential hazard to human health or the environment when improperly treated, stored, transported, disposed of, or otherwise managed.
The types of infectious or physically dangerous medical or biological waste are:

1- **Sharps waste:** Discarded medical/research articles that may cause puncture or cuts, including but not limited to all, used and discarded hypodermic needles and syringes, Pasteur pipettes, broken medical glassware, scalpel blades, disposable razors, and suture needles.
2- **Microbiological Wastes:** Laboratory wastes containing or contaminated with concentrated forms of infectious agents. Such waste includes discarded specimen cultures, stocks of etiologic agents, discarded live and attenuated viruses, blood or body fluids known to contain infectious pathogens, wastes from the production of biological and serums, disposable culture dishes, and devices used to transfer, inoculate and mix cultures .
3- **Human blood and blood products:** All human blood, blood products (such as serum, plasma, and other blood components) in liquid or semi-liquid form. Items contaminated with blood that, if compressed, would release blood in a liquid or semi-liquid form, or items caked with dried blood capable of being released during handling. Other body fluids or tissues containing visible blood.
4- **Human Body Fluids:** Human body fluids in a liquid or semi-liquid state, including semen, vaginal secretions, cerebral spinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, and saliva from dental procedures. Also includes any other human body fluids visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids.
5- **Pathological waste:** All human tissues, organs, and body parts, including waste biopsy materials, tissues, and anatomical parts from surgery, procedures, or autopsy.

6- **Animal waste:** All animal carcasses, body parts, and any bedding material from animals known to be infected with, or that have been inoculated with human pathogenic microorganisms infectious to humans.
7- **Recombinant DNA Disposal:** Recombinant DNA -containing materials (i.e. cultures, microbes, plasmids, plants) , cultures and stocks of recombinant DNA.

The Bloodborne Pathogens Standard uses the term, "regulated waste," to refer to the following categories of waste: liquid or semi-liquid blood or other potentially infectious materials (OPIM); items contaminated with blood or OPIM and which would release these substances in a liquid or semi-liquid state if compressed; items that are caked with dried blood or OPIM and are capable of releasing these materials during handling; contaminated sharps; and pathological and microbiological wastes containing blood or OPIM.
Different Names for Medical Waste
Medical waste goes by several names that all have the same basic definition. All the terms below refer to waste created during the healthcare process that is either contaminated or potentially contaminated by infectious material.

- Medical Waste
- Biomedical Waste
- Clinical Waste
- Biohazardous Waste
- Regulated Medical Waste (RMW)
- Infectious Medical Waste
- Healthcare waste

The terms are used interchangeably, but there's a distinction between general healthcare waste and hazardous medical waste. The WHO classified the medical waste into the following categories of medical waste:

1. **Infectious waste:** Discarded materials and biological waste products that may transmit infection from virus, bacterial, parasites to human, i.e.: lab cultures, tissues, swabs, equipment and excreta.
2. **Sharps waste:** Sharps are anything that can pierce or cut the skin. This includes needles, syringes with needles, lancets, scalpels, wires, staples, broken glass, etc.

3. **Pathological waste:** Any recognizable human body part, organs and tissue, or any animal body part, organ or tissue contaminated or suspected to be contaminated by a zoonotic disease.
4. **Radioactive –** Unused liquid in radiotherapy or lab research, contaminated glassware, etc.

5. **Pharmaceuticals –** Expired and contaminated medicines, residual chemotherapy medications (less than 3% of the original amount), syringes, and items utilized in the preparation of chemotherapy infusions are all required to be disposed of as regulated medical waste.

6. **Genotoxic Waste.** This is a highly hazardous form of medical waste that's either carcinogenic, teratogenic, or mutagenic. It can include cytotoxic drugs intended for use in cancer treatment.

7. **Chemical –** Expired lab reagents, film developer, disinfectant. solvents used for laboratory purposes, batteries, and heavy metals from medical equipment such as mercury from broken thermometers.

8. **Pressurized containers –** Gas cylinders and gas cartridges.

9. **General waste –** No risk to human health because no blood or any related bodily fluid, i.e.: office paper, wrapper, kitchen waste, general sweeping, etc.

Collection and Segregation of Biohazardous Waste

Waste collection is a component of waste; it eliminates the possibility of interaction with humans, animals or the environment hence minimizing exposures to medical waste. During waste collection, ensure;

- Wastes do not accumulate at the point of production.
- Waste bags are tightly closed or sealed when they are about three-quarters full before
- transportation and should not be closed by stapling
- Sharps containers are puncture resistant closable, leak proof and should be sealed and disposed when ¾ full.

Segregation is the separation of different types of wastes based on risks levels, it is the most crucial step in regulated medical waste management. Regulated medical waste should be segregated by placing it in color-based code bags supported in bins in matching color to minimize damage and retain spillage.

Effective segregation alone can ensure:

- effective regulated medical waste management,
- reduces the amount of waste needs special handling and treatment,
- prevents the mixture of medical waste like sharps with the general municipal waste,
- prevents illegally reuse of certain components of medical waste like used syringes, needles, and other plastics.
- Provides an opportunity for recycling certain components of medical waste like plastics after proper and thorough disinfection.
- Recycled plastic material can be used for non-food grade applications.
- Of the general waste, the biodegradable waste can be composted within the hospital premises and can be used for gardening purposes.
- Recycling is a good environmental practice, which can also double as a revenue generating activity.
- Reduces the cost of treatment and disposal.

Containers for Regulated Medical Waste

i. Obtaining and Discarding Containers

The collection of regulated biomedical wastes (RMW) involves use of different types of container from various sources of regulated biomedical wastes. Color-coded waste holding bags or containers can be used to differentiate between types of waste. All biohazardous

waste must be labeled with the universal biohazard symbol (see below) and the word `Biohazard.' Additional information, such as the type of waste (such as "sharps" or "liquid waste") and origin of the waste, is recommended.

Colored bags made of non-chlorinated plastic with biohazard sign and labels mentioning date and details of waste are to be used. Containers must be appropriate for the contents; not leak; be properly labeled; and maintain their integrity if chemical or thermal treatment is used.

For laboratories, the preferred method of collection for solid RMW at the lab bench is a small, red bag, or a rigid container lined with a small red bag that can be closed (either by tying the bag, or lid covering the container). Small, bench top containers must be emptied into the larger solid waste containers either when full or not in use.

For clinical areas, the solid RMW does not need to be autoclaved or chemically disinfected. RMW can be collected in small/temporary containers or can be placed directly into the large RMW bin/ box as appropriate.

When temporary containers (bench top or floor model) are full, the bags must be pulled out by lab or clinic personnel, closed/sealed, labeled with an inner container label and placed in a RMW bin/ box. The RMW bin or box is then closed/sealed.

Metal sharps should be Placed in a rigid, puncture resistant container (heavy walled plastic is recommended) and labeled " SHARPS". Broken glassware should be place in a rigid, puncture resistant container (plastic, heavy cardboard or metal), seal securely and clearly label "BROKEN GLASS".

Liquids should be placed in leak-proof containers able to withstand thermal or chemical treatment, culture transfer devices, blood-soaked items, and other paper or cloth related items must be collected in autoclave bags or red RMW liner bags. Do not chop, bend, break or otherwise destroy hypodermic needles or syringes before discarding them into the sharp's container.

For nonhazardous material heavy duty plastic bags or other appropriate container without a Biohazard label are preferred. Red or orange biohazard bags or containers should not be used for nonhazardous material. Containers of biohazardous material should be kept closed.

The latest guidelines for segregation of biomedical waste recommend the following color coding:

- **Red Bag -** Syringes (without needles), soiled gloves, catheters, IV tubes etc. should be all disposed of in a red colored bag, which will later be incinerated.

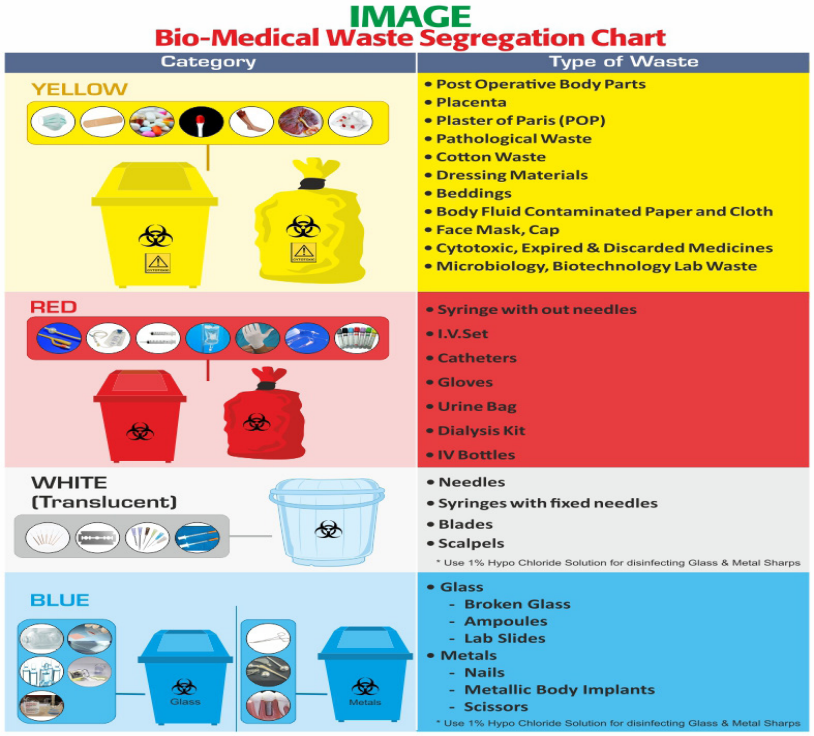
- **Yellow Bag -** All dressings, bandages and cotton swabs with body fluids, blood bags, human anatomical waste, body parts are to be discarded in yellow bags.
-

- **Cardboard box with blue marking -** Glass vials, ampules, other glass ware is to be discarded in a cardboard box with a blue marking/sticker.

- **White Puncture Proof Container (PPC) -** Needles, sharps, blades are disposed of in a white translucent puncture proof container.
- **Chemotherapy Sharps Container -** Cytotoxic and Genotoxic Waste include chemotherapy needles, syringes, iv catheter, sutures, broken glasses & scalpels

- **Black Bags -** These are to be used for non-biomedical waste. In a hospital setup, this includes stationary, vegetable and fruit peels, leftovers, packaging including that from medicines, disposable caps, disposable masks, disposable shoe-covers, disposable teacups, cartons, sweeping dust, kitchen waste etc.

Waste bags should be supported in bins in matching color to minimize damage and retain spillage. See chart below for biomedical waste segregation.



ii. Storage and Containment

Generally, it is not necessary to treat RMW before placing it in the outer container (RMW box/bin) for ultimate disposal. However, the laboratories working with human pathogens regulated by the CDC or NIH at Biosafety Level 2 or higher must autoclave or chemically disinfect their waste prior to placing the waste into RMW boxes/bins for collection by the RMW vendor.

Outer containers must be stored in a secure area protected from the elements, high temperatures, vandalism, insects, and rodents. Unauthorized personnel must be denied access to this designated storage area.

This place should at the minimum have the following: -

a. Store waste in a designated location with limited access.
b. Floors should be impervious to liquid and room with good ventilation to control odors.
c. Keeping storage area clean will keep vermin and other vectors away.
d. Post the area prominently with the universal biohazard symbol.
e. Storage of regulated waste should be at a minimum amount of time and should be specified in the institution waste management plan.

Containers must be sealed securely, and stored in a freezer, refrigerator, or cold room to prevent spillage, putrescence, or the leaking of vapors. Liquids (e.g. blood) must be put into containers that are packaged with a sufficient amount of surrounding absorbent material to absorbent leakage. Volumes of liquid may not exceed 20cc per individual container. Storage of non-inactivated waste is restricted to within the generating laboratory. The material may not be stored longer than 24 hours prior to inactivation.

Packaging

Packaging of regulated waste can be described as containment of waste and is used to ensure the safety, protection of personnel and environment. This can be achieved by: -

a. Ensuring all regulated waste is sealed properly with tape.
b. Placing bags in upright position to prevent spillage of liquid.
c. Storing regulated waste in approved red plastic bags that are impervious to moisture, puncture resistant, and display the distinctive biohazard symbol.
d. Using durable reusable containers for storage of regulated waste and ensure containers are cleaned and decontaminated with approved disinfectant each time they are emptied.
e. Using packaging that maintains its integrity during storage and transport.

See Appendix 2: Proper Regulated Medical Waste Packaging Procedures.

Many types of biohazardous material can simply be decontaminated or disinfected and subsequently handled as normal waste for disposal through the municipal waste stream or down the sewer. The preferred method is steam sterilization (autoclaving).

Transportation

The waste should be transported for treatment either in trolleys or in covered wheel-barrow. The bags / Containers containing biomedical waste should be tied/ lidded before transportation. Before transporting the bag containing biomedical waste should be accompanied with a signed document mentioning date, shift, quantity, and destination. The workers transporting the waste use PPEs like boots, gloves, masks and aprons. The

collected waste is not stored for more than 48hrs. at collection site .

Figure: Storage and Transport of biomedical waste

Final Transport of biomedical waste must be by authorized vehicle with appropriate documentation for further record. Special vehicles must be used to prevent access to, and direct contact with, the waste by the transportation operators, the scavengers and the public. The effects of traffic accidents should be considered in the design, and the driver must be trained in the procedures he must follow in case of an accidental spillage.

Figure: Vehicle for transporting Biomedical Waste

See Table 1 for Summary of medical waste storage and treatment.

Table 1: Summary of medical waste storage and treatment

Type	Container	Storage	Treatment
Sharp waste	Sharps container. Do not recap or bend needles.	Dispose of closed containers within 7 days.	Off-site treatment.
Solid waste	Place two red biohazard bags in a secondary container.	Dispose of biohazard waste within 7 days of accumulation	Off-site treatment.
Liquid waste	Collect liquid waste in a flask with a stopper. Store flask in a container capable of holding entire volume of flask if outside of biosafety cabinet	Maximum storage time is 7 days.	Treat with household bleach (1 bleach : 9 water) with 30 minute contact time
Animal waste	Place carcass in a clear colorless bag. Transfer to red pathological waste container with a red bag liner and tight-fitting lid	Store in refrigerator or freezer. Maximum storage time is 7 days.	Off-site incineration
Pathological waste	Place not recognizable tissue in a clear colorless bag. Transfer to red pathological waste container with a red bag liner and tight-fitting lid.	Store in refrigerator or freezer. Maximum storage time is 7 days.	Off-site incineration

Disposal of Biohazardous Material or Waste

i. Introduction

A local waste management plan for the disposal of biohazardous waste should be developed. Standard operating procedures should include Safe Work Procedures for waste handling and segregation, spills management, training of staff and regular review and update of waste management plan, all contaminated materials, solid or liquid, must be decontaminated before disposal or reuse. Waste material treated correctly is rendered non-infectious and may be discarded through the sewer, in the case of liquids, or through the regular garbage disposal for solid wastes.

Disposal occurs off-site, at a location that is different from the site of generation. Treatment may occur on-site or off-site. On-site treatment of large quantities of biohazardous waste usually requires the use of relatively expensive equipment and is generally only cost effective for very large hospitals and major universities who have the space, labor and budget to operate such equipment. Off-site treatment and disposal involve hiring of a biohazardous waste disposal service whose employees are trained to collect and haul away biomedical waste in special containers for treatment at a facility designed to handle biomedical waste.

ii. Procedures for Disposing of Biohazardous Medical Waste

It is the responsibility of the research unit to develop suitable procedures for treating the biohazardous waste it produces, bearing in mind that each research program has unique requirements and problems. The method used in each lab should be proven effective in that lab.

The primary methods for disposing of biohazardous waste include:

a) Sanitary Landfill

An earth pit isolated from ground water to protect it from pollution. The base is equipped with a network for water discharging resulting from rain and decomposition of organic materials. A layer of gravel and sand is placed over the network to facilitate entry of water into the drainage network. Waste is primary treated, distributed on the base of the hole and pressed to reach the amount of 0.8 - 1.0 tons per m2.

Advantages:

1. Low cost and ease of application, no high-tech.
2. Absorb massive amounts of solid wastes.

3. Replanting the area with trees.
4. Access to methane.

Disadvantages:

1. Leakage of air pollutant gases: methane, carbon dioxide.
2. Possibility of contamination of water sources by wastewater resulting from landfill.

b) Incineration

Able to treat many types of medical waste, except: Radioactive materials, mercury, and compressed containers. The best way is to burn at a temperature of 1200 C for two seconds only, burning in bad incinerators or burn randomly should be avoided, this leads

to the formation of dangerous dioxin. Before 1997, over 90% of all infectious medical waste was disposed of by incineration. later this is still the only method used on pathological waste, for example body parts and recognizable tissues.

Advantages of Incineration

1. Complete destruction of pathogens
2. No odour trouble

Disadvantages:

1. Incineration is not a solution to the problem; it is a simple transfer of pollutants from waste itself to the smoke and ash.
2. The ash contaminates soil and groundwater.

c) Sterilization

The recent methods for safe disposal of medical wastes. This method has become a trend in the world

Sterilization ways:

i. **Autoclaving is** usually the most convenient choice for labs since autoclaves are readily available throughout most research laboratory buildings. Environmentally safe for all medical wastes except anatomical parts and animal contaminated bodies because the steam cannot penetrate.

Autoclaving renders biohazardous waste non-infectious. After it's been sterilized, the waste can be disposed of normally in solid waste landfills, or it can be incinerated under less-stringent regulation.

ii. **Microwaving:** Another way to render biohazardous waste non-hazardous is to microwave it with high-powered equipment . As with autoclaving, this method opens up the waste to normal landfill disposal or incineration afterward.

d) **Chemical disinfection** is a treatment option for liquid biological waste. An example is household bleach, the disinfectant must be effective against the biological material it is treating. The appropriate contact time must be allowed for effective disinfection / inactivation.

Categories of Biological Waste and Acceptable Treatment:

- **Solid Waste**

Solid biohazardous waste (e.g. contaminated plastic flasks, tubes, etc.) should either be surface decontaminated by chemical means, or autoclaved or incinerated as appropriate. (e.g., contaminated plastic bottles may be decontaminated by soaking in bleach prior to disposal as non-biohazardous waste).

Solid tissues must be incinerated. Human cadaver material has special provisions to be buried.

- **Liquid Waste**

Most aqueous liquid biohazardous waste generated from biological research activities (after disinfection) are suitable for sewer disposal. Most liquid biohazardous waste must be:

a. decontaminated, this can be in one of two ways:
   i. In a 10% dilution (final) of household bleach for 30 minutes, after which waste can be disposed of down the drain.
   ii. By autoclaving, but such waste must not contain chemical hazards incompatible with autoclaving – do not autoclave bleach.

b. and it must be covered securely during transport,
c. labeled clearly with a contact name and identification of contents.
d. When liquid waste is transported in the hallways, it should be covered and placed in an unbreakable container on a cart with sides to prevent contamination of hallways.

Liquid waste including bulk blood and blood products, cultures and stocks of etiologic agents and viruses, cell culture material and products of recombinant DNA technology should be disinfected by thermal or chemical treatment then discharged into the Sewer System.

- **Metal sharps**

Discarded sharps (contaminated or not) that may cause puncture or cuts, must be contained, encapsulated, and disposed of in a manner that prevents injury to laboratory, custodial and landfill workers. needles, blades, etc., are considered biohazardous even if they are sterile, capped and in the original container.

Disposal Method:

1) Encapsulate (solidify) in a properly labeled, puncture resistant container; sent to Landfill site.
2) Needles, such as those used for gas chromatography, should be thoroughly rinsed to remove hazardous chemicals, then disposed with non-contaminated broken glassware.
3) Do not attempt to recap, bend, break or cut discarded needles.

- **Pasteur pipets and broken glassware:**

i. Contaminated with biohazardous material:

a. Place in a properly labeled, leak proof and puncture resistant container; disinfect by thermal or chemical treatment; sent to Landfill site.

; or

b. Encapsulate in a properly labeled, rigid, puncture resistant container, and sent to Landfill site.

Note: Encapsulation is required if glass is commingled with metal sharps.

ii. Not contaminated with biohazardous material:

a) Place in a puncture resistant container, then sent to Landfill site. The container must be clearly labeled to indicate that it contains broken glass.
b) Do not incinerate glassware.

- **Plastic waste:**

i. Contaminated with biohazardous material:
Place in a properly labeled, leak proof container; disinfect by thermal or chemical treatment; sent to Landfill site.

ii. Not contaminated: sent to Landfill site .

- Animal carcasses and body parts:

Research animal carcasses and parts must be disposed via incineration. Animal diagnostic specimen shipping containers, if contaminated, must be treated by autoclaving or chemical treatment prior to disposal, incinerated.

All animal waste, including bedding, that is infectious or harmful to animals, humans, or the environment, should be appropriately treated prior to disposal, regardless of the origin of contamination. The following disposal methods are acceptable:

1. Preferred Method: incineration followed by deposition of the residual ash in the Landfill.
2. Thermal or chemical disinfection followed by deposition in the Landfill.

- **Human pathological waste:**
  1. Human cadavers, recognizable body parts: must be cremated or buried.
  2. Other pathological waste from human and higher primates must be incinerated.

- **Disposal of Radioactive Wastes:** The radioactive material kept in an insulated and sealed bowl, then set aside to lose half-radioactive life. The material Kept in a more compact and insulating box, and then buried in the soil.

Mixed Waste

Certain research protocols may generate hybrid waste materials that are mixtures of two or more categories of waste: biological, chemical, and/or radioactive wastes. Some lab analyses may involve treatment or exposure of biological materials to chemical compounds or radioactive materials. Examples may include radioisotope labeling of genetic material in culture or cells, and exposure of cells or research animals to carcinogens or diagnostic processes involving radiation hazards. In these situations, mixed wastes are likely to be produced that will require special consideration for collection, handling, and disposal.

Biohazardous waste treatment and disposal techniques alone are not likely be suitable for "mixed" wastes. When planning studies that will generate "mixed wastes", the requirements for collection and decontamination of mixed wastes should be discussed prior to their generation.

For waste which is a mixture of chemical/radioactive and biohazardous waste, it is might be possible to destroy the biohazard first by chemical means and then treat and/or

dispose of the waste as appropriate for chemical or radioactive waste. However, when some chemical hazards are present, disinfectant (eg. bleach) will create a worse chemical hazard. Mixed waste should not be autoclaved and should not be sent for incineration without consultation.

The following strategies should be considered when dealing with mixed wastes:

- endeavor to minimize the production of mixed wastes.
- assess the risks associated with the hazards.
- minimize the hazard – e.g. use water-based chemicals instead of solvent-based chemicals.
- choose an appropriate disposal option, and if possible, a single option (e.g. incineration);
- identify multiple statutory requirements.
- ensure that laboratory staff are adequately trained in waste management.

Treatment and disposal of mixed waste must ensure that all hazards are appropriately addressed.

Requirements for Recombinant DNA Disposal

Recombinant DNA waste includes the following categories:

- Cultures and stocks of rDNA, and
- culture dishes and devices used to transfer, inoculate, and mix cultures of rDNA.

Recombinant DNA -containing materials (i.e. cultures, microbes, plasmids, plants) must be decontaminated or inactivated before disposal. Although generally non-infectious, biosafety level 1 recombinant DNA waste requires adequate decontamination prior to disposal. Similarly, rDNA and transgenic organisms must be treated the same as medical or infectious waste before disposal.

Chemical treatment with an appropriate disinfectant is an acceptable method of inactivation. Autoclaving of recombinant nucleic acid -containing materials is also acceptable.

Appropriate waste handling practices are detailed below:

i. **Solid wastes** contaminated by microorganisms or rDNA, including used gloves,

pipette tips, petri dishes, and paper products, must be placed in an autoclave bucket lined with paper towels or in an autoclavable biohazard waste bag. After autoclaving, solid wastes should be transferred to the green bin for disposal.

ii. **Liquid cultures** should be decontaminated by autoclaving. Alternatively, small quantities of liquid culture may be decontaminated by addition of one volume of bleach followed by soaking overnight. Decontaminated liquid waste may be washed down the drain.

iii. **Sharps** such as scalpel blades and glass waste must be placed in a sharp's container.

iv. **Chemical wastes** must be placed in appropriate waste containers. Liquid wastes should be segregated as either aqueous, halogenic organic (like chloroform), or flammable organic waste. Solid wastes should be segregated as hazardous or non-hazardous. Gels containing ethidium bromide or similar mutagens should be placed in a labeled plastic bag and allowed to dry. Buffers containing ethidium bromide or similar mutagens should be passed through a sealed charcoal filter. Used filters and dried gels should be disposed as hazardous wastes.

v. Soils and other plant-related materials (i.e. leaves, shoots, stalks, etc.) that contains recombinant nucleic acid or is contaminated with plant pathogens must be biologically inactivated prior to disposal. Inactivation by autoclaving requires sufficient run time and specific temperatures and pressures. Soil is more difficult to inactivate, as it is very dense.

Disposing of Infectious Waste

Infectious wastes include:

- Cultures
- Etiological agents
- Specimens
- Stocks
- Related contaminated wastes
- Vaccine vials

  Other Potentially Infectious Material (OPIM)
- Any body fluid with visible blood
- Amniotic fluid

- Cerebrospinal fluid
- Pericardial fluid
- Peritoneal fluid
- Pleural fluid
- Saliva in dental procedures
- Semen/vaginal secretions
- Synovial fluid
- Anywhere body fluids are indistinguishable

Infectious medical wastes must be collected at the point of generation in the appropriate color-coded bags. Orange bags for autoclaved waste, Red bags for all other treatment methods. Biohazard bags must be labeled with the international biohazard symbol and appropriate wording; "biohazard," "biomedical waste," "infectious medical waste," or "regulated medical waste.

Solid waste should be placed in a properly labeled, leak proof container; disinfected by thermal or chemical treatment; then sent to Landfill site.

Liquid Infectious Medical Waste should be disinfected by thermal or chemical treatment then discharged into the Sewer System. Liquid waste could be solidified using an approved disinfectant solidifier and discarded in the solid waste.

Isolation wastes that do not meet the definition of infectious medical waste should be separated and disposed in the general waste stream
(disposable gowns, face masks & shoe covers). All waste from an isolation room should be treated with caution and the appropriate Personal Protective Equipment (PPE) must be worn during handling and disposal.

Fixed Pathological wastes are not Infectious Medical Waste. Unfixed Pathological wastes must be incinerated, wastes containing pathological items must be appropriately labeled to ensure they are incinerated.

Waste disposal recording

appropriate records of waste disposal must be kept by the waste generator for a period of at least three years as follows:

- name, address, and license number of the authorized contractor.
- copy of agreement for waste disposal.
- accurate identity of waste type and advice to authorized contractor of details for each load; date of collection; receipt of waste disposal or incineration from the authorized contractor for each load.

See Appendix3: Proper Regulated Medical Waste Disposal.

Disinfection and Sterilization

It is a basic biosafety principle and a critical component of containment that all contaminated material is decontaminated prior to disposal. The principles of sterilization, disinfection, and decontamination are critical for reducing the risk of pathogen release within containment zones, to the environment, and within the community.

a. Definitions

Cleaning is a process which removes foreign material (e.g. soil, organic material, microorganisms) from an object.

Sterilization is a process that completely eliminates all living microorganisms, including bacterial spores. The probability of a microorganism surviving a sterilization process is considered to be- less than one in one million (i.e., 10^{-6}), and is referred to as "sterility assurance". A sterilization procedure is one that kills all microorganisms, including high numbers of bacterial endospores. Sterilization can be accomplished by heat, ethylene oxide gas, hydrogen peroxide gas, plasma, ozone, and radiation (in industry).

Disinfection is a less lethal process than sterilization that eliminates most forms of living microorganisms. The effectiveness of the disinfection process is affected by a number of factors, including the nature and quantity of microorganisms(especially , the presence of bacterial spores); the amount of organic matter present (e.g., soil, feces, and blood); the type and state of items being disinfected, water hardness, and the temperature.

Many different terms are used for disinfection and sterilization. The following are among the more common in biosafety:

Antimicrobial: An agent that kills microorganisms or suppresses their growth and multiplication.

Antiseptic: A substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.

Biocide: A general term for any agent that kills organisms.

Chemical germicide: A chemical or a mixture of chemicals used to kill microorganisms.
Antisepsis: Is the application of a liquid antimicrobial chemical to skin or living tissue to inhibit or destroy microorganisms. It includes using germicidal solutions for swabbing an injection site on a person or animal and for handwashing.
Disinfectant: A chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.
Microbicide: A chemical or mixture of chemicals that kills microorganisms. The term is often used in place of "biocide", "chemical germicide" or "antimicrobial".
Sporocide – A chemical or mixture of chemicals used to kill microorganisms and spores.

b. Decontamination

Decontamination is the process by which materials and surfaces are rendered safe to handle and reasonably free of microorganisms or toxins. The primary objective of decontamination is to protect containment zone personnel and the community from exposure to pathogens that may cause disease. Depending on the situation, decontamination may require disinfection or sterilization.
The specific requirements for decontamination for biosafety will depend on the type of work and the nature of the infectious agent(s) being handled. Laboratories should prepare and implement a specific disinfection and sterilization protocol. For an effective disinfection protocol, consideration should be given to the microorganism being targeted, the characteristics of a specific disinfectant, and environmental issues. Additionally, the health and safety of personnel and environment are always an important consideration. Decontamination procedures should be included in personnel training on the hazards and exposure/release mitigation strategies associated with the work being done. This includes information on the products used, and the factors influencing their effectiveness.

c. Methods of Decontamination

There are different types of decontamination methods:

a. Chemical methods

There are many types of chemicals which can be used as disinfectants and antiseptics. Formulations must therefore be carefully selected for specific needs, and stored, used and disposed off as directed by the manufacturer. The germicidal activity of many chemicals is faster and better at higher temperatures. However, high temperatures can reduce their activity due to faster degradation. Particular care is therefore needed in their use and

storage in tropical regions, because of high ambient temperatures. Many germicides are harmful to humans and the environment therefore they should be selected, handled, and disposed off with care. For personal safety, proper PPE should be used.
Numerous types of disinfectants are available; however, the active components of disinfectants belong to relatively few classes of chemicals. Commonly used classes of chemical germicides are described below:

i. Acids

Examples: acetic acid, citric acid.

Acidic disinfectants function by destroying the bonds of nucleic acids and precipitating proteins. Acids also change the pH of the environment making it detrimental to many microorganisms. Concentrated solutions of acids can be caustic, cause chemical burns, and can be toxic at high concentrations in the air. These characteristics limit their use. The antimicrobial activity of acids is highly pH dependent.
Acetic acid is usually sold as glacial acetic acid (95% acetic acid) which is then diluted with water to make a working solution concentration of 5%. The concentrated form is corrosive to the skin and lungs, but the typical dilution (5%) is considered non-toxic and non-irritating.

ii. Alcohols

Examples: ethanol, isopropanol.

Alcohols are broad spectrum antimicrobial agents that damage microorganisms by denaturing proteins, causing membrane damage and cell lysis. Alcohols are used for surface disinfection, topical antiseptic, and hand sanitizing lotions. Alcohols are rapidly bactericidal rather than bacteriostatic against vegetative forms of bacteria; Alcohols are capable of killing most bacteria within five minutes of exposure and they also are tuberculocidal, fungicidal, but are limited in virucidal activity and do not destroy bacterial spores.

Ethanol is considered virucidal; isopropanol is not effective against non-enveloped viruses. An important consideration with alcohols is the concentration used, their cidal activity drops sharply when diluted below 50% concentration, and the optimum bactericidal concentration is 60%–90% solutions in water (volume/volume). Higher concentrations (95%) are actually less effective because some degree of water is required for efficacy (to denature proteins). Alcohols evaporate quickly but leave behind no residue. The activity of alcohols is limited in the presence of organic matter.

Alcohols are highly flammable and consequently must be stored in a cool, well-ventilated area. Alcohols can cause damage to rubber and plastic and can be very irritating to injured skin and should not be used near open flames.
Mixtures with other agents are more effective than alcohol alone, e.g. 70% (v/v) alcohol with 100 g/l formaldehyde, and alcohol containing 2 g/l available chlorine. A 70% (v/v) aqueous solution of ethanol can be used on skin, work surfaces of laboratory benches and biosafety cabinets, and to soak small pieces of surgical instruments. Since ethanol can dry the skin, it is often mixed with emollients. Alcohol-based hand-rubs are recommended for the decontamination of lightly soiled hands in situations where proper handwashing is inconvenient or not possible. However, it must be remembered that ethanol is ineffective against spores and may not kill all types of non-lipid viruses.

iii. Aldehydes

Examples: formaldehyde, gluteraldehyde.

Aldehydes are highly effective, broad spectrum disinfectants, which typically achieve sterilization by denaturing proteins and disrupting nucleic acids. The most commonly used agents are formaldehyde and gluteraldehyde. Aldehydes are effective against bacteria, fungi, viruses, mycobacteria, and spores. Aldehydes are noncorrosive to metals, rubber, plastic, and cement. These chemicals are highly irritating, toxic to humans or animals with contact or inhalation, and are potentially carcinogenic; therefore, their use is limited. Personal protective equipment (i.e., nitrile gloves, fluid resistant gowns, eye protection) should be worn if using these chemicals.

Formaldehyde is a gas that kills all microorganisms and spores at temperatures above 20 C. Formaldehyde is relatively slow-acting and needs a relative humidity level of about 70%. it is used as a disinfectant and sterilant in both its gaseous and liquid states. Principally formaldehyde is sold and used as a water-based solution called formalin, which is 37% formaldehyde by weight containing methanol (100 ml/l) as a stabilizer.
Formaldehyde inactivates microorganisms by alkylating the amino and sulfhydral groups of proteins and ring nitrogen atoms of purine bases. It is used as a surface disinfectant and a fumigant and has been used to decontaminate wooden surfaces, bricks, and crevices of electronic and mechanical equipment. Its use must occur in an airtight building, which must remain closed for at least 24 hours after treatment. OSHA indicated that formaldehyde

should be handled in the workplace as a potential carcinogen and set an employee exposure standard for formaldehyde that limits an 8-hour time-weighted average exposure concentration of 0.75 ppm. formaldehyde is a dangerous, irritant gas that has a pungent smell and its fumes can irritate eyes and mucous membranes. It must therefore be stored and used in a fume-hood or well-ventilated area. Ingestion of formaldehyde can be fatal, and long-term exposure to low levels in the air or on the skin can cause asthma-like respiratory problems and skin irritation, such as dermatitis and itching. For these reasons, employees should have limited direct contact with formaldehyde, and these considerations limit its role in sterilization and disinfection processes.

Paraformaldehyde, a solid polymer of formaldehyde, can be vaporized by heat for the gaseous decontamination of enclosed volumes such as rooms and safety cabinets when maintenance work or filter changes require access to the sealed portion of the cabinet.

Glutaraldehyde is primarily used as a disinfectant for medical equipment (e.g. endoscopes) but can provide sterilization at prolonged contact times. Aqueous solutions of glutaraldehyde are acidic and generally in this state are not sporicidal. Only when the solution is "activated" (made alkaline) by use of alkalinating agents to pH 7.5–8.5 does the solution become sporicidal. Once activated, these solutions have a shelf-life of minimally 14 days because of the polymerization of the glutaraldehyde molecules at alkaline pH levels. This polymerization blocks the active sites (aldehyde groups) of the glutaraldehyde molecules that are responsible for its biocidal activity.
Novel glutaraldehyde formulations (e.g., glutaraldehyde-phenol-sodium phenate, potentiated acid glutaraldehyde, stabilized alkaline glutaraldehyde) have overcome the problem of rapid loss of activity (e.g., use-life 28–30 days) while generally maintaining excellent microbicidal activity. The biocidal activity of glutaraldehyde results from its alkylation of sulfhydryl, hydroxyl, carboxyl, and amino groups of microorganisms, which alters RNA, DNA, and protein synthesis. It is also active against vegetative bacteria, spores, fungi, and lipid- and nonlipid-containing viruses.
The use of glutaraldehyde-based solutions in health-care facilities is widespread because of their advantages, including excellent biocidal properties; activity in the presence of organic matter (20% bovine serum); and noncorrosive action to endoscopic equipment, thermometers, rubber, or plastic equipment. A 2% concentration is used for high level disinfection. Its efficacy is highly dependent on pH and temperature, working best at a pH

greater than 7 and high temperatures. It is considered more efficacious in the presence of organic matter, soaps, and hard water than formaldehyde.

iv. Alkalis

Examples: sodium or ammonium hydroxide, sodium carbonate, calcium oxide.

Alkaline agents work by saponifying lipids within the envelopes of microorganisms. The activity of alkali compounds is slow but can be increased by raising the temperature. Alkalis have good microbicidal properties but are very corrosive agents and personal protection precautions should be observed.

Sodium hydroxide (lye, caustic soda, soda ash) is a strong alkali used to disinfect buildings but is highly caustic. Protective clothing, rubber gloves, and safety glasses should be worn when mixing and applying the chemical. Lye should always be carefully added to water. Never pour water into lye; a very violent reaction will occur as well as the production of high heat that can melt plastic containers. Sodium hydroxide is corrosive for metals.
Ammonium hydroxide is an effective disinfectant against coccidial oocysts however strong solutions emit intense and pungent fumes. This substance is not considered effective against most bacteria. General disinfection should follow the use of this compound.

v. Biguanides

Example: chlorhexidine.

Biguanides are detrimental to microorganisms by reacting with the negatively charged groups on cell membranes which alters the permeability. Biguanides have a broad antibacterial spectrum, however they are limited in their effectiveness against viruses and are not sporicidal, mycobacteriocidal, or fungicidal. Biguanides can only function in a limited pH range (5-7) and are easily inactivated by soaps and detergents. These products are toxic to fish and should not be discharged into the environment

vi. Halogens

Examples: chlorine or iodine compounds.

Halogen compounds are broad spectrum compounds that are considered low toxicity, low cost and easy to use. They do lose potency over time and are not active at temperatures above 43C^0 or at high pHs (>9). Since these compounds lose activity quickly in the presence of organic debris, sunlight, and some metals, they must be applied to thoroughly cleaned surfaces for disinfection.

Chlorine compounds function through their electronegative nature to denature proteins and are considered broad spectrum. The exact mechanism by which free chlorine destroys microorganisms has not been elucidated. Inactivation by chlorine can result from a number of factors: oxidation of sulfhydryl enzymes and amino acids; ring chlorination of amino acids; loss of intracellular contents; decreased uptake of nutrients; inhibition of protein synthesis; decreased oxygen uptake; oxidation of respiratory components; decreased adenosine triphosphate production; breaks in DNA; and depressed DNA synthesis. The actual microbicidal mechanism of chlorine might involve a combination of these factors or the effect of chlorine on critical sites.

Hypochlorites, the most widely used of the chlorine disinfectants, are available as liquid (e.g., sodium hypochlorite) or solid (e.g., calcium hypochlorite). Aqueous solutions of 5.25%–6.15% sodium hypochlorite, usually called household bleach. They have a broad spectrum of antimicrobial activity, are effective against bacteria, enveloped and non-enveloped viruses, mycobateria and fungi , do not leave toxic residues, are unaffected by water hardness, are inexpensive and fast acting , remove dried or fixed organisms and biofilms from surfaces, and have a low incidence of serious toxicity. Bleach is not recommended as an antiseptic but may be used as a general-purpose disinfectant. A general all-purpose laboratory disinfectant should have a concentration of 1 g/l available chlorine. A stronger solution, containing 5 g/l available chlorine, is recommended for dealing with biohazardous spillage and in the presence of large amounts of organic matter.

Sodium hypochlorite at the concentration used in household bleach (5.25-6.15%) can produce ocular irritation or oropharyngeal, esophageal, and gastric burns. Other disadvantages of hypochlorites include corrosiveness to metals in high concentrations (>500 ppm) and rapidly inactivated by light and some metals so fresh solutions should always be used. Hypochlorites should never be mixed with acids or ammonia as this will result in the release of toxic chlorine gas.

Granules or tablets of calcium hypochlorite ($Ca(ClO)_2$) generally contain about 70% available chlorine. Solutions prepared with granules or tablets, containing 1.4 g/l and 7.0 g/l, will then contain 1.0 g/l and 5 g/l available chlorine, respectively.
Alternative compounds that release chlorine and are used in the health-care setting include chlorine dioxide, sodium dichloroisocyanurate, and chloramine-T. The advantage of these compounds is that they retain chlorine longer and so exert a more prolonged bactericidal effect.

Sodium dichloroisocyanurate in powder form contains 60% available chlorine. Solutions prepared with sodium dichloroisocyanurate powder at 1.7 g/l and 8.5 g/l will contain 1 g/l or 5 g/l available chlorine, respectively. Tablets of sodium dichloroisocyanurate generally contain the equivalent of 1.5 g available chlorine per tablet.
Sodium dichloroisocyanurate at 2,500 ppm available chlorine is effective against bacteria in the presence of up to 20% plasma, compared with 10% plasma for sodium hypochlorite at 2,500 ppm.
Sodium dichloroisocyanurate tablets are stable, and for two reasons, the microbicidal activity of solutions prepared from sodium dichloroisocyanurate tablets might be greater than that of sodium hypochlorite solutions containing the same total available chlorine.

- First, with sodium dichloroisocyanurate, only 50% of the total available chlorine is free (HOCl and OCl^-), whereas the remainder is combined (monochloroisocyanurate or dichloroisocyanurate), and as free available chlorine is used up, the latter is released to restore the equilibrium.
- Second, solutions of sodium dichloroisocyanurate are acidic, whereas sodium hypochlorite solutions are alkaline, and the more microbicidal type of chlorine (HOCl) is believed to predominate.

Chloramines are available as powders containing about 25% available chlorine. Chloramines release chlorine at a slower rate than hypochlorites. Higher initial concentrations are therefore required for efficiencies equivalent to those of hypochlorites.
Chlorine dioxide-based disinfectants are prepared fresh as required by mixing the two components (base solution (citric acid with preservatives and corrosion inhibitors) and the activator solution (sodium chlorite)). It is a strong and fast-acting germicide, disinfectant agent and oxidizer, often reported to be active at concentrations levels lower than those

needed by chlorine as bleach. In vitro suspension tests showed that solutions containing about 140 ppm chlorine dioxide achieved a reduction factor exceeding 10^6 of S. aureus in 1 minute in the presence of 3 g/L bovine albumin. The potential for damaging equipment requires consideration because long-term use can damage the outer plastic coat of the insertion tube.

Iodophors are iodine complexes that have increased solubility and sustained release of iodine. Iodine can penetrate the cell wall of microorganisms quickly, and the lethal effects are believed to result from disruption of protein and nucleic acid structure and synthesis. iodophors are bactericidal, mycobactericidal, and virucidal but can require prolonged contact times to kill certain fungi and bacterial spores. One of the more commonly used iodophors if povidone-iodine. Tey are good for general use and are less readily inactivated by organic matter than elemental iodine compounds. The dilution of iodophors actually increases the free iodine concentration and antimicrobial activity.

vii. Oxidizing Agents

Examples: hydrogen peroxide, peracetic acid.

Oxidizing agents are broad spectrum, peroxide based compounds that function by denaturing the proteins and lipids of microorganisms. Peroxygen compounds vary in their microbiocidal range but are considered effective on hard surfaces and equipment. In their diluted form, these agents are relatively safe but may be irritating and damage clothing when concentrated.

Hydrogen peroxide (at a 5-20% concentration) is considered bactericidal, virucidal (nonenveloped viruses may be resistant), fungicidal and at high concentrations sporicidal. Its activity against mycobacteria is limited. Hydrogen peroxide is supplied either as a ready-to-use 3% solution or as a 30% aqueous solution to be diluted to 5–10 times its volume with sterilized water. However, such 3–6% solutions of hydrogen peroxide alone are relatively slow and limited as germicides. Products now available have other ingredients to stabilize the hydrogen peroxide content, to accelerate its germicidal action and to make it less corrosive. Hydrogen peroxide can be used for the decontamination of work surfaces of laboratory benches and biosafety cabinets, and stronger solutions may be suitable for disinfecting heat-sensitive medical/dental devices. Hydrogen peroxide and peracids can be corrosive to metals such as aluminium, copper, and zinc. They should always be stored away from heat and protected from light.

Peracetic acid is a strong oxidizing agent and is a formulation of hydrogen peroxide and acetic acid. It is considered bactericidal, fungicidal, sporicidal and virucidal. It is also effective against mycobacteria and algae and has some activity in the presence of organic material. peracetic acid denatures proteins, disrupts the cell wall permeability, and oxidizes sulfhydryl and sulfur bonds in proteins, enzymes, and other metabolites
The use of vaporized hydrogen peroxide or peracetic acid (CH3COOOH) for the decontamination of heat-sensitive medical/surgical devices requires specialized equipment.

Two chemical sterilants are available that contain peracetic acid plus hydrogen peroxide (i.e., 0.08% peracetic acid plus 1.0% hydrogen peroxide (no longer marketed); and 0.23% peracetic acid plus 7.35% hydrogen peroxide. The combination of peracetic acid and hydrogen peroxide inactivated all microorganisms except bacterial spores within 20 minutes. The 0.08% peracetic acid plus 1.0% hydrogen peroxide product effectively inactivated glutaraldehyde-resistant mycobacteria.

viii. Phenols

Phenols are broad spectrum disinfectants that function by denaturing proteins and inactivating membrane-bound enzymes to alter the cell wall permeability of microorganisms ,in high concentrations, phenol acts as a gross protoplasmic poison, penetrating and disrupting the cell wall and precipitating the cell proteins. Low concentrations of phenol and higher molecular-weight phenol derivatives cause bacterial death by inactivation of essential enzyme systems and leakage of essential metabolites from the cell wall.
Phenols are typically formulated in soap solutions to increase their penetrative powers and at 5% concentrations are considered bactericidal, tuberculocidal, fungicidal and virucidal for enveloped viruses. Phenols are not effective against non-enveloped viruses and spores. Phenols do maintain activity in hard water and in the presence of organic matter and have some residual activity after drying. Phenolic disinfectants are generally safe for humans but prolonged exposure to the skin may cause irritation.

ix. Quaternary Ammonium Compounds (QACs)

The quaternary ammonium compounds are widely used as disinfectants, these compounds are cationic detergents that are attracted to the negatively charged surfaces of microorganisms, where they irreversibly bind phospholipids in the cell membrane and denature proteins impairing permeability. QACs can be from different "generations"

depending on their chemistry, with later generations being more germicidal, less foaming, and more tolerate to organic loads. QACs are highly effective against Gram positive bacteria, and have good efficacy against Gram-negative bacteria, fungi, and enveloped viruses. They are not effective against non-enveloped viruses or mycobacteria and are considered sporostatic but not sporocidal. QACs have some residual effect, keeping surfaces bacteriostatic for a brief time. They are more active at neutral to slightly alkaline pH but lose their activity at pH less than 3.5. QACs are considered stable in storage but are, in general, easily inactivated by organic matter, detergents, soaps and hard water (this may vary with the "generation").

Selection of Chemical Disinfectants

The selection of an appropriate chemical disinfectant is dependent on a variety of factors, including:

- the resistance of the infectious material or toxin,
- the method of application (e.g., liquid, or gaseous), and
- the nature of the material to be disinfected (e.g., hard surface, porous materials).
- Organic load, concentration, contact time, temperature, relative humidity, pH, and stability can also impact the efficacy of a chemical disinfectant.

See Appendix 4: Decontamination Of Used Instruments, Equipment And Surfaces, Appendix 5: Susceptibility Of Microorganisms To Chemical Disinfectants and Appendix 6: Disadvantages of Chemical Disinfectants.

b. Heat:

Heat is the most common among the physical agents used for the decontamination of pathogens. The heat method can be further divided into two:

a. Moist Heat (Autoclave or Steam Sterilizer)

The use of an autoclave or steam sterilizer is the preferred method for treating biohazardous waste as well as decontaminating labware.

- Autoclave

Autoclaves use pressurized steam to destroy microorganisms and are the most dependable systems available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents.

Principle

When water boils, its vapor pressure is equal to surrounding atmospheric pressure. When boiling is done in a closed vessel, there is increase in the inside pressure of vessel which raises the temperature of boiling water above 100 C. Saturated steam under pressure is more efficient way of sterilization as compared to dry heat because it provides greater lethal action. It is quicker in heating up the exposed articles. It penetrates the porous material such as cotton wool, stoppers, paper, cloth wrapper etc.

Saturated steam under pressure (autoclaving) is the most effective and reliable means of sterilizing laboratory materials. For most purposes, the following cycles will ensure sterilization of correctly loaded autoclaves:

1. 3 min holding time at 134 C
2. 10 min holding time at 126 C
3. 15 min holding time at 121 C
4. 25 min holding time at 115 C.

Type of Autoclaves

The two basic types of steam sterilizers (autoclaves) are the gravity displacement autoclave and the high-speed prevacuum sterilizer.

In the gravity displacement autoclave, steam is admitted at the top or the sides of the sterilizing chamber and, because the steam is lighter than air, forces air out the bottom of the chamber through the drain vent. In order for this system to function efficiently, care should be taken to ensure that the valves remain unobstructed and that the chamber is not overfilled.

The gravity displacement autoclaves are primarily used to process laboratory media, water, pharmaceutical products, regulated medical waste, and nonporous articles whose surfaces have direct steam contact.

For gravity displacement sterilizers the penetration time into porous items is prolonged because of incomplete air elimination. For example, to decontaminate 4 Kg of microbiological waste requires at least 45 minutes at 121 C because the entrapped air remaining in a load of waste greatly retards steam permeation and heating efficiency. The high-speed prevacuum sterilizers are similar to the gravity displacement sterilizers except they are fitted with a vacuum pump (or ejector) to ensure air removal from the sterilizing

chamber and load before the steam is admitted. Pre-vacuum autoclaves resolve the air entrapment problems that are often encountered in gravity displacement autoclaves. Autoclaves can be designed with a single door or with double doors. Double-door autoclaves are installed on the containment barrier, typically in high containment zones, to facilitate the decontamination and movement of waste and other contaminated material out of the containment zone.

Fuel-heated pressure cooker autoclaves. These should be used only if a gravity displacement autoclave is not available. They are loaded from the top and heated by gas, electricity or other types of fuels.

When using an autoclave, the following guidelines should be taken into consideration:

- Biohazardous materials should not be placed in autoclaves overnight in anticipation of autoclaving the next day.
- Autoclaves should not be operated by untrained personnel.
- 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 hypochlorite, or any other strong oxidizing material, must not be autoclaved with organic materials such as paper, cloth, or oil.

Three factors in combination determine the effectiveness of autoclaving:

1. **Temperature**

An autoclave uses steam under a pressure of approximately 15 psi to achieve a chamber temperature of at least 121 C. Although the autoclave chamber may reach 121 C, this does not necessarily mean that the interior of the load will reach this temperature.

2. **Time**

A minimum autoclave cycle time of 20 minutes at a chamber temperature of 121 C (time does not begin as soon as the autoclave cycle is initiated) is commonly recommended for sterilization of clean items.

However, the total processing time required to achieve decontamination depends on several loading factors, including:

- the load container (heat transfer properties).
- the amount of water added to the load.
- and the weight of the load.

For increased loads, an increased cycle time will be required to ensure effective decontamination.

3. **Contact**

Steam saturation is essential for maximum heat transfer. Steam must contact all areas of the load. Autoclave bags and other containers should be left partially open (or otherwise permit entry of steam) to ensure adequate contact. Studies have shown that adding water to the interior of the bag improves the time-temperature profile of the autoclave cycle, thereby increasing the autoclave's sterilization efficiency.

See Appendix7: Recommended Procedures for The Use of Autoclaves and for Efficacy Monitoring of Autoclaves.

b. Dry Heat

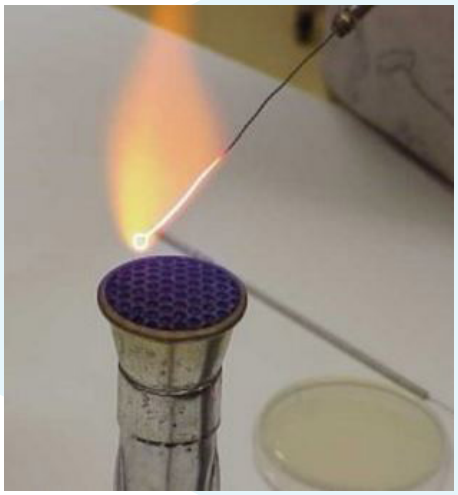
The use of dry heat for the decontamination of biohazardous materials and contaminated

items is less efficient than autoclaving and requires a longer exposure time with higher temperature. It may be possible to decontaminate materials or soiled items by exposing them to 160 C (320 F) or higher for 2-4 hours. This is suitable for destruction of viable agents on impermeable non-organic material such as glass but is not reliable in even shallow layers of organic material that can act as insulation. If items are heat sensitive, a temperature of 120 C (248 F) can be used and the exposure time necessary for decontamination is usually greater than 24 hours.

The commonly used methods to sterilize the material are as follows:

a. Flaming

Used to sterilize material, such as, inoculating wire/ loop, tip of the forceps, searing iron, scalpel etc.



b. Hot air sterilization

The method is used for sterilizing the material like dry glass test tubes, Petri dishes, flasks, glass pipettes, all glass syringes etc. and instruments like forceps, scalpels etc. Dry heat destroys microorganisms by dehydration and oxidation or even incineration.

Principle of Dry heat sterilization using HOT AIR OVEN

Sterilizing by dry heat is accomplished by conduction. The heat is absorbed by the outside surface of the item, then passes towards the center of the item, layer by layer. The entire item will eventually reach the temperature required for sterilization to take place.

Dry heat does most of the damage by oxidizing molecules. The essential cell constituents are destroyed, and the organism dies. The temperature is maintained for almost an hour to kill the most difficult of the resistant spores.

The most common time-temperature relationships for sterilization with hot air sterilizers are

1. 170 C (340 F) for 30 minutes,
2. 160 C (320 F) for 60 minutes, and
3. 150 C (300 F) for 150 minutes or longer depending up the volume.

There are two types of dry-heat sterilizers:

1. the static-air type and
2. the forced-air type.

The **static-air type** is referred to as the oven-type sterilizer as heating coils in the bottom of the unit cause the hot air to rise inside the chamber via gravity convection. This type of dry-heat sterilizer is much slower in heating, requires longer time to reach sterilizing temperature, and is less uniform in temperature control throughout the chamber than is the forced-air type.

The **forced-air or mechanical convection** sterilizer is equipped with a motor-driven blower that circulates heated air throughout the chamber at a high velocity, permitting a more rapid transfer of energy from the air to the instruments.

Advantages of dry heat sterilization

1. A dry heat cabinet is easy to install and has relatively low operating costs.
2. It penetrates materials
3. It is nontoxic and does not harm the environment.
4. And it is noncorrosive for metal and sharp instruments.

Disadvantages for dry heat sterilization

1. Time consuming method because of slow rate of heat penetration and microbial killing.
2. High temperatures are not suitable for most materials e.g. plastic and rubber items cannot be dry-heat sterilized because temperatures used (160–170 C) are too high for these materials.
3. The time and temperature required will vary for different substances and overexposure may ruin some substances.

Room Decontamination

Decontamination of the laboratory space, its furniture and its equipment require a combination of liquid and gaseous disinfectants. Surfaces can be decontaminated using a solution of sodium hypochlorite (NaOCl); a solution containing 1 g/l available chlorine may be suitable for general environmental sanitation, but stronger solutions (5 g/l) are recommended when dealing with high-risk situations. For environmental decontamination, formulated solutions containing 3% hydrogen peroxide (H2O2) make suitable substitutes for bleach solutions.

Rooms and equipment can be decontaminated by fumigation with formaldehyde gas generated by heating paraformaldehyde or boiling formalin. This is a highly dangerous process that requires specially trained personnel. All openings in the room (i.e. windows, doors, etc.) should be sealed with masking tape or similar before the gas is generated. Fumigation should be conducted at an ambient temperature of at least 21 ºC and a relative humidity of 70%. After fumigation, the area must be ventilated thoroughly before

personnel are allowed to enter. Appropriate respirators must be worn by anyone entering the room before it has been ventilated. Gaseous ammonium bicarbonate can be used to neutralize the formaldehyde. Fumigation of smaller spaces with hydrogen peroxide vapour is also effective but requires specialized equipment to generate the vapour.
After the decontamination, the remains of microorganisms often stay present. This is why it is important to use hot water and soap to clean the work surfaces, floors and doorknobs during the weekly cleaning of the laboratory and there is a need to decontaminate twice in order to decontaminate successfully.

Incineration

Incineration can be defined as a controlled combustion process for burning solid, liquid and gaseous combustible wastes to gases and residue containing noncombustible material. Incinerators are used for the process of incineration.
Biohazardous waste that must be disposed of by incineration includes human or animal anatomical waste, material soaked with blood, biohazard sharps containers, and biohazardous waste that is contaminated with chemicals that would not be compatible with autoclave decontamination.
Effective incineration depends on proper equipment design, time, temperature, turbulence, and air required for complete oxidation, as well as careful loading of the unit. Many incinerators, especially those with a single combustion chamber, are unsatisfactory for dealing with infectious materials, animal carcasses and plastics. Such materials may not be completely destroyed and the effluent from the chimney may pollute the atmosphere with microorganisms, toxic chemicals and smoke.
Modern incinerators that have two chambers, with an ideal temperature of at least 800oC in the primary chamber and at least 1000oC in the secondary chamber, may be effective. Loads with high moisture content may lower the processing temperature, and sawdust may be added to enhance stability. There are no microbial standards for stack emissions, but there are for emission of particulate matter and selected chemical contaminants. Provincial or territorial regulatory authorities should be consulted for additional requirements related to incinerator operations and emissions.

Autoclaving is the preferred method to decontaminate materials, equipment, and waste at the containment barrier prior to its removal from high containment zones for transporting to an incinerator. Material to be incinerated should be packaged in leak-proof plastic bags, even if previously decontaminated. Incinerator attendants should receive proper instructions about loading and temperature control. It should also be noted that the efficient operation of an incinerator depends heavily on the right mix of materials in the waste being treated.

It is important that written protocols for the packaging, labeling, storage, and transportation of waste materials destined for the incinerator be developed and followed by all personnel. There are ongoing concerns regarding the possible negative environmental effects of existing or proposed incinerators, and efforts continue to make incinerators more environmentally friendly and energy efficient.

New Alternative Technologies for Incineration

Non-incineration treatment includes four basic processes: thermal, chemical, irradiative, and biological. The main purpose of the treatment technology is to decontaminate waste by destroying pathogens. Modern technology invented mechanics that would allow clinical laboratories and hospitals to dispose medical waste in an environmentally friendly way; such as: autoclaving, plasma pyrolysis, chemical methods, and microwave irradiation. These alternatives are also highly versatile and can be used for all different types of waste.

1. **irradiation**

There are 2 general types of radiation used for sterilization, ionizing radiation, and non-ionizing radiation.

Ionizing radiation is the use of short wavelength, high-intensity radiation to destroy microorganisms. This radiation can come in the form of gamma or X-rays that react with DNA resulting in a damaged cell.

- **Gamma irradiation** (e.g., Cobalt-60) is a low-temperature sterilization method can be used for the decontamination of heat-sensitive materials and is effective at decontaminating the chemicals and solvents that may be used in higher containment zones; however, it may not be capable of effectively decontaminating certain pathogens (e.g., bacterial spores). The efficacy of this process is dependent on the penetration of the materials by gamma irradiation, which is a function of the density of the treated substance and the strength of the irradiation source.

Non-ionizing radiation uses longer wavelength and lower energy. As a result, non-ionizing radiation loses the ability to penetrate substances, and can only be used for sterilizing surfaces. The most common form of non-ionizing radiation is ultraviolet light, which is used in a variety of manners throughout industry.

- **Breakdown of ozone (O_3)** is one industrial application of non-ionizing radiation. By adding ozone to water, bacteria are unable to sustain life. Unfortunately, ozone also destroys process media. Therefore, ozone must be broken down so water can be used for its designated purpose. Since ozone is very sensitive to ultraviolet light, pass the water stream under UV bulbs. This breaks the oxygen-oxygen bonds and results in safe process water. Here is a simple representation of the system.

Advantages:

1. No degradation of media during sterilization, thus it can be used for thermally labile media.
2. Leaves no chemical residue
3. Administration of precise dosage and uniform dosage distribution
4. Immediate availability of the media after sterilization

Disadvantages:

1. This method is a more costly alternative to heat sterilization
2. Requires highly specialized equipment

- **Ultraviolet irradiation (UV)** should not be relied upon as the sole method of decontamination for materials to be removed from containment equipment (biological safety cabinets) or facilities. UV has limited penetrating power and is primarily effective against unprotected microbes on exposed surfaces or in the air. It can be effective in reducing airborne and surface contamination provided that the lamps are properly cleaned, maintained, and checked to ensure that the appropriate intensity is being emitted.

  UV may be recommended in certain situations, however it is important to note that, the accumulation of dust, dirt, grease or clumps of microorganisms reduce its germicidal effects; UV light is not effective against all organisms; and exposure to UV light is hazardous, it may result in severe eye damage and burns to the skin.

2. Microwave

Microwave radiation , are a form of electromagnetic radiation with wavelengths ranging from about 1 mm to 1 m, a range that includes television and police radar wavelengths. Microwave irradiation is not widely used as a means of decontamination in containment zones. Similar to autoclaving, this process is based on the use of heat to eliminate viable microorganisms and, for this reason, autoclaving is usually the technology of choice. The efficacy of microwave irradiation is dependent on the wavelength of the irradiation, the duration of exposure, and the moisture content of the material to be decontaminated.

Sterilization by Microwave Oven:

A specialized microwave oven has recently become available that can be used to sterilize media in just 10 minutes. It has 12 pressure vessels, each of which holds 100 ml of medium. Microwave energy increases the pressure of the medium inside the vessels until sterilizing temperatures are reached.

Applications of Microwaves:

Microwave oven frequencies are tuned to match energy levels in water molecules. In the liquid state, water molecules quickly absorb the microwave energy and then release it to surrounding materials as heat. The molecules are set into high-speed motion, and the heat of friction is transferred of foods, which become hot rapidly. Thus, materials that do not

contain water, such as plates made of paper, china, or plastic, remain cool while the moist food on them becomes heated.

Limitations of Microwave Ovens:

Other than the heat generated, there is no specific activity against microorganisms. For this reason, the home microwave cannot be used to sterilize items such as bandages and glassware. Conduction of energy is metals leads to problems such as sparking, which makes most metallic items also unsuitable for microwave sterilization. Moreover, bacterial endospores, which contain almost no water, are not destroyed by microwaves.

3. Pyrolysis / Vitrification

Pyrolysis is defined as heating the solid waste at very high temperature in absence of air. It is the thermal decomposition of materials at elevated temperatures in an inert atmosphere. It involves a change of chemical composition and is irreversible. Pyrolysis is carried out at a temperature between 500 ^{0} C to 1000 ^{0}C to produce three component streams:

- Gas: It is a mixture of combustible gases such as hydrogen, carbon dioxide, methane, carbon mono-oxide and some hydrocarbons.
- Liquid: It contains tar, pitch, light oil, and low boiling organic chemicals like acetic acid, acetone, methanol etc.
- Char: It consists of elemental carbon along with inert material in the waste feed.

Plasma pyrolysis technology has been recognized as a non-incineration process. It is an environment-friendly mechanism, which converts organic waste into commercially useful byproducts. The intense heat generated by the plasma enables it to dispose all types of waste including municipal solid waste, biomedical waste and hazardous waste in a safe and reliable manner. It is one of the technologies which could be opted for disposal of plastic waste.

In Plasma pyrolysis, high temperature is produced using plasma torch in oxygen starved environment to destroy plastic waste efficiently and in an ecofriendly manner, high destruction performance and releases dioxins and furans in the range of 0.005-0.009 ng/m3, which is well below the set limit of environmental standard in the world .

Vitrification is the transformation of a substance into a glass, that is to say a non-crystalline amorphous solid. Vitrification is usually achieved by heating materials until they liquidize, then cooling the liquid, often rapidly, so that it passes through the glass transition to form a vitrified solid. Certain chemical reactions also result in glasses.

Until recently, vitrification technology cost too much to treat low-level wastes."The advantage of vitrification is that it converts a waste product into recyclable, reusable glass. During vitrification, contaminants are subjected to extremely high temperatures in the melter. The organic compounds are destroyed, and the remaining organic elements become part of the glass's molecular structure. Hazardous metal components in the waste are converted to nonhazardous oxides.

Vitrification is a process used to stabilize and encapsulate high-level radioactive waste. In the vitrification process, radioactive waste is mixed with a substance that will crystallize when heated (e.g., sugar, sand) and then calcined. Calcination removes water from the waste to enhance the stability of the glass product. The calcinated materials are continuously transferred into a heated furnace and mixed with fragmented glass. Upon mixing, the radioactive waste is bonded to the glass material. The melted product is subsequently poured into an encapsulation container (e.g., stainless steel liner). Once the contents cool down, the melt solidifies into the glass matrix. The encapsulation container is ultimately sealed, and the waste stored in disposal repositories. Vitrification allows the immobilization of the waste for thousands of years.

4. Other

Chemical. Some kinds of chemical waste may be neutralized by applying reactive chemicals that render it inert. This is generally reserved for waste that is chemical in nature.

Biological. This experimental method of treating biomedical waste uses enzymes to neutralize hazardous, infectious organisms. It is still under development and rarely used in practice.

Macrowave. The Macrowave pasteurization system uses a high-frequency electric field to control pathogens. Material is conveyed through a high-frequency electrode array where alternating electrical energy causes material to heat rapidly and uniformly throughout the product thickness. The system operates at 40 MHz to optimize depth and uniformity of heating, and runs "instant-on, instant-off" to use energy only during the treatment process.

The Radio Frequency Macrowave RF disinfestation system can be used to disinfest and/ or pasteurize products such as flour, oat and cornmeal, nuts, spices, and pasta, either packaged or in bulk.

Sewage Treatment

Much of the hospital and clinical laboratories sewage has similar characteristics as domestic sewage but may also contain various potential hazardous materials including , microbiological pathogens, radioactive isotopes, disinfectants, drugs, chemical compounds and pharmaceuticals. The major aim of sewage treatment is to remove as much of the suspended solids as possible before the remaining water, called effluent, is discharged back to the environment. The selection of suitable treatment technology and proper treatment of sewage is essential.

1. Connection to a municipal sewage treatment plant

In countries that do not experience epidemics of enteric disease and that are not endemic for intestinal helminthiasis, it is acceptable to discharge the sewage of the hospital and clinical laboratories to municipal sewers without pretreatment, provided that the following requirements are met:

- The municipal sewers are connected to efficiently operated sewage treatment plants that ensure at least 95% removal of bacteria; the sludge resulting from sewage treatment is subjected to anaerobic digestion, leaving no more than one helminth egg per litre in the digested sludge.

- The waste management system of the health-care establishment maintains high standards, ensuring the absence of significant quantities of toxic chemicals, pharmaceuticals, cytotoxic drugs, and antibiotics in the discharged sewage; excreta from patients being treated with cytotoxic drugs may be collected separately and adequately treated (as for other cytotoxic waste).

In normal circumstances, the usual secondary bacteriological treatment of sewage, properly applied, complemented by anaerobic digestion of sludge, can be considered as

sufficient. During outbreaks of enteric disease, however, or during critical periods (usually in summertime because of warm weather, and in autumn because of reduced river water flow), effluent disinfection by chlorine dioxide (ClO_2) or by any other efficient process is recommended.

When the final effluents or the sludges from sewage treatment plants are reused for agricultural, the safety recommendations of the relevant WHO guidelines should be respected.

2. On-site treatment or pretreatment of sewage

Many health-care establishments, in particular those that are not connected to any municipal treatment plant, have their own sewage treatment plants.

Efficient on-site treatment of hospital sewage should include the following operations:

- Primary treatment.
  Minimal treatment involving flotation and settlement to separate solids and liquids, and disposal of the clarified but still contaminated effluent.

- Secondary biological purification.
  More advanced treatment using air and biological processes to decompose the solids and chlorine to disinfect the liquid to a standard safe for irrigation (such as aerated wastewater treatment systems). Most helminths will settle in the sludge resulting from secondary purification, together with 90-95% of bacteria and a significant percentage of viruses; the secondary effluent will thus be almost free of helminths but will still include infective concentrations of bacteria and viruses.

- Tertiary treatment.
  Highly advanced systems that use air and biological processes as well as membranes to treat sewage to a very high standard (such as membrane filtration and advanced aerated sewage treatment systems).

- Chlorine disinfection.
  To achieve pathogen concentrations comparable to those found in natural waters, the tertiary effluent will be subjected to chlorine disinfection to the breakpoint. This may be done with chlorine dioxide (which is the most efficient), sodium hypochlorite, or chlorine gas. Another option is ultraviolet light disinfection.

Sludge treatment

The sludge from the sewage treatment plant requires anaerobic digestion to ensure thermal elimination of most pathogens. Alternatively, it may be dried in natural drying beds and then incinerated together with solid infectious health-care waste. On-site treatment of hospital sewage will produce a sludge that contains high concentrations of helminths and other pathogens.

Personal Protective Equipment (PPE)

It has long been recognized that working with biohazardous materials may be inherently dangerous. Personal Protective Equipment (PPE) may act as barrier to minimize the risk of exposure. The clothing and equipment selected is dependent on the nature of work performed, type of the pathogen and its transmissibility. PPE should be worn when working in the laboratory. It should be removed, and hands should be washed before leaving the laboratory.

The employee must understand the biohazard procedure or process, the nature of the biohazard and the need for the equipment; and must understand the functioning, proper use, and limitations of the PPE used.

Types of PPE

The types of Personal Protective Equipment may include:

a. Gloves

Gloves must be worn whenever handling clinical specimens, human blood or body fluids, culture dishes or other equipment potentially contaminated with BSL-2 or BSL-3 pathogens, infected animals, or infectious waste.

Gloves are also specifically recommended for recombinant DNA work involving organisms from risk group 2/ BSL-2 or DNA sequences derived from these organisms. For all other work involving recombinant DNA, gloves may be worn as a method of protecting experiments from personal and environmental contamination.

The type of glove that can be selected ranges from rubber gloves for minimum protection to other types of gloves (e.g., nitrile gloves) for maximum protection against blood borne pathogens, animals, or other types of physical hazard. It should be noted that for those individuals with latex allergies, nitrile gloves may be used for protection against biohazards. Those who prefer latex should use only powder-free gloves that are designated "low protein" by the manufacturer. Corrosives and solvents may penetrate latex or nitrile gloves or diminish their protective ability; it may be necessary to stock more than one type of glove for the full range of a laboratory's activities.

- Natural Latex or Rubber Gloves (No powder):

Provide protection from most water solutions of acids, alkalis, salts, and ketones. These gloves have excellent wearing qualities, pliability, and comfort and are a good general-purpose glove.

- Nitrile Rubber Gloves:

Provide protection from chlorinated solvents (trichloroethylene, perchloroethylene). They are intended for jobs requiring dexterity and sensitivity, yet they stand up under mechanical use even after prolonged exposure to substances that cause other glove materials to deteriorate. They also resist abrasion, puncturing, snagging, and tearing.

Gloves should be removed, and hands thoroughly washed after handling infectious materials, working in a biological safety cabinet and before leaving the laboratory. Used disposable gloves should be discarded with infected laboratory wastes. Gloves should not be worn outside the laboratory areas.

Protective gloves should be worn when handling hazardous materials, chemicals of unknown toxicity, corrosive materials, rough or sharp-edged objects, and very hot or very cold materials. Stainless steel mesh gloves should be worn when there is a potential exposure to sharp instruments e.g. during postmortem examinations. Such gloves protect against slicing motion but do not protect against puncture injury.

b. Eye and Face Protection

The choice of equipment to protect the eyes and face from splashes or spray of infectious or other hazardous material will depend on the activity performed.

-Safety glasses

Safety glasses look very much like normal glasses but have lenses that are impact resistant and frames that are far stronger than standard street wear glasses. Safety glasses must have side shields and should be worn whenever there is the possibility of objects striking the

eye, such as splashes, particles, glass, or metal shards. Safety Spectacles may be adequate when the potential splash is minimal; Goggles must be used for activities with any splash hazard or when working with organisms transmissible through mucous membrane exposure; and Goggles with a face shield should be worn when an elevated risk of large quantity splashes exists.

b. Face Shields

Face shields are made of shatterproof plastic, fit over the face and are held in place by head straps or caps .Face shields are in order when working with large volumes of hazardous materials, either for protection from splash to the face or flying particles. Face shields must be used in conjunction with safety glasses or goggles.

The following apply to the use of eye and face protection in the laboratory:

1. Select eye and face protection that is appropriate to the task being performed. Do not put on or remove face/eye protection while wearing gloves that are potentially contaminated.
2. Individuals who wear contact lenses in the laboratory should also wear eye protection. Contact lenses do not provide protection to the eyes. Foreign material splashed into the eye may become trapped under the contact lens and result in more serious injury.
3. If eye protection is deemed necessary in a laboratory, then an emergency eyewash station should also be available.
4. Eye and face protection must be decontaminated and cleaned before reuse or disposed with other contaminated laboratory waste.
5. Safety glasses, goggles or face shields should not be worn outside the laboratory areas.

c. Lab Coats

Laboratory coats are available in a variety of different materials and fabrics and are principally worn to protect clothing and arms from inadvertent contamination while working in the laboratory. It is important to choose a coat based on the level of protection that is needed. For example, a lab coat worn to protect against spilled or splashed liquids should be capable of preventing liquids from soaking through and contaminating an individual's

clothing or skin. If there are specific concerns about a splash to the front of the coat, a solid front gown or an apron worn in conjunction with a laboratory coat may be a more appropriate choice.
Laboratory coveralls, gowns or uniforms must be worn at all times for work in the laboratory. Laboratory coats should preferably be fully buttoned. However, long-sleeved, back-

opening gowns or coveralls give better protection than laboratory coats and are preferred in microbiology laboratories and when working in the biological safety cabinet. Aprons should be worn over laboratory coats or gowns where necessary to give further protection against spillage of chemicals or biological materials such as blood or culture fluids.
Laboratory coats should be discarded or laundered when they become soiled or contaminated. Laboratory coats that are grossly contaminated with infectious material should be disinfected immediately with either a chemical disinfectant or by autoclaving before they are laundered or disposed of as decontaminated waste. Disposable lab coats are recommended in situations where there are no departmental or laboratory facilities for laundering. Lab coats should not be taken home for laundering.

d. Foot Protection

Closed-toed shoes should be worn at all times in buildings where chemicals are stored or used. Open toed shoes, sandals and other open footwear is prohibited. Shorts are forbidden in laboratories using potentially infectious materials.
Chemical resistant overshoes or boots may be used to avoid possible exposure to corrosive chemical or large quantities of solvents or water that might penetrate normal footwear (e.g., during spill cleanup). Leather shoes tend to absorb chemicals and may have to be discarded if contaminated with a hazardous material.
Steel-toed safety shoes may be necessary when there is a risk of heavy objects falling or rolling onto the feet, such as in bottle-washing operations or animal care facilities.

e. Surgical Masks

A surgical mask is a loose-fitting, disposable device that creates a physical barrier between the mouth/nose of the wearer and potential contaminants in the immediate environment. It also prevents patients from direct transmission of infectious agents from health care professionals or the surgical area.

Surgical masks do not provide respiratory protection against harmful/infectious aerosols, smoke or chemical fumes, but if used correctly may protect the user from aerosol/droplet borne/air-borne infection. As with other PPE, their use in the laboratory is based upon a risk assessment of the hazards associated with the specific procedure being performed.
Two types of masks are recommended for various categories of personnel depending upon the work environment.

-Triple layer Surgical Mask
-Surgical N95 Respirators
N-95 and triple layer mask is used while handling of patient `s specimens who are suspected of novel influenza viruses. will help prevent ingestion and protect the mucous membrane of the nose and mouth from splashes. They do not provide protection against inhalation of organisms transmitted by aerosols.

f. Respirators

Respiratory protection may be used when carrying out high-hazard procedures (e.g. cleaning up a spill of infectious material). The choice of respirator will depend on the type of hazard(s). Respirators are available with interchangeable filters for protection against gases, vapours, particulates and microorganisms. It is imperative that the filter is fitted in the correct type of respirator.
Respirators are used when there is the risk of airborne exposure to infectious organisms, specifically BSL3 organisms (ex. Mycobacterium tuberculosis) that can be transmitted by inhalation. To achieve optimal protection, respirators should be individually fitted to the operator's face and tested. Fully self-contained respirators with an integral air supply provide full protection.
Respirators may only be worn after the employee has been medically certified, trained, and fit tested. Respirators should not be worn outside the laboratory areas.

Classification of Respirators

- FFP1 respirators – Suitable for lower filtering efficiency and protection factor.
- FFP3 respirators - For highest levels of filtering efficiency and protection factor.

Specifications of Respirators

- N95 respirator is the most common of the seven types of particulate filtering facepiece respirators. This product filters at least 95% of airborne particles but is not resistant to oil.
- N99 respirator filters at least 99% of airborne particles. Not resistant to oil.
- N100 respirators remove at least 99.97% of airborne particles. Not resistant to oil.
- R95 respirator filters at least 95% of airborne particles and is somewhat resistant to oil.
- P95 respirator filters at least 95% of airborne particles and is strongly resistant to oil.

- P99 respirator filters at least 99% of airborne particles and is strongly resistant to oil.
- P100 respirator filters at least 99.97% of airborne particles and is

Good laboratory Practice (GLP)

Introduction

Most laboratory accidents, injuries and work-related infections are caused by human error, poor laboratory techniques and misuse of equipment. A major proportion of the work in the laboratory involves handling infectious biological materials. It is, therefore, important for laboratories to establish standard policies and procedures necessary for safe laboratory conduct, handling laboratory hazards, and contingency planning for safety issues as part of a safety program. Laboratory personnel must have knowledge of safe laboratory procedures and an awareness of potential hazards.

Work Practice

This chapter contains a minimum set of procedures and recommendations required to maintain a safe working environment. It is responsibility of the Principal Investigator to develop specific standard operating procedures (SOPs) for the laboratory. The SOPs must identify the hazards of the protocol, as well as measures to be taken to mitigate those hazards. Standard operating procedures must be readily available to all laboratory employees. The following general safety procedures have been established to minimize or eliminate hazards in the laboratory. These procedures have also been provided to maintain a safe laboratory environment. It is the responsibility of each person that enters into the laboratory to understand the safety and health hazards associated with potential hazardous materials and equipment in the laboratory. All staff of the laboratory should follow the **Good Microbiological Techniques (GMTs)** which include the following:

- **Laboratory Access**

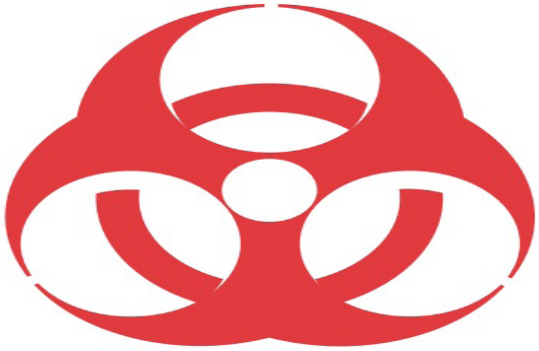
1. The Principal Investigator or laboratory supervisor authorizes access to the laboratory and is responsible for the safety of individuals working in the laboratory.
2. Individuals who make requests to use the laboratory or laboratory equipment and who are not directly affiliated with the lab must be advised of the potential risks associated with the laboratory and receive training appropriate to the work that will be performed.
3. Visitors must be accompanied by an individual with authorization to work in the laboratory.

4. Children under age 16 are not permitted in laboratories. Exceptions may be granted for supervised youth participating in University sponsored programs.
5. The doors of unoccupied laboratories shall be locked, to prevent unauthorized access.
6. The increased risks to individuals undergoing immunosuppressive therapy, pregnant women, etc., must be evaluated and addressed before they enter or work in laboratories where dangerous materials such as infectious agents, toxins or radioactive materials are used.

- **Signage**

A "caution" sign will be posted on or near the entrance door to biological laboratories. The sign shall include the following information:

1. The biosafety level of the laboratory.
2. A listing of the hazards associated with work in the laboratory (infectious agents, toxins radioactive materials, etc.,).
3. The name(s) and contact information of the Principal Investigator, laboratory supervisor and /or other responsible persons. See figure below.



BIOHAZARD

AUTHORIZED PERSONNEL ONLY!!

HAZARD: _____

BIOSAFETY LEVEL: _____

LOCATION: _____

INSTRUCTIONS: _____

_____

	NAME	ROOM	CAMPUS	HOME PHONE
RESPONSIBLE INVESTIGATOR				
ALTERNATE				

In addition to cautions posted at the entrance to the laboratory, appropriate universal warning signs or symbols shall be placed on all freezers, refrigerators, centrifuges, incubators, waste containers, etc., where hazardous materials are used or stored. See figure below.

- **General Biosafety instructions for laboratory workers**

i. Attend and complete all required laboratory safety training prior to the start of your research assignment.
ii. Follow all outlined safety instructions.
iii. Be familiar with the agents, processes, and equipment in the laboratory.
iv. Post appropriate warning signs within the laboratory.
v. Read all posted signs.
vi. Smoking, eating, drinking, applying cosmetics, and taking medicine in laboratories is strictly prohibited.
vii. Storing food, beverages or tobacco in laboratories is not permitted.
viii. Laboratory water sources and deionized water should not be used for drinking water and laboratory materials should never be consumed or tasted.
ix. Wear appropriate protective clothing and proper eye protection in chemical work, handling, and storage areas. Contact lenses should normally not be worn.
x. Open shoes, such as sandals, high-heeled shoes or shoes made of woven material should never be worn in the laboratory.
xi. Confine long hair and loose clothing.
xii. Keep workplaces clean and free of unwanted chemicals, biological specimens, and idle equipment. Avoid leaving reagent bottles, empty or with content, on the floor.
xiii. Work only with materials once you know their flammability, reactivity, toxicity, safe handling, storage, and emergency procedures.
xiv. Consult material safety data sheets (MSDS) before working with hazardous chemicals or infectious material. Read all labels carefully.
xv. Never remove chemicals, biological agents, or radioactive materials from the facility without proper authorization.
xvi. Label reagents, materials, and storage containers legibly and according to regulations.
xvii. Keep equipment back from the edge of the lab bench to prevent spillage and support all beakers and flasks with clamps. Do not use cracked or chipped glassware.
xviii. Use a hood whenever there is a possibility of poisonous or irritating fumes being emitted.
xix. Inform co-workers of any potential health hazard associated with the work performed Prepare and maintain a chemical inventory for the laboratory.

xx. Never pipette by mouth; use mechanical transfer devices.
xxi. Do not run in the laboratory.
xxii. Keep exits and passageways clear at all times.
xxiii. Be familiar with the location and operation of safety and emergency equipment such as fire extinguishers, eye wash and emergency shower, first aid and spill response kits, fire alarm pull stations and emergency exits.
xxiv. Ensure that access to emergency equipment (eyewashes, emergency showers and fire extinguishers) is not blocked.
xxv. Be familiar with the emergency spill response procedures for the materials you will handle.
xxvi. Report accidents and incidents ("near-misses") promptly to your supervisor.
xxvii. Wash your hands before leaving the laboratory.
xxviii. Leave your laboratory coats in the laboratory.
xxix. Procedures involving release of volatile toxic or flammable materials should be conducted in a chemical fume hood or in a well aerated room.
xxx. Perform procedures that liberate infectious aerosols in a biological safety cabinet or ventilated workstations or good laboratory techniques.
xxxi. Handle all human specimen as if potentially infectious.
xxxii. Avoid working alone in a laboratory, especially outside of regular business hours.
xxxiii. New or unfamiliar procedures should never be performed without supervision. Only work of relatively low risk should be performed without supervision.

- **Hand Washing**

Wash hands after laboratory procedures and before leaving the laboratory, this is essential to avoid becoming exposed to chemical irritants and infectious agents. Hand washing is one of the most important (and easiest) practices used to prevent transmission of pathogens. Hands or other exposed skin should be washed as soon as possible following an exposure incident. Use soft, antibacterial soap, if possible. Avoid harsh, abrasive soaps, as these may open fragile scabs or other sores. See figure below.

Source: World Health Organization

- **Good Housekeeping**

Housekeeping practices in the laboratory are essential in every workplace for keeping things clean and organized can help provide a safer environment. They are especially important in the laboratory environment, where spills from broken reagent containers, sample bottles, reaction vessels, etc. can create unnecessary exposure to potentially hazardous substances. The following items are steps to take to ensure the upkeep of a laboratory.

1. The work area should be kept clean and uncluttered, with hazardous materials and equipment properly stored. Clean the work area upon completion of a task and at the end of the day.
2. The custodial staff is only expected to perform routine duties such as cleaning the floor and emptying the general trash.
3. Drawers and cabinet doors must keep closed and electrical cords off the floor to avoid tripping hazards, aisles must keep clear of obstacles such as boxes, chemical containers, and other storage items that might be put there.
4. Slipping hazards should avoid by cleaning up spilled liquids promptly and by keeping the floor free of loose equipment such as stirring rods, glass beads, stoppers, and other such hazards.
5. Use the required procedure for the proper disposal of chemical wastes and solvents.

- **Use of Pipettes and Pipetting Aids**

Pipetting aids minimize the risk of aerosol generation when used properly; they also eliminate the risk of ingestion of infectious material through oral pipetting, which is prohibited at all containment levels. The following points highlight some requirements and recommendations for the safe use of pipettes and pipetting aids:

i. Use a Bio Safety Cabinet (BSC) when pipetting infectious material.
ii. work over plastic-backed absorbent material; the droplets will be absorbed rather than "splash".
iii. All pipettes should have cotton plugs to reduce contamination of pipetting devices.
iv. Use plastic pipettes instead of glass pipettes whenever possible
v. Air should never be blown through liquid containing infectious agents.
vi. Infectious materials should not be mixed by alternate suction and expulsion through a pipette.
vii. Liquids should not be forcibly expelled from pipettes.
viii. Use pipettes calibrated "to deliver", which reduces the risk of creating aerosols by retaining the last drop in the tip.
ix. Use appropriate decontamination procedures for pipette aids and micropipettors.
x. Contaminated pipettes should be completely submerged in a suitable disinfectant contained in an unbreakable container. They should be left in the disinfectant for 18-24 h before disposal.
xi. A discard container for pipettes should be placed close to the work area.
xii. Syringes fitted with hypodermic needles must not be used for pipetting. Blunt cannulas should be used instead of needles. There are devices for opening septum-capped bottles that allow pipettes to be used and avoid the use of hypodermic needles and syringes.

- **Handling Glassware:**

Glass breakage is a common cause of injuries in laboratories.

i. Only glass in good condition will be used.
ii. Discard all broken, chipped, starred or badly scratched glassware in marked "broken glass" containers only.
iii. Hand protection should be used when picking up broken glass.
iv. Protect hands with leather gloves when inserting glass tubing.
v. Do not store glassware near the edge of shelves.
vi. Store large or heavier glassware on lower shelves.
vii. Use glassware of the proper size. Allow at least 20% free space. Grasp a three-neck flask by the middle neck, not a side neck.
viii. Conventional laboratory glassware must never be pressurized.

- **Safe Use of Laboratory Equipment**

- **Fume Hoods**

Fume hoods are designed to evacuate dangerous fumes away from the laboratory worker. Fume hoods must be operating while in use (work, short term reagent holding). Fume hoods are certified by annually by an outside provider. To be certified, a fume hood must be operating within certain established parameters with respect to operating FPM flow. Fuming chemicals must always be handled in the fume hood. Do not tamper with fume hook measurement apparatus. Confirm that there is power to the unit and that it is operational. If you have reason to believe the equipment is not functioning properly, contact your lab manager and/or environmental health and safety (EHS) office before proceeding with any work that requires the protection of the hood/cabinet. Do not tamper with the measurement apparatus that might be connected to the hood/cabinet.

- **Laminar Flow Hoods**

Laminar flow hoods are designed to protect sensitive samples and filtered air is gently pushed out of the hood towards the laboratory worker. Laminar flow hoods must be operating while in use. Laminar flow hoods are certified by annually by an outside vendor. To be certified, a laminar flow hood must be operating within certain established parameters with respect to operating flow. Confirm that there is power to the unit and that it is operational. If you have reason to believe the equipment is not functioning properly, contact your lab manager

and/or environmental health and safety (EHS) office before proceeding with any work that requires the protection of the hood/cabinet. Do not tamper with the measurement apparatus that might be connected to the hood/cabinet.

- **Biological Safety Cabinets (BSC)**

General considerations:

i. Determine the biological safety level of the laboratory, type of infectious agents or biochemical hazard that may be present, and the nature of the work performed.
ii. Use a biological safety cabinet when performing procedures that may create an inhalation or aerosol hazard.
iii. Use the correct classification of a biological safety cabinet (BSC) when working with infectious agent depending on its risk classification.
iv. Biological Safety Cabinets must be inspected and certified when installed or relocated and certified annually thereafter. Documentation of certification consists of a sticker with the certification date affixed to each BSC. Also, the next due date for certification.

- **Use of Biological Safety Cabinets**

i. The use and limitations of biological safety cabinets should be explained to all potential users. Written protocols or safety or operations manuals should be issued to staff. It must be made clear that the cabinet will not protect the operator from spillage, breakage or poor technique.
ii. The cabinet must not be used unless it is working properly.
iii. The glass viewing panel must not be opened when the cabinet is in use.
iv. Apparatus and materials in the cabinet must be kept to a minimum. Air circulation at the rear plenum must not be blocked. Materials should be surface decontaminated before placing them inside the working area of the cabinet.
v. Bunsen burners must not be used in the cabinet. The heat produced will distort the air flow and may damage the filters. An electric micro-incinerator is permissible but sterile disposable transfer loops are better.
vi. All work must be carried out in the middle or rear part of the working surface and be visible through the viewing panel.
vii. Do not work in the BSC while the ultraviolet light is on. Ultraviolet light can quickly injure the eye.

viii. Do not place anything on the air intake grills, as this will block the air supply.

ix. Prevent unnecessary opening and closing of door because this will disrupt the airflow of the cabinet.

x. The operator should not disturb the air flow by repeated removal and reintroduction of his or her arms.

xi. The surface of the biological safety cabinet shall be wiped using an appropriate disinfectant at the beginning of a shift, after work is completed and at the end of the shift.

xii. The cabinet fan should be run for at least 5 min before beginning work and after completion of work in the cabinet.

- **Use of Centrifuges**

There is a risk of infectious aerosol generation when a centrifuge is used (e.g., tube breakage, improper use of safety cups or rotors, or lack of proper maintenance). The following points highlight some requirements and recommendations for centrifuge use when working with infectious material:

i. The outside surface of cups and rotors should be decontaminated, as required.

ii. Equipment should be used in accordance with the manufacturer's instructions, which includes the balancing of rotors to prevent rotor damage or explosion.

iii. Plastic tubes that are suitable for centrifugation should be used

iv. Centrifuges shall be placed on flat and firm surface to reduce vibrations and shall not share a bench with other laboratory equipment.

v. Centrifuges should be placed at such a level that workers of less than average height can see into the bowl to place trunnions and buckets correctly. Buckets and trunnions should be paired by weight and, with tubes in place, correctly balanced.

vi. Sealed centrifuge cups or rotors are to be used to prevent the release of aerosols during centrifugation, and the integrity of the cup or rotor seal regularly inspected.

vii. The buckets must be loaded, equilibrated, sealed, and opened in a biological safety cabinet or let stand for 30 minutes before opening.

viii. The amount of space that should be left between the level of the fluid and the rim of the centrifuge tube should be given in manufacturer's instructions.

ix. Distilled water or alcohol (Iso-propanol, 70%) shall be used for balancing empty buckets. Saline or hypochlorite solutions should not be used as they corrode metals.
x. Sealable centrifuge buckets (safety cups) shall be used for microorganisms of Risk Groups 3 and 4.
xi. When using angle head centrifuge rotors, care shall be taken to ensure that the tube is not overloaded as it might leak.
xii. The interior of the centrifuge bowl shall be inspected daily for staining or soiling at the level of the rotor. If staining or soiling is evident then the centrifugation protocols should be re-evaluated.
xiii. Centrifuge rotors and buckets shall be inspected daily for signs of corrosion and for hairline cracks.
xiv. Buckets, rotors and centrifuge bowls shall be decontaminated after each use.
xv. After use, buckets should be stored in an inverted position to drain the balancing fluid.
xvi. Infectious airborne particles may be ejected when centrifuges are used. These particles travel at speeds too high to be retained by the cabinet air flow if the centrifuge is placed in a traditional open fronted Class I or Class II biological safety cabinet. However, good centrifuge technique and securely capped tubes offer adequate protection against infectious aerosols and dispersed particles.

- **Blenders, Grinders, and Mortar and Pestle**

All these devices release considerable aerosols during their operation. For maximum protection to the operator during the blending of biohazards, the following practices should be observed:

a. Operate blending, cell disruption, and grinding equipment in a BSC.

OR

b. Use a heat-sealed flexible plastic film enclosure for a grinder or blender. The grinder or blender must be opened in a BSC.

- **Water Baths and Incubators**

After use, decontaminate water baths and incubators with an appropriate decontaminant. Maintenance service on water baths and incubators that appear to be improperly used and/or contaminated may be denied. It is not the responsibility of maintenance personnel to clean up after laboratory personnel.

- **Sonicators, Homogenizers, Shaking Incubators, and Mixers**

The operation of sonicators, homogenizers, mixers, shaking incubators, and other similar equipment can generate aerosols. The following points highlight some requirements and recommendations when using these types of equipment:

i. Laboratory equipment and associated accessories specially designed to contain infectious aerosols can be used for manipulations of pathogens and toxins. For example, cup horn sonicators allow sonication of samples within a contained vessel without direct contact with the material being processed.

ii. When equipment designed to contain infectious aerosols is not available, equipment should be operated in a BSC (only if the equipment does not disrupt airflow patterns) or another primary containment device.
iii. Time for aerosols to settle should be allowed before opening or removing the covers.
iv. Domestic (kitchen) homogenizers should not be used in laboratories as they may leak or release aerosols. Laboratory blenders and stomachers are safer.
v. Caps and cups or bottles should be in good condition and free from flaws or distortion. Caps should be well-fitting, and gaskets should be in good condition.
vi. At the end of the operation the containers should be opened in a biological safety cabinet or let to stand for 30 minutes before opening.
vii. Hearing protection should be provided for people using sonicators.

- Care and Use of Refrigerators and Freezers

i. Refrigerators, deep-freezers, and solid carbon dioxide (dry ice) chests should be defrosted and cleaned periodically, and any ampoules, tubes, etc. that have broken during storage removed. Face protection and heavy-duty rubber gloves should be worn during cleaning. After cleaning, the inner surfaces of the cabinet should be disinfected as per manufacturer's instructions.
ii. All containers stored in refrigerators, etc. should be clearly labeled with the scientific name of the contents, the date stored and the name of the individual who stored them. Unlabeled and obsolete materials should be autoclaved and discarded.
iii. An inventory must be maintained of the freezer's contents.
iv. Flammable solutions must not be stored in a refrigerator unless it is explosion-proof.

Notices to this effect should be placed on refrigerator doors.

- Microtomes and Cryostats

The microtome and the cryostat are used for cutting thin sections of fixed and unfixed tissue. The use of microtomes and cryostats in the laboratory presents a laceration hazard

in addition to generating potentially infectious aerosols. Unfixed tissues should be considered capable of causing infection and should be treated with care. Observe the following procedures when using microtomes/cryostats:

a. Always keep hands away from blades.

b. Position the sample first and then put in the blade with the blade edge positioned away from hands.
c. Use engineering controls like forceps, tweezers, dissecting probes, and small brushes to retrieve samples, change blades, dislodge blocks, or clean equipment.
d. Use protectors/guards for knife-edges that may extend beyond the microtome knife holder.
e. Wear appropriate personal protective equipment (PPE) such as gloves, lab coat or gown, mask, and safety glasses or goggles. Consider the use of surgical grade Kevlar gloves when using a cryostat to provide additional protection from cuts and scrapes.
f. Do not leave motorized microtomes running unattended.
g. Discard and handle trimmings and sections of tissue as biohazardous waste.
h. Do not move or transport a microtome with the knife in position.
i. Always lock the chuck rotating mechanism (wheel) to immobilize the block when not actively cutting tissue and before insertion or removal of the blade.
j. Never walk away from an exposed blade.
k. At the end of each session with the microtome or cryostat, either dispose of the blade immediately in a sharp's container or secure reusable blades in a container.

- **Safe Handling of Specimens in the Laboratory**

Improper collection, internal transport, and receipt of specimens in the laboratory carry a risk of infection to the personnel involved. Plastic specimen containers are preferable to glass due to biosafety reasons and should not leak when the cap or stopper is correctly applied. No material should remain on the outside of the container. Containers should be correctly labeled to facilitate identification (figure below). If the outside of the container is visibly contaminated with blood it should be cleaned with disinfectant. All blood specimens

should be placed in small leakproof impervious plastic tubes for transportation to the laboratory. Preferably blood specimens should be collected in vacutainer tubes. Specimen request or specification forms should not be wrapped around the containers but placed in separate, preferably waterproof envelopes/ zip locks whenever the specimen needs to be transported.

- **Specimen transport within the facility**

To avoid accidental leakage or spillage, secondary leak proof containers, should be used so that the specimen containers remain upright. The secondary containers may be of metal

or plastic, should be autoclavable or resistant to the action of chemical disinfectants, and the seal should preferably have a gasket. They should be regularly decontaminated. The outer container should be rigid and sturdy.

- **Opening specimen packages**

Personnel who receive and unpack specimens should be aware of the potential health hazards involved, and should be trained to adopt standard precautions, particularly when dealing with broken or leaking containers. Primary specimen containers should be opened preferably in a biological safety cabinet if not available must be opened while wearing proper PPE.

- **Avoiding the Dispersal of Infectious Materials**

i. To avoid the premature shedding of their loads, microbiological transfer loops should have a diameter of 2–3 mm and be completely closed. The shanks should not be more than 6 cm in length to minimize vibration.
ii. The risk of spatter of infectious material in an open Bunsen burner flame should be avoided by using an enclosed electric micro incinerator to sterilize transfer loops. Disposable transfer loops, which do not need to be re-sterilized, are preferable.
iii. Catalase tests should not be performed on slides to avoid bubbling and dispersal of aerosols. The tube, capillary tube or cover-glass methods should be used instead.
iv. Discarded specimens and cultures for autoclaving and/or disposal should be placed in leak proof containers, e.g. laboratory discard bags.
v. Working areas must be decontaminated with a suitable disinfectant, at the beginning of shift, after every procedure and at the end of shift.

- **Avoiding Ingestion of Infectious Materials and Contact with Skin and Eyes**

Large particles and droplets (> 5µm in diameter) released during microbiological manipulations settle rapidly on bench surfaces and on the hands of the operator.

i. Disposable gloves should be worn.
ii. Laboratory workers should avoid touching their mouth, eyes and face.
iii. Food and drink must not be consumed or stored in the laboratory.
iv. There should be no gum-chewing in the laboratory and cosmetics should not be applied in the laboratory.

v. The face, eyes and mouth should be shielded or otherwise protected during any operation that may result in the splashing of potentially infectious materials.

- **Avoiding Injection of Infectious Materials**

i. Accidental inoculation with broken or chipped glassware can be avoided through careful practices and procedures. Glassware should be replaced with plastic ware whenever possible.
ii. Injections may result from accidents with hypodermic needles (needle-sticks), glass Pasteur pipettes and broken glass.
iii. Needle-stick accidents can be reduced by (a) taking particular care, and (b) minimizing the use of syringes and needles; for many techniques, syringes with blunt cannulas may be used instead.
iv. Simple devices are available for opening septum-stoppered bottles so that pipettes can be used.
v. Needles should never be recapped. Without disconnecting them from the syringe (if available), disposable articles should be discarded into puncture-proof containers fitted with covers.
vi. Plastic Pasteur pipettes should replace those made of glass.

- **Separation of Serum and Plasma**

i. Only properly trained staff should be employed for this work.
ii. Gloves and eye and mucous membrane protection should be worn.
iii. Splashes and aerosols can only be avoided or minimized by good laboratory technique. Blood and serum should be pipetted carefully, not poured. Pipetting by mouth must be forbidden.
iv. After use, pipettes should be completely submerged in hypochlorite or other suitable disinfectant. They should remain in the disinfectant for at least 18 h before disposal or washing and sterilization for reuse.
v. Discarded specimen tubes containing blood clots, etc. (with caps replaced) should be placed in suitable leak proof containers for autoclaving and/or incineration.
vi. A solution of hypochlorite, freshly prepared daily, should be available for clean-up of splashes and spillages.

- **Opening of Ampoules Containing Lyophilized Infectious Materials**

Care should be taken when ampoules of freeze-dried materials are opened, as the contents may be under reduced pressure and the sudden in rush of air may disperse some of the materials into the atmosphere. Ampoules should always be opened in a biological safety cabinet.

The following procedures are recommended for opening ampoules.

i. First decontaminate the outer surface of the ampoule.
ii. Make a file mark on the tube near to the middle of the cotton or cellulose plug, if present.
iii. Hold the ampoule in a wad of alcohol-soaked cotton to protect hands before breaking it at a file scratch.
iv. Remove the top gently and treat as contaminated material.
v. If the plug is still above the contents of the ampoule, remove it with sterile forceps
vi. Add liquid for re-suspension slowly to the ampoule to avoid frothing.

- **Storage of Ampoules Containing Infectious Materials**

i. Ampoules containing infectious materials should never be immersed in liquid nitrogen because cracked or imperfectly sealed ampoules may break or explode on removal.
ii. If very low temperatures are required, ampoules should be stored only in the gaseous phase above the liquid nitrogen.
iii. Infectious materials should be stored in mechanical deep-freeze cabinets or on dry ice.
iv. Laboratory workers should wear eye and hand protection when removing ampoules from cold storage.
v. The outer surfaces of ampoules stored in these ways should be disinfected when the ampoules are removed from storage.

- **Inventory Control**

Laboratories should have a process for controlling inventory of infectious agents. Document and label all microorganisms stored in the lab. Properly decontaminate and dispose of any stocks or cultures that are not needed.

- **Biohazardous Waste**

Decontaminate all biohazardous liquid or solid wastes before disposal. This includes waste from research with all forms of rDNA. Do not fill sharps containers to more than two-thirds full.

Post-work Safeguards and Procedures

The safeguards and procedures specific to the facility to ensure that they are using the appropriate equipment and practices for the conditions of their laboratory including, but not limited to:

1. Engineering controls such as containment equipment; including, but not limited to:
    - Biological safety cabinets
    - Animal caging systems
    - Centrifuge safety containers
2. Administrative Controls such as vaccinations and the creation of biosafety plans and procedures.
3. Work Practices such as procedures that describes safe and proper work practices.
4. Personal protective equipment (PPE)

- **Engineering controls**

The basic concept behind engineering controls is that, to the extent feasible, the work environment and the biosafety/biocontainment risk associated with the laboratory procedures should be designed to eliminate hazards or reduce exposure to hazards. Engineering controls should be based on the following principles:

- If feasible, design the facility, equipment, or process to remove the hazard.
- If removal is not feasible, enclose the hazard to prevent exposure during normal operations.
- Where complete enclosure is not feasible, establish barriers or local ventilation to reduce exposure to the hazard during normal operations.

The basic types of engineering controls are:

- Process control
- Enclosure and/or isolation of source
- Ventilation

Some examples of engineering controls may include:

- Building ventilation/exhaust or HVAC (heating, ventilation, and air conditioning) must provide safe, comfortable, breathable environments for all employees and the public, and to minimize exposures to hazardous air contaminants. At BSL-3 and BSL-4, exhaust laboratory air must be directly exhausted to the outside since it is considered potentially contaminated. The exhausted room air can be high-efficiency particulate air (HEPA)-filtered to prevent the hazards from being released to the outside environment. The HVAC exhaust system must be sized to handle both the room exhaust and the exhaust requirements of all containment devices that may be present. Adequate supply air must be provided to ensure proper function of the exhaust system.
- The containment equipment should focus on biological safety cabinets, animal/arthropod caging systems, plant growth chambers and centrifuge safety containers. Secondary containment may include separation of the laboratory work area from public access, availability of decontamination equipment (e.g., autoclave), separate clean and dirty corridors, double entry ways, air locks, hand washing facilities, etc.
- Effluent Decontamination System (EDS) is defined as a system that sterilizes biohazardous liquid waste generated from biocontainment laboratories or other facilities prior to discharge.
- Pathological incinerators, alkaline hydrolysis digesters, or other approved means, must be provided for the safe disposal of the large carcasses of infected animals. Redundancy and the use of multiple technologies need to be considered and evaluated.
- Anaerobic digesters use a biochemical process in which organic matter is decomposed by bacteria in the absence of oxygen. Digesters must be airtight (no oxygen) for anaerobic digestion to occur.

- **Enclosure and Isolation**

An enclosure keeps a selected hazard "physically" separated from workers. Enclosed equipment, for example, is tightly sealed and it is typically only opened for moving samples/cultures or for cleaning and maintenance. Examples include closed animal caging, "glove boxes" or Class III biosafety cabinets. Care must be taken when the enclosure is opened for maintenance as exposure could occur if adequate precautions are not taken. The enclosure itself must be well maintained to prevent leaks.

Isolation places the hazardous process "geographically" away from most of the workers. Common isolation techniques are to create a contaminant-free area either around the equipment or around the employee workstations.

- **Administrative Controls**

Administrative controls are those that modify workers' work schedules and tasks in ways that minimize their exposure to workplace hazards. Examples include vaccinations and developing plans and procedures to reduce the risk to the worker. The plan should explain the following:

- Process controls should be appropriate for the activities performed and the select agent or toxin in use. Biosafety /biocontainment levels are dependent on the risks of the work being performed.
  For example, the BMBL recommends BSL-3 practices, containment equipment and facilities for all manipulations of suspect cultures of Francisella tularensis. In contrast, BSL-2 practices, containment equipment, and facilities are recommended for diagnostic activities involving infectious cultures of Bacillus anthracis, Burkholderia mallei, Burkholderia pseudomallei, and Yersinia pestis, where there is no propagation of the agent or risk of aerosol or droplet formation only. All other activities with these agents are to be conducted at BSL-3.

- Describe detailed safety measures to ensure that primary and secondary containment are maintained during especially hazardous procedures (e.g., intentional production of select agent infectious aerosols or select toxin aerosols).

- **Personal protective equipment (PPE)**

In determining the PPE and other safety equipment needed, consider the hazardous characteristics of each agent or toxin listed on the entity's registration and the risk associated with laboratory procedures related to the select agent or toxin. There may be hazards that require specialized personal protective equipment in addition to safety glasses, laboratory gowns, and gloves. For example, a procedure that presents a splash hazard may require the use of a mask and a face shield to provide adequate protection. Inadequate training

in the proper use of personal protective equipment may reduce its effectiveness, provide a false sense of security, and could increase the risk to the laboratory worker.

Employees should be educated that PPE must not be worn outside the containment laboratory except when transporting samples between laboratories within containment. It must not be worn (or stored) in break rooms, office areas, toilets, or outside the building. Employees must be properly instructed on how don (put on) required PPE before entering an area with a potential hazard that requires the use of the PPE. Procedures for employees to remove (doff) required PPE before leaving the area of potential exposure should be structured to prevent transfer of infectious material outside laboratory room, as well as protect workers from exposure to infectious agents during exit procedures.

Gloves can reduce the incidents of contamination of hands but cannot prevent penetrating injuries by needles and other sharp instruments. Gloves should be worn while collecting/ handling blood specimens, blood soiled items or whenever there is a possibility of exposure to blood or body fluids containing blood and gloves should worn while disposing laboratory waste.

Laboratory gowns prevent contamination of clothing. Laboratory gowns or uniforms (preferably wrap-around gowns) should be worn when in the laboratory and should be removed before leaving. Front opening lab coats/gowns must be buttoned up while working in the laboratory and must be with full sleeves. Plastic aprons should be used while cleaning infected re-usable items and during disposing wastes.

Facial protection reduces the impact and splash on face/eyes/mouth. Simple protective glasses/goggles or face shields may be worn if splashing or spraying of blood/body fluids is expected. These should not be worn outside the laboratory. Masks if used correctly may protect the user from aerosol/droplet borne/air-borne infection. The mask to be used is related to particular risk profile of the category of personnel and his/her work.

Two types of masks are recommended for various categories of personnel depending upon the work environment: triple layer surgical mask and N 95 Respirator.

See table 1 for biological safety - personal protective equipment (PPE) requirements.

See appendix 8: Steps to put on and take off personal protective equipment (PPE) including gown.

Table 1: Biological Safety - Personal Protective Equipment (PPE) Requirements

BSL-1	BSL-4	BSL-3	BSL-4
•Protective laboratory coats, gowns, or uniforms recommended to prevent contamination of personal clothing. •Protective eyewear worn when conducting procedures that have the potential to create splashes of microorganisms or other hazardous materials.	•Protective laboratory coats, gowns, smocks, or uniforms must be worn while working with hazardous materials. •Eye and face protection (goggles, mask, face shield or other splatter guard) must be used for anticipated splashes or sprays of infectious or other hazardous materials when the microorganisms are handled outside the Biological Safety Cabinet (BSC) or physical containment device.	• Protective laboratory clothing with a solid-front, such as tie-back or wrap-around gowns, scrub suits, or coveralls must be worn. • Eye and face protection (goggles, mask, face shield or other splash guard) must be used for anticipated splashes or sprays of infectious or other hazardous materials. (All procedures involving the manipulation of infectious materials must be	•The class III biosafety cabinet serves as engineering control to prevent worker exposure to infectious agents and material. • Protective Suit Laboratory: All work is conducted within a class II biosafety cabinet with the Use of a positive pressure suit connected to a HEPA filtered airline. The positive pressure suit completely isolates the laboratory worker from the laboratory environment, ensuring there is no
•Personnel who wear contact lenses in laboratories should also wear eye protection. • Gloves must be worn to protect hands from exposure to hazardous materials.	•Personnel who wear contact lenses in laboratories should also wear eye protection. • Gloves must be worn to protect hands from exposure to hazardous materials. • Eye, face and respiratory protection should be used in rooms containing infected animals.	conducted within a BSC, or other physical containment devices.) •Personnel who wear contact lenses in laboratories must also wear eye protection. • Gloves must be worn to protect hands from exposure to hazardous materials. • Eye, face, and respiratory protection must be used in rooms containing infected animals	contact with potentially hazardous material. Laboratory personnel who work in positive pressure suits require significant training.

Biosafety Aspects of Transmissible Agents

Clinical laboratories, especially those in health care facilities, receive clinical specimens with requests for a variety of diagnostic and clinical support services. Typically, the infectious nature of clinical material is unknown, and specimens are often submitted with a broad

request for microbiological examination for multiple agents (e.g., sputa submitted for "routine," acid-fast, and fungal cultures). It is the responsibility of the laboratory director to establish standard procedures in the laboratory that realistically address the issue of the infective hazard of clinical specimens. All occupational exposure to blood or other potentially infectious materials is regulated under the Occupational Safety and Health Administration (OSHA) standards for bloodborne pathogens (BBP, 29 CFR 1910.1030). The BBP standard applies when workers have occupational exposure to human blood or other potentially infectious materials (OPIM).

Employers and workers should be familiar with several key approaches to infection control, including universal precautions, standard precautions and transmission-based precautions.

- **Universal precautions (UP)**, originally recommended by the CDC in the 1980s, was introduced as an approach to infection control to protect workers from HIV, HBV, and other bloodborne pathogens in human blood and certain other body fluids, regardless of a patients' infection status. UP is an approach to infection control in which all human blood and certain human body fluids are treated as if they are known to be infectious. Although the BBP standard incorporates UP, the infection control community no longer uses UP on its own.
- **Standard precautions (SP)**, introduced in 1996 in the CDC/Healthcare Infection Control and Prevention Advisory Committee's "1996 Guideline for Isolation Precautions in Hospitals," added additional infection prevention elements to UP in order to protect healthcare workers not only from pathogens in human blood and certain other body fluids, but also pathogens present in body fluids to which UP does not apply. SP includes hand hygiene; the use of certain types of PPE based on anticipated exposure; safe injection practices; and safe management of contaminated equipment and other items in the patient environment. SP is applied to all patients even when they are not known or suspected to be infectious.

- **Transmission-based precautions (TBP)** for contact-, droplet-, and airborne-transmissible diseases augment SP with additional controls to interrupt the route(s) of transmission that may not be completely interrupted using SP alone. The different types of TBP are applied based on what is known or suspected about a patient's infection.

The BBP standard requires the use of UP, and extends UP to protect workers against pathogens found in saliva during dental procedures and body fluids in situations where it is difficult or impossible to differentiate between body fluids (e.g., vomit mixed with blood). During recent outbreaks of emerging infectious diseases, other body fluids to which UP and the BBP standard do not apply have been identified as potential sources of worker exposures and infections. For example, the CDC identified contact with urine, saliva, feces, vomit, and breast milk as potential sources of Ebola virus exposure. Studies also found that urine of individuals with Zika can contain high concentrations of infectious virus that could persist in urine longer than it is detectable in serum, a component of blood.

Special Precautions with Blood and Other Body Fluids, Tissues and Excreta

The precautions outlined below are designed to protect laboratory workers against infection by blood borne pathogens and infectious agents.

- **Collection, labeling and transport of specimens**

i. Universal precautions shall always be followed.
ii. Samples shall be collected from patients and animals in accordance with SOPs.
iii. For blood collection, conventional needle and syringe systems should be replaced by single-use safety vacuum devices that allow the collection of blood directly into stoppered transport and/or culture tubes, automatically disabling the needle after use.
iv. The tubes shall be placed in adequate containers for transport to the laboratory within the hospital facility.
v. Request forms should be placed in separate water-proof bags or envelopes.
vi. Reception staff shall not open these bags. Opening Specimen Tubes and Sampling Contents.
vii. Specimen tubes shall be opened in a Class I or Class II biological safety cabinet.
viii. Gloves should be worn for all procedures. Eye and mucous membrane protection is also recommended (goggles or shield (visor)).
ix. Protective clothing should be supplemented with a plastic apron.
x. The stopper should be grasped through a piece of paper or gauze to prevent splashing.
xi. Gloves should be changed immediately if contamination is visible.

xii. The heavy-duty gloves may be decontaminated and reused but should be discarded if they are peeling, cracked, discolored, or if they have puncture, tears etc.

xiii. Should be removed before handling doorknobs, telephones, pens, performing office work and leaving the laboratory. Remove disposable gloves without contacting the soiled part of the gloves and dispose of them in a proper container (see figure below).

xiv. Thoroughly wash your hands and other areas immediately after providing care. Use alcohol-based hand sanitizer where hand-washing facilities are not available if your hands are not visibly soiled. When practical, wash your hands before providing care.

Removing Disposable Gloves

To remove gloves without spreading germs, never touch your bare skin with the outside of either glove.

1- Pinch the palm side of one glove near your wrist. Carefully pull the glove off so that it is inside out

2- Hold the glove in the palm of your gloved hand. Slip two fingers under the glove at the wrist of the remaining gloved hand.

3- Pull the glove until it comes off, inside out. The first glove should end up inside the glove you just removed.

4- After removing the gloves:

- **Dispose of gloves and other personal protective equipment (PPE) in a proper biohazard container.**
- **Wash your hands thoroughly with soap and running water, if available. Otherwise, rub hands thoroughly with an alcohol-based hand sanitizer if hands are not visibly soiled.**

- **Glass and "Sharps"**

i. Plastics shall replace glass wherever possible. Only laboratory grade (borosilicate) glass should be used, and any article that is chipped or cracked should be discarded.

ii. Hypodermic needles must not be used as pipettes. Blunt cannulas are permitted.

- **Films and Smears for Microscopy**

Fixing and staining of blood, sputum and fecal samples for microscopy does not necessarily kill all organisms or viruses on the smears. These items should be handled with forceps, stored appropriately, and decontaminated and/or autoclaved before disposal.

- **Tissues**

i. Formalin fixatives shall be used. Small specimens, e.g. from needle biopsies, can be fixed

and decontaminated within a few hours, but larger specimens may take several days.

ii. Frozen sectioning shall be avoided. Should it be essential, the cryostat shall be shielded and the operator shall wear a safety shield (visor)

iii. For decontamination, the temperature of the instrument should be raised to at least 20˚C.

Laboratory Animal Handling Practices

Standard procedures should be designed to protect personnel and students that have contact with animals. These procedures should be consulted and followed when working with animals that may harbor biohazardous agents.

For security reasons, the animal house should be an independent, detached unit. If it adjoins a laboratory, the design should provide for its isolation from the public parts of the laboratory should such need arise, and for its decontamination and disinfestation.

Four standard biosafety levels are described for activities involving infectious disease work with commonly used experimental animals. These four combinations of practices, safety equipment, and facilities are designated Animal Biosafety Levels 1, 2, 3, and 4, and provide increasing levels of protection to personnel and the environment.

Working with animals in research, caring for animals in animal care facilities, or coming in contact with animals or vectors in the field may cause zoonotic or other diseases. A

zoonosis or zoonose is an infectious disease that can be transmitted (in some instances, by a vector) from nonhuman animals, both wild and domestic, to humans, or from humans to nonhuman animals (the latter is sometimes called reverse zoonosis). Human diseases caused by a noninfectious, etiological agent derived from animals or their vectors are not considered a zoonosis (e.g., allergic reactions to animal products such as dander or urine). Work involving animals may expose workers to etiologic agents in a variety of ways such as wound infections, inhalation of aerosols (e.g., dust from animal bedding), and animal bites or scratches.

The requirements for design features, equipment and precautions increase in stringency according to the animal biosafety level. The standard practices are:

1. Doors to animal rooms are self-closing and are kept closed when experiments are in progress.
2. Work surfaces are decontaminated following use or spills of biohazardous materials.
3. Eating, drinking, smoking, and storing food are not permitted in animal rooms.
4. Personnel wash their hands after handling viable cultures and animals and before leaving the animal room.
5. All procedures are carefully conducted to minimize the creation of aerosols.
6. An insect and rodent control program are in effect.

I. Animal biosafety level 1

Animal Biosafety Level 1 is suitable for work involving well characterized agents that are not known to cause disease in immunocompetent adult humans, and present minimal potential hazard to personnel and the environment.

A. Special practice

1. Bedding materials from cages used for animals infected with agents transmissible to humans are decontaminated (preferably by autoclaving) before being discarded.
2. Cages used for animals infected with agents transmissible to humans are washed and/or rinsed with water heated to at least 180°F for at least 20 minutes.
3. The wearing of laboratory coats, gloves, gowns, or a uniform in the animal room is recommended. Coats and gloves worn in the animal room are not worn in the laboratory or in other animal housing areas.

B. Biosafety equipment

Special containment equipment is generally not required for animals infected with agents assigned to Biosafety Level 1.

C. Animal facilities

1. The animal facility should be designed and constructed to facilitate cleaning and housekeeping.
2. A hand washing sink is available in the animal facility.
3. If the animal facility has windows that open, they are fitted with fly screens.
4. It is recommended, but not required, that the animal facility be provided within ward directional airflow and that exhaust air be discharged to the outside without being recirculated to other rooms.

II. Animal biosafety level 2

ABSL-2 is suitable for work involving laboratory animals infected with agents associated with human disease and pose moderate hazards to personnel and the environment. It also addresses hazards from ingestion as well as from percutaneous and mucous membrane exposure.

A. Special practices

1. Cages are decontaminated, preferably by autoclaving, before being cleaned and washed.
2. Surgical-type masks are worn by all personnel entering animal rooms housing non-human primates.
3. Laboratory coats, gowns, or uniforms are worn while in the animal room. Protective clothing is not worn elsewhere.
4. Access to the animal room is restricted by the laboratory or animal facility supervisor to personnel who have been advised of the potential hazard and whose presence is required when experiments are in progress. In general, persons who may be at increased risk of acquiring infection or for whom infection might be unusually hazardous are not allowed in the animal room. Persons at increased risk may include children, pregnant women, and individuals who are immunodeficient or immunosuppressed. The supervisor has the final responsibility for assessing individual circumstances and determining who may enter or work in the animal room.

5. The laboratory supervisor will assure that only persons who have been advised of the potential hazard and meet any specific requirements (e.g., immunization, if available) enter the animal room.
6. Hazard warning signs incorporating the universal biohazard warning symbol are posted on access doors to animal rooms when materials containing, or animals infected with agents assigned to Biosafety Level 2 or higher are present. The hazard warning sign should identify the agent(s) in use, list the name of the animal room supervisor or other responsible person(s) and indicate any special conditions of entry into the animal room (e.g., immunizations, respirators).
7. Special care is taken to avoid contaminating skin with biohazardous material; gloves are worn when handling infected animals and when skin contact with biohazardous materials is unavoidable.
8. All wastes from the animal room are appropriately decontaminated (preferably by autoclaving) before being disposed of. Infected animal carcasses are incinerated after being transported from the animal room in leak-proof, sealed containers.
9. Hypodermic needles and syringes are used only for the parenteral injection oraspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) are used for the injection or aspiration of biohazardous materials .Needles are not bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe are promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before being discarded or reused.
10. If floor drains are provided, the drain traps are always filled with water.
11. When appropriate, considering the agents handled, baseline serum samples from animal-care and other at-risk personnel are collected and stored. Additional serum samples may be collected periodically, depending on the agents handled or the function of the facility.

B. Containment equipment

Biological safety cabinets (Class II), other physical-containment devices, and/or personal protection devices (e.g., respirators, face shields) are used when procedures with a high potential for creating aerosols are conducted. These include necropsy of infected animals, harvesting of infected tissues or fluid from animals or eggs, intranasal inoculation of animals, and manipulation of high concentrations or large volumes of biohazardous materials.

C. Animal facilities

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping.
2. A hand washing sink is available in the room where infected animals are housed.
3. If the animal facility has windows that open, they are fitted with fly screens.
4. It is recommended, but not required, that the direction of airflow in the animal facility be inward and that exhaust air be discharged to the outside without being recirculated to other rooms.
5. An autoclave to decontaminate biohazardous laboratory waste is available in the same building that contains the animal facility.

III. Animal biosafety level 3

Animal Biosafety Level 3 involves practices suitable for work with laboratory animals infected with indigenous or exotic agents, agents that present a potential for aerosol transmission and agents causing serious or potentially lethal disease. ABSL-3 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-2. ABSL-3 laboratory has special engineering and design features.

A. Special practices

1. Cages are autoclaved before bedding is removed and before they are cleaned and washed.
2. NIOSH approved respiratory protection devices are worn by personnel entering rooms that house animals infected with agents assigned to Biosafety Level 3.
3. Wrap-around or solid-front gowns or uniforms are worn by personnel entering the animal room. Front-button laboratory coats are unsuitable. Protective gowns must remain in the animal room and must be decontaminated before being laundered.
4. The supervisor or other responsible person limits access to the animal room only to personnel who have been advised of the potential hazard and who need to enter

the room for program service purposes when infected animals are present .In general, persons who may be at increased risk of acquiring infection or for whom infection might be unusually hazardous are not allowed in the animal room.

5. The laboratory supervisor or other responsible person will assure that only persons who have been advised of the potential hazard and meet any specific requirements (e.g., immunization, if available) may enter the animal room.
6. Hazard warning signs incorporating the universal biohazard warning symbol are posted on access doors to animal rooms containing animals infected with or materials containing agents assigned to Biosafety Level 3. The hazard warning sign should identify the agent(s) in use, list the name and telephone number of the supervisor or other responsible person(s), and indicate any special conditions of entry into the animal room (e.g., the need for immunizations or respirators).
7. Personnel wear gloves when handling infected animals or biohazardous agents. Gloves are removed aseptically and autoclaved with other animal room waste before being disposed of or reused.
8. All wastes from the animal room are autoclaved before being disposed. All animal carcasses are incinerated. Carcasses are transported from the animal room to the incinerator in leak-proof, sealed containers.
9. Hypodermic needles and syringes are used only for gavage or parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles .Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) are used. A needle should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before being discarded or reused. When possible, cannulas should be used instead of sharp needles (e.g., gavage).
10. If floor drains are provided, the drain traps are always filled with water or a suitable disinfectant.
11. If vacuum lines are provided, they are protected with HEPA filters and liquid traps.
12. Boots, shoe covers, or other protective footwear, and disinfectant footbaths are available and used when indicated.

B. Containment equipment

1. Personal protective clothing and equipment and/or other physical containment devices are used for all procedures and manipulations of biohazardous materials or infected animals.
2. Animals are housed in partial-containment caging systems, such as open cages placed in ventilated enclosures (e.g., laminar-flow cabinets), solid wall and bottom cages covered by filter bonnets or other equivalent primary containment systems.

C. Animal facilities

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping and is separated from areas that are open to unrestricted personnel traffic within the building. Passage through two sets of doors is the basic requirement for entry into the animal room from access corridors or other contiguous areas. Physical separation of the animal room from access corridors or from other activities may also be provided by a double-door change room(showers may be included), airlock, or other access facility that require espassage through two sets of doors before entering the animal room.
2. The interior surfaces of walls, floors, and ceilings are water resistant and easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate fumigation or space decontamination.
3. A foot, elbow, or automatically operated sink for hand washing is provided near each animal-room exit door.
4. Windows in the animal room are closed and sealed.
5. Animal room doors are self-closing and self-locking and are kept closed when infected animals are present.
6. An autoclave for decontaminating wastes is available, preferably within the animal facility. Materials to be autoclaved outside the animal room are transported in a covered, leak-proof container.
7. An exhaust-air ventilation system is provided. The system creates inward directional airflow that draws air into the animal room through the entry area. The building exhaust can be used for this purpose if the exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. Personnel must verify that the direction of the airflow

is proper (i.e., into the animal room). The exhaust air from the animal room that does not pass through biological safety cabinets or other primary containment equipment can be discharged to the outside without being filtered or otherwise treated.

8. The HEPA-filtered exhaust air from Class II biological safety cabinets or other primary containment devices is discharged directly to the outside or through the building's exhaust system. Exhaust air from these primary containment devices may be recirculated within the animal room if the cabinet is tested and certified at least every 12 months. If the HEPA-filtered exhaust air from Class II biological safety cabinets is discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble-unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system.

IV. Animal biosafety level 4

Animal Biosafety Level 4 is required for work with animals infected with dangerous and exotic agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring ABSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or to re-designate the level.

A. Standard practices

a. Access must be strictly controlled; only staff designated by the director of the establishment should have authority to enter.
b. Individuals must not work alone: the two-person rule must apply.
c. Personnel must have received the highest possible level of training as microbiologists and be familiar with the hazards involved in their work and with the necessary precautions.
d. Housing areas for animals infected with Risk Group 4 agents must maintain the criteria for containment described and applied for maximum containment laboratories – Biosafety Level 4.
e. The facility must be entered by an airlock anteroom, the clean side of which must be separated from the restricted side by changing and showering facilities.

f. Staff must remove street clothing when entering and put on special, protective clothing. After work they must remove the protective clothing for autoclaving, and shower before leaving.
g. All animal bedding and waste must be autoclaved before removal from the facility.

B. Animal facilities

There are two models for ABSL-4 laboratories:

1. A Cabinet Laboratory where all handling of agents, infected animals and housing of infected animals must be performed in Class III BSCs.

2. A Suit Laboratory where personnel must wear a positive pressure protective suit ; infected animals must be housed in ventilated enclosures with inward directional airflow and HEPA filtered exhaust; infected animals should be handled within a primary barrier system, such as a Class II BSC or other equivalent containment system.

ABSL-4 builds upon the standard practices, procedures, containment equipment, and facility requirements of ABSL-3. However, ABSL-4 cabinet and suit laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment and personnel. The ABSL-4 cabinet laboratory is distinctly different from an ABSL-3 laboratory containing a Class III BSC.

Spill Procedures

Planning for spills

All spills of biohazardous materials do not represent the same risk to personnel and the environment, making each spill somewhat unique.

Factors other than volume that must be considered in spill risk assessment include:

- Location
- Nature
- Volatility and viscosity of spilled material
- Other properties of material
- Nature of affected surfaces
- Complicating materials
- Susceptibility of spilled material to neutralization/disinfection

a- Chemical Spill

The range and quantity of hazardous substances used in laboratories require preplanning to respond safely to chemical spills. The cleanup of a chemical spill should only be done by knowledgeable and experienced personnel.

The first line of action to have safe environment in the laboratories is to minimize and contain chemical spills which may occur at any time. Sometimes, even a small spill of chemical can cause much harm to the public health. All chemical spills irrespective whether it is a major or minor spill must be officially reported to the chairman of the Department. A chemical spill is considered serious and urgent to care for if it causes injuries, fire, breathing problems, airborne contaminants, or requires an extended cleaning effort.

Most manufacturers of laboratory chemicals issue charts describing methods for dealing with spills. Appropriate charts should be displayed in a prominent position in the laboratory. The following equipment should also be provided:

1. Chemical spill kits
2. Protective clothing, e.g. heavy-duty rubber gloves, overshoes or rubber boots, respirators.
3. Scoops and dustpans
4. Forceps for picking up broken glass
5. Mops, cloths, and paper towels
6. Buckets
7. Soda ash (sodium carbonate, Na2CO3) or sodium bicarbonate (NaHCO3) for neutralizing acids and corrosive chemicals
8. Sand (to cover alkali spills)
9. Non-flammable detergent.

The most occurring chemical spills are:

- Inorganic acids or bases

Small amounts: use neutralizing agent as soda or an adsorbent like vermiculite, dry sand or towels.

Large amounts: flush with large amounts of water if water does not pose any danger like reacting violently with other chemicals which might occur in the area of the spill. Make sure that the floors must be dry after the cleanup process is over.

-Volatile materials

With fully functional hood equipped with appropriate filters, allow the spill to evaporate.

-Solids

If the spilled solid is not dangerous and does not pose any threat to the health, the spill should be swept and placed in the proper chemical waste container for appropriate treatments. High power vacuum cleaner with appropriate filter must be used to clean up the solid toxic spill. If the solid spill has caused staining, always give priority to the manufacturer's stain removal guideline if available.

-Mercury spills

Due to its severe effect to public health, special care must be taken when dealing with mercury spills. Mercury can get into the body through inhaling its vapor or getting absorbed through the skin. The use of mercury must be kept to the minimum. Mercury spills must be covered completely with adsorbent and properly and fully cleaned. Mercury spill kits are available in all chemistry labs. Protecting clothing, cloves, and breathing devices must be used when with mercury spills.

Since students breaking Mercury Thermometers is one of the main reasons of Mercury spills in laboratories, we need special precautions when cleaning up broken mercury thermometers:

• Remove students from the area.

• Clean up the spill promptly to avoid accumulation on surfaces and evaporation causing inhalation. Because of the very small amount of mercury involved (less than 0.5 ml), it is usually not necessary to use a respirator in these cases. Use a mercury absorption sponge to collect the liquid beads from your Mercury clean-up kit. Wash the affected area with a detergent soap and allow to air dry before it is safe to reuse the area.

• Do not touch the liquid mercury with your hand and do not flush down the drain nor use a broom or vacuum to clean up mercury.

The following actions should be taken in the event of a significant chemical spill:

- Minor Chemical Spill

1. Alert people in immediate area of spill. All electrical and gas supplies must be turned off.
2. Wear protective equipment, including safety goggles, gloves, and long-sleeve lab coat.
3. If there is chemical exposure to others, respond as quickly as possible in administering First Aid.

4. Avoid breathing vapours from spill (wear appropriate breathing apparatus or protection).
5. Confine spill to small area.
6. Use appropriate kit to neutralize and absorb inorganic acids and bases. Collect residue, place in container, and dispose as chemical waste.
7. Clean spill area with water.
8. Review area when decontamination is complete. Check surrounding areas.
9. Report incident.

- Major Chemical Spill

Protect yourself and, if safe to do –

1. Attend to injured or contaminated persons and remove them from exposure.
2. Alert people in the laboratory to evacuate (use Break glass alarm if needed)
3. If spilled material is flammable, turn off ignition and heat sources.
4. Call Security
5. Cordon of the immediate area and close doors to affected area.
6. Have person knowledgeable of incident and laboratory assist emergency personnel.
7. Report incident.

Chemical Spill on Body

1. Flood exposed area with running water from faucet or safety shower for at least 5 minutes.
2. Remove contaminated clothing at once.
3. Make sure chemical has not accumulated in shoes.
4. Obtain medical attention, if necessary.
5. Report incident.

b- Spill Clean Up of Biological Agents

It is important to be aware that there may be potential risks in handling and processing biological materials (especially those of human), microorganisms such as bacteria and viruses (e.g. Hepatitis A, B and C virus, HIV), recombinant DNA, tissue culture and oncogenic viral systems and other human pathogens that are blood-borne, etc. All spills involving biological material and agents should therefore be treated as potentially infectious.

As part of the biosafety program, Laboratory has a spill response kit with appropriate personal protective clothing and clean up materials located in the laboratory and is available for use at all times. Spill kits should include absorbent material appropriate to contain the spill and reduce aerosols.

All biological spills must be attended to immediately. The approach that take will depend on the risk group of the biological material, the volume and location of the spill.

Biological Spill Kit

A basic biological spill kit should include:

Personal Protective Equipment

Materials: Appropriate disinfectant , bottle for making dilutions of disinfectant , paper towels or other suitable absorbent materials , forceps or tongs , sharps container for broken glass or needle, dust pan with broom , yellow biohazard trash bags , biohazard spill alert notice , biological spill response procedure and emergency contact numbers.

1- General Spills Clean-up Procedure (outside of biological safety cabinet)

- **Small spill of Risk group 1 or 2 material (< 100mls)**

1. Alert people in immediate area of spill.
2. At a minimum, wear disposable gloves and face protection.
3. Cover spill with paper towel or other absorbent material.
4. Carefully pour a freshly prepared 1:10 dilution of household bleach (or other effective disinfectant) around the edges of the spill and then into the spill. Avoid splashing.
5. Allow a 20-minute contact period for bleach (or as indicated as effective time for different disinfectant). If broken glass is present, use forceps to remove and place glass in sharps collection container.
6. Use paper towels to wipe up the spill, working from the outer edges into the center.
7. Clean spill area again as indicated in steps 4 and 5.
8. Depending on the size and concentration of the spill, a third disinfection (steps 4 and 5) may be warranted.
9. Discard disinfected disposal materials. Items that do not contain large amounts of bleach may autoclaved according to the Medical Waste Management Policy before disposal.

• **Spill Involving Concentrated Microorganisms Required BSL 3 Containment (e.g. Mycobacterium tuberculosis, (TB) cultures)**

Attend to injured or contaminated persons and remove them from exposure. Alert people in the area to evacuate. Close doors to affected area; do not enter area for at least one hour. Have a person knowledgeable of the incident and area assist in proper clean-up. Wearing gowns, gloves, respirator, and shoe covers, clean up spills as indicated for biohazard spill clean-up.

• **Large spill of Risk group 1 material (> 100mls)**

1. Get help if required.
2. Clean up procedure is the same as for small risk group 1 spills only on a larger scale.
3. Notify the lab manager or supervisor that there has been a spill.

• **Large spill of Risk group 2 material (> 100mls)**

1. Get Help.
2. Contact the lab manager immediately.
3. Keep people out of the area
4. If the spill is potentially infectious the area must be vacated for 30 minutes to allow aerosols to settle before the clean- up procedure can commence.
5. Follow the instructions inside the Biohazard Spill kit.
6. Remove and decontaminate any PPE (including lab coats) according to laboratory protocols.
7. Report the incident.

2- Spill in a Biological Safety Cabinet

1. Ensure that the cabinet is on and continues to operate during the clean-up procedure
2. Put on PPE
3. Remove any sharp objects with forceps
4. Cover the spill with absorbent material and dispose of in the biological waste bin

5. All surfaces must be decontaminated. Cover the affected area with suitable disinfectant (e.g. 1:10 dilute of sodium hypochorite) and leave for the appropriate contact time (e.g. 30 minutes).

6. Ensure that the surfaces below the work area are also treated.
7. After decontamination thoroughly rinse the surface to remove any remaining bleach because it can corrode stainless steel.
8. Items in the BSC at the time of the spill must be thoroughly cleaned with 70% ethanol prior to removal from the BSC and/or bagged for removal and autoclaved.
9. Wipe the inside of the cabinet with 70% ethanol and allow BSC to run for 10 minutes prior to resuming work.
10. Remove and decontaminate any PPE (including lab coats) according to laboratory protocols.
11. Report incident.

3- Spill in a centrifuge

A biological spill in a centrifuge has the potential for creating aerosols. As soon as the operator becomes aware of a spill immediate action is required.

1. Turn off centrifuge. Do not open the centrifuge for 30 minutes to allow the aerosols to disperse and settle; place a note warning other not to open it.
2. Allow 30 minutes settling time before cleaning up procedures commence.
3. Put on PPE. Remove all debris.
4. Place contaminated equipment in leak proof bag and if possible, transfer to Biological Safety cabinet for disinfection
5. Disinfect (1:10 dilute of sodium hypochorite) the interior of the centrifuge and the head (or cups).
6. All debris must be collected, bagged, autoclaved, and disposed of appropriately
7, Remove and decontaminate any PPE (including lab coats) according to laboratory protocols.
8. Report spill.

4- Biological Spill on Body

1. Remove contaminated clothing.
2. Vigorously wash exposed area with soap and water for 1 minute.
3. Obtain medical attention, if necessary.
4. Report incident

5- Hazardous Material Splashed in Eye

1. Immediately rinse eyeball and inner surface of eyelid with water continuously for 15 minutes.
2. Forcibly hold eye open to ensure effective wash behind eyelids.
3. Obtain medical attention.
4. Report incident.

6- Minor Cuts and Puncture Wounds

1. Vigorously wash injury with soap and water for several minutes.
2. Obtain medical attention.
3. Report incident.

Laboratory Emergencies

Emergencies resulting from fire, natural disasters, riots, wars, utility failures or loss of services (e.g. electric power, heat, air conditioning, water, etc.) can have a disastrous impact on laboratory equipment, materials, tests, and research. By taking appropriate precautions, we will avoid losses and releases of hazardous materials.

Prepare a lab contingency plan that meets your specific needs. This plan should be shared with your lab, your department, and your management for inclusion in the Institution Emergency Plan. The plan should be implemented whenever a major emergency event has been issued. Remember, you must take responsibility to protect your laboratory and research.

The plan should be kept relevant by needed updates and by regularly calling telephone numbers of contacts listed in the plan and protocols to ensure both numbers and contact persons are current and accurate. All procedures should be tested on a regular basis as well as randomly.

➢ Laboratory Shutdown Procedures

These procedures should be implemented whenever a severe event threatens laboratory operations, or when directed by the Institution's Emergency Management system.

- Shutdown experiments that could be affected by the loss of electricity, water, gas or other services.
- Close the sash on all chemical fume hoods in the event that ventilation is lost.
- Remove all infectious materials from biosafety cabinets, and autoclave, disinfect, or safely store them as appropriate.
- Ensure that all chemical, biological, radioactive materials and hazardous waste containers are properly covered and sealed.
- Ensure that all gas valves are closed.
- Turn off all appliances, computers, hot plates, ovens, and other equipment.
- Review storage of perishable items. Consolidate valuable items within storage units that have backup systems or store items in duplicate locations as appropriate. Review safety precautions for the use of alternate cooling methods (e.g. liquid nitrogen, dry ice, etc.), if used.

- Ensure that water reactive chemicals are in sealed containers and stored in areas that are unlikely to become wet.
- Check that all gas cylinders are secured. Remove regulators and install transport caps where possible.
- Elevate equipment, materials, and supplies, including electrical wires and chemicals, off the floor, particularly in lower elevations that are prone to flooding.
- Close all doors, including cabinets, storage areas, offices, and utility chase-ways.
- Lock all exterior lab doors before leaving.
- Secure lab notebooks and backup critical data on computers.

➢ In case of Major High Hazard Emergencies

Major high hazards emergencies are situations that pose such as an immediate threat to health, property or the environment and require

- immediate attention due to imminent danger
- evacuation of employees in the area
- response from outside the immediate release area

Releases of hazardous materials are major emergency situations which normally require the assistance of outside specialists. The release may cause high levels of exposure to toxic substances and may pose a serious threat of fire and explosion, conditions that are immediately dangerous to life and health.

RESPONDING TO MAJOR EMERGENCIES

- Notify your supervisor and call Safety Unit
- Identify yourself and the reason you are calling.
- Identify the exact location (building, room number) of the emergency.
- Identify the nature of the emergency, any injuries or symptoms involved, and the identity of any hazardous materials involved if you know them.
- For situations that threaten fire or explosion and spills in which hazardous vapors are present:
  - Evacuate the area and tell others to evacuate.
  - Close, but do not lock doors behind you to isolate the area.
  - If you have time to do so safely, close fume hood sashes.

- If you have time to do so safely, post a sign to warn others not to enter the area.
- Call Safety Unit from a safe location nearby.
- If fire, smoke, gases, or vapors are spreading to other areas:
  - Pull the fire alarm to evacuate the building.
  - Call Safety Unit from a remote location and inform them of the spill.
  - Be available to advise emergency response personnel by identifying yourself when they arrive.
  - Someone responsible for that room or building should be present to provide details of the incident to emergency responders. This individual should be able to identify the types and quantities of chemicals stored there, and their locations within the rooms.

➢ Fires

Be prepared for fires. Participate in the annual building evacuation drills. Know where your emergency exits, and nearest fire alarms are. Learn how to use fire extinguishers. Fire-fighting equipment should be placed near room doors and at strategic points in corridors and hallways. This equipment may include hoses, buckets (of water or sand) and a fire extinguisher. Fire extinguishers should be regularly inspected and maintained, and their shelf-life kept up to date. Your ability to respond quickly and competently with the appropriate fire extinguisher can keep a minor flame from turning into a major conflagration. Fire warnings, instructions and escape routes should be displayed prominently in each room and in corridors and hallways.

It is essential that all electrical installations and equipment are inspected and tested regularly, including earthing and grounding systems. All laboratory electrical equipment and wiring should conform to national electrical safety standards and codes.

Common causes of fires in laboratories:

1. Electrical circuit overloading
2. Poor electrical maintenance, e.g. poor and perished insulation on cables
3. Excessively long gas tubing or long electrical leads
4. Equipment unnecessarily left switched on
5. Equipment that was not designed for a laboratory environment
6. Open flames
7. Deteriorated gas tubing
8. Improper handling and storage of flammable or explosive materials

9. Improper segregation of incompatible chemicals
10. Sparking equipment near flammable substances and vapors
11. Improper or inadequate ventilation.

In the event of a fire

- Pull the fire alarm first.
- Then, if you have been trained and the fire is very small, attempt to extinguish it with an appropriate fire extinguisher.
- If you manage to completely extinguish the fire, inform safety office.
- If you decide to fight the fire, do so from a position where you can escape.
- A fire contained in a small vessel can usually be suffocated by covering the vessel with a lid.
- While attempting to control the fire, continually assess the situation.
- If you doubt your ability to quickly extinguish the fire with an extinguisher, get out of the building.
- If possible, prior to evacuation shut down any equipment that may add fuel to the fire.
- Do not turn off any hoods in the immediate area as they will work to keep the area free from smoke and flames but close the door behind you to help prevent the fire's spread.
- When you evacuate, move well away from the building to allow firefighters room to work.
- Move upwind of the building.

Do not reenter the building until permission is given by the Fire Department.

Types and uses of fire extinguishers

TYPE	USE FOR	DO NOT USE FOR
Water	Paper, wood, fabric	Electrical fires, flammable liquids, burning metals
Carbon dioxide (CO_2) extinguisher gases	Flammable liquids and gases, electrical fires	Alkali metals, paper
Dry powder	Flammable liquids and gases, alkali metals, electrical fires	Reusable equipment and instruments, as residues are very difficult to remove
Foam	Flammable liquids	Electrical fires

➢ Explosions

Many experiments release tremendous amounts of energy. There are many fuel sources in a lab that can aggravate the situation. An explosion is a High Hazard Emergency.

Beware of secondary explosions, fires, and spills or releases of toxic chemicals due to glass container damage triggered by the first blast.

Stay clear of windows that may shatter.

Preparing for a Power Failure in the Lab

Learning what to do and how to prepare for a laboratory power failure provides substantial savings in budget, supplies, personnel resources, and equipment damage while bringing the operation back up to its normal capacity.

The following guidelines are general suggestions for laboratory preparedness before, during and after a power outage or loss event. Consult with your Risk Management Team to develop an approved plan.

➢ Before the Power Fails

- Equip your emergency/spill kit with a battery powered flashlight.
- Do not leave open chemicals in the fume hood when the fume hood is unattended. Always safely store chemicals after use.
- Put essential equipment on emergency power circuits. These circuits should have red cover plates and are powered by an emergency generator at each lab building.

- Install appropriately sized surge protection devices for all sensitive or expensive electronics.
- Consult with Facilities Management if you need to install an uninterruptible power source (ups) or other backup electrical systems or equipment. Make a list of equipment that must be reset, reprogrammed, restarted, or recalibrated once power returns.
- Post the list in a conspicuous place.
- Program equipment that operates unattended to shut down safely during a power failure and not restart automatically when power returns.
- Identify an emergency source of dry ice if you have items that must be kept cold. Refrigerators and freezers will maintain their temperature for several hours if they are not opened.
- Do not use dry ice in walk-in refrigerators or other confined areas. Designate an emergency contact person for your lab. This person should be available for contact 24 hours a day.
- Give the contact's information to your Safety Coordinator. Post emergency contact phone numbers on the lab safety sign in the hallway outside your lab.
- Consult with Facilities Management if you need to install an uninterruptible power source (ups) or other backup electrical systems or equipment.
- Make a list of equipment that must be reset, reprogrammed, restarted, or recalibrated once power returns.
- Post the list in a conspicuous place.
- Program equipment that operates unattended to shut down safely during a power failure and not restart automatically when power returns.
- Identify an emergency source of dry ice, if you have items that must be kept cold. Note: Refrigerators and freezers will maintain their temperature for several hours if they are not opened.
- Do not use dry ice in walk-in refrigerators or other confined areas

➢ When the Power Returns
- Check equipment.
- Reset and restart equipment.

- If building systems, including fume hoods, fail to restart or operate correctly, contact designated person.
- If non-building equipment fails to restart or operate correctly, contact designated person the manufacturer or service provider.
- Confirm air flow in your fume hood is restored.
- Recalibrate and reprogram equipment, as necessary.
- Keep doors closed on refrigerators and freezers that failed until they have been repaired and returned to safe working temperature.
  Note: Some refrigerators and freezers require a manual restart.
- If system or equipment failures create hazardous conditions, immediately notify designatedperson

Summary

- Ensure experiments, equipment and machinery are stabilized and safe.
- Cease work, close containers in fume hoods and close the sash. In most buildings, the fume hoods are not connected to generator power. Do not use hazardous materials or enter areas that require mechanical ventilation during the outage.
- Avoid opening environmental room, refrigerator or freezer doors until power is restored.
- Maintain a log of equipment that must be reset, restarted or that requires special attention following an outage.
- For an extended power outage, consider consolidating materials in freezers and using dry ice. Order additional dry ice supplies immediately.
- Equipment that runs unattended should have been programmed to shut down safely and not restart when power returns.
- After the outage, reset or restart equipment. Check air flow in hoods.

Shipping of Infectious Substances

The shipping of infectious materials is governed by national laws and administrations. Internationally, the International Air Transport Association (IATA) and the International Civil Aviation Organization (ICAO) as well as the World Health Organization and the United Nations have input on the transportation of infectious substances and biological specimens. Because of the numerous and varied organizations involved, a person who ships infectious materials should stay aware of the annual updates and changes made in the requirements to avoid shipping delays and fines. Nobody is immune.

Below is a description of the procedure that may be required in the shipment of an infectious material or biological specimen. These guidelines are not all inclusive to all situations and shipments. The shipper should consult with the relevant guidelines for updates and additions to the applicable regulation to ensure full compliance. International shipments entering and leaving the country will require import export documentation and further approvals. The approval process may be lengthy; plan ahead, the approval process can be lengthy.

- Shipping Regulations

United Nations (UN) - Committee of Experts (UNCETDG)
Different transport modes (air, sea, rail, road)
International Civil Aviation Organization (ICAO)
International Air Transport Association (IATA)
International Maritime Organization (IMO)
Universal Postal Union (UPU)
Others (ADR/RID)

- Useful Definitions from IATA (Dangerous Goods Regulations, 49th edition)

Infectious substance

Infectious substances are defined as substances that are known or are reasonably expected to contain pathogens. Pathogens are defined as microorganism (including bacteria, viruses, rickettsiae, parasites, fungi) and other agents such as prions, which can cause disease in humans and animals.

Biological products

Biological products are those products derived from living organisms that are manufactured and distributed in accordance with the requirements of appropriate national authorities, which may have special licensing requirements, and are used either for prevention, treatment, or diagnosis of disease in humans or animals, or for development, experimental or investigational purposes related thereto. They include, but are not limited to, finished or unfinished products such as vaccines.

Category A

Category A is an infectious substance which is transported in a form that, when an exposure to it occurs, is capable of causing permanent disability, life threatening or fatal disease in otherwise healthy humans or animals. Category A infectious substances have two shipping names: "Infectious substances, affecting humans" (UN 2814) or "Infectious substances, affecting animals" (UN 2900).

Category B

An infectious substance which does not meet the criteria for inclusion in Category A. Category B infectious substances have the proper shipping name "Biological Substance, Category B" and the identification number UN 3373.

Cultures

Cultures are the result of a process by which pathogens are intentionally propagated. This definition does not include patient specimens as defined below.

Patient specimens

Patient specimens are those collected directly from humans or animals, including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluid swabs, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention.

Medical or clinical waste

Medical or clinical waste is waste derived from the medical treatment of animals or humans or from bioresearch.

➢ Shipping of Infectious Materials via Express Couriers

Infectious substances are assigned to Classification 6.2. In this classification there are two categories, A and B. Any biological specimen that may have a likelihood of causing a possible infection must be assigned to one of the two categories. A level of judgment must be used in these instances. An organism that may be doubtful in meeting the criteria must be included as a Category A organism.

Category A Infectious substance

UN 2814 Infectious substance affecting humans

Bacillus anthracis
Brucella abortus
Brucella melitensis
Brucella suis
Burkholderia mallei– Pseudomonas mallei– Glanders cultures
Burkholderia pseudomallei– Pseudomonas
Chlamydia psittaci– avian strains
Clostridium botulinum
Coccidioides immitis
Coxiella burnetii
Crimean-Congo hemorrhagic fever virus
Dengue virus
Eastern equine encephalitis virus
Escherichia coli, verotoxigenic
Ebola virus
Flexal virus
Francisella tularensis
Guanarito virus
Hantaan virus

Hantavirus causing hemorrhagic fever with renal syndrome
Hendra virus
Hepatitis B virus
Herpes B virus
Human immunodeficiency virus
Highly pathogenic avian influenza virus
Japanese Encephalitis virus
Junin virus
Kyasanur Forest disease virus
Lassa virus
Machupo virus
Marburg virus
Monkeypox virus
Mycobacterium tuberculosis
Nipah virus
Omsk hemorrhagic fever virus
Poliovirus
Rabies virus
Rickettsia prowazekii
Rickettsia rickettsii
Rift Valley fever virus
Russian spring-summer encephalitis virus
Sabia virus
Shigella dysenteriae type 1
Tick-borne encephalitis virus
Variola virus
Venezuelan equine encephalitis virus
West Nile virus
Yellow fever virus
Yersinia pestis

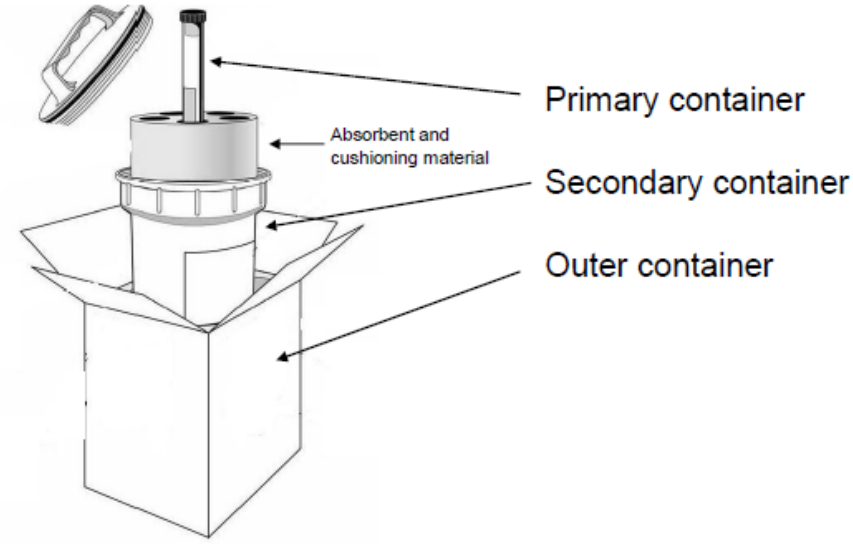
UN 2900 Infectious substances affecting animals

African swine fever virus
Avian paramyxovirus Type 1 – Velogenic Newcastle disease virus
Classical swine fever virus
Foot and mouth disease virus
Lumpy skin disease virus
Mycoplasma mycoides– Contagious bovine pleuropneumonia
Peste des petits ruminants virus
Rinderpest virus
Sheep-pox virus
Goatpox virus
Swine vesicular disease virus
Vesicular stomatitis virus

- Packaging

Triple packaging method

The packaging of a shipment of an infectious material or biological material should follow the triple packaging method. The material that is to be shipped is held in a vessel that will not leak its contents. Preventing leaking may be accomplished with a self-sealing container or by additional means like taping or using wax film to create a leak-proof seal. This primary container is then placed into a secondary container that also contains absorbent material. The absorbent material should be of adequate amount to absorb the entire contents from the primary contain in the event of loss of containment of the primary container. See graphic below.



The third layer is that of the outside packaging. This outer packaging should be durable, rigid, and able to with stand transportation. Depending on the category of material being shipped there may be specific requirements to the individual components. For example, the secondary container for the Category A infectious substances must be able to withstand an internal pressure of 95 kPa.

Outside shipping container markings

The outside of any container used to ship infectious substances must be marked in accordance with the pertinent guidelines. There are differences in the requirements for a Category A, category B and other packages.

Category A shipments must be labeled with the following:

- UN Packing instruction 602
- Inner packaging:
  - Watertight primary container
  - Watertight secondary container
  - Primary or secondary container must be able to withstand without leaking, internal pressure differential of no less than 95kPa (0.95 bar) and a temperature range of – 40 C to 55 C.
  - Absorbent material able to absorb the contents of the primary container(s), can be used to separate individual primary containers

- Itemized list of contents, inserted between secondary container and outer packaging
- Rigid outer packaging, smallest external dimension cannot be less than 100mm.
- Only same type materials are to be shipped together. E.g.: Category A and chemical hazard are not permitted to be shipped together.
- If the infectious substance being shipped is unknown but suspected of being included into category A the words "Suspected Category A Infectious Substance" must be shown in parenthesis following the proper shipping name on the documents inside the package on the list of contents.
- The package must be marked on the outside with a name and telephone number of a responsible person.
- If solid carbon dioxide is used as a refrigerant internal support must keep the secondary container in place as the dry sublimates away.
- If wet ice is used the packaging or over- pack must be leak proof.

Infectious substance Cargo aircraft only Dry Ice, UN1845
Note, The weight in Kg of dry ice must be noted on the outside of the packaging.

Category A shipments UN 2814 and 2900

The shipping of Category A infectious substances requires a high level of packaging. The packaging must meet specific requirements that are outlined in the UN packing instruction 602.

The packaging must include: (From IATA Dangerous Goods Regulations, 49th edition)

- Inner packaging comprising of:
    - Watertight primary receptacle(s)
    - A watertight secondary packaging

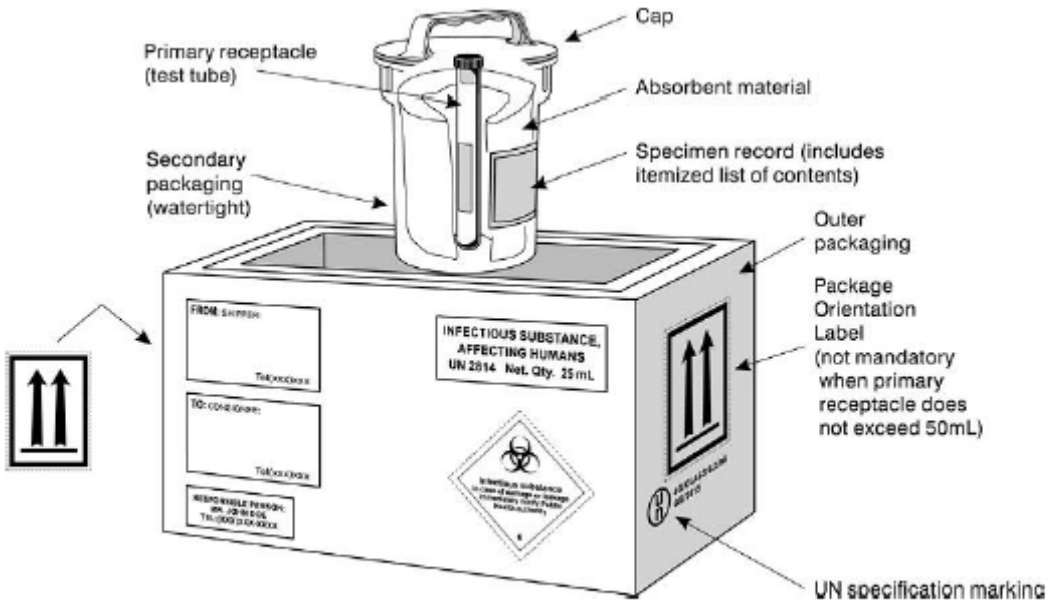
Other than for solid infectious substances, absorbent material, such as cotton wool, in sufficient quantity to absorb the entire contents placed between the primary receptacle(s)

and secondary packaging; if multiple fragile primary receptacles are placed in a single secondary packaging, they must either be individually wrapped or separated so as to prevent contact between them.

- An itemized list of contents, enclosed between the secondary packaging and the outer packaging; and
- A rigid outer packaging of adequate strength for its capacity, weight and intended use. The smallest external dimension must be not less than 100mm.

Whatever the intended temperature of the consignment, the primary receptacle or the secondary packaging must be capable of withstanding, without leakage, an internal pressure producing a pressure differential of not less than 95kPa and temperatures in the range of -40 C to 55 C. All packages containing infectious substances must be marked durably and legibly on the outside of the package with the NAME and TELEPHONE NUMBER OF A PERSON RESPONSIBLE.

Below is an example of a Category A package (P620):



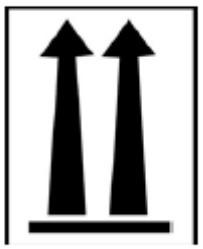
The outside markings for a Category A shipment need to include the following:

1. Infectious substance diamond

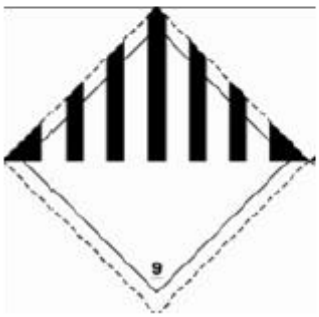
2. Proper shipping name, *Infectious substance, affecting humans UN 2814* or *Infectious substance, affecting animals, UN2900.*

3. To and from labels, include responsible person with telephone number

4. Package orientation label, if volume is over 50ml.



5. If using dry ice, use the Class 9 Miscellaneous Dangerous goods diamond. Include the weight of the dry ice on the outside of the package.



Include the weight of the dry ice on the outside of the package.

Category B infectious substances UN 3373

Category B infectious substances are materials that are not included on the Category A list and do not met exceptions. Category B infectious substances also are packaged under the triple packaging system. The outer packaging must be of suitable construction to with stand normal shipping. The packaging must be composed of the triple packaging model (primary container, secondary container, and rigid outer packaging). The secondary packaging must contain enough absorbent material to contain the contents of the primary container in the event of loss of containment by the primary container. The secondary container must have ample packaging material to prevent movement within the outer packaging. For packages containing liquids the following must be followed (IATA Dangerous Goods Regulations, 49th edition):

Liquid Substances

- The primary receptacle(s) must be leak proof, cannot contain more than 1L
- The secondary container must be leak proof
- If multiple fragile primary receptacles are placed in a single secondary packaging, they must either individually wrapped or separated to prevent contact between them.
- Absorbent material must be placed between primary receptacle and the secondary packaging. The absorbent material, such as cotton wool, must be in sufficient quantity to absorb the entire contents of the primary receptacle(s) so that any release of liquid substance will not compromise the integrity of the cushioning material or of the outer packaging.
- The primary receptacle or the secondary packaging must be capable of withstanding, without leakage, an internal pressure of 95kPA in the range of -40 C to 55 C
- The outer packaging must no contain more than 4L. This quantity excludes ice, dry ice or liquid nitrogen when used to keep the specimens cold.

Solid Substances

- The primary receptacle(s) must be sift proof and must not exceed the outer packaging weight limit.
- The secondary packaging must be sift proof
- If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them.

- Except for packages containing body parts, organs or whole bodies, the outer packaging must not contain more than 4Kg. This quantity excludes ice, dry ice or liquid nitrogen when used to keep specimens cold.
- If there is any doubt as to whether or not residual liquid may be present in the primary receptacle during transport then a package suitable for liquids, including absorbent materials must be used.

An itemized list of contents must be enclosed between the secondary packaging and the outer packaging. At least one surface of the outer packaging must have a minimum dimension of 100mm X 100mm.

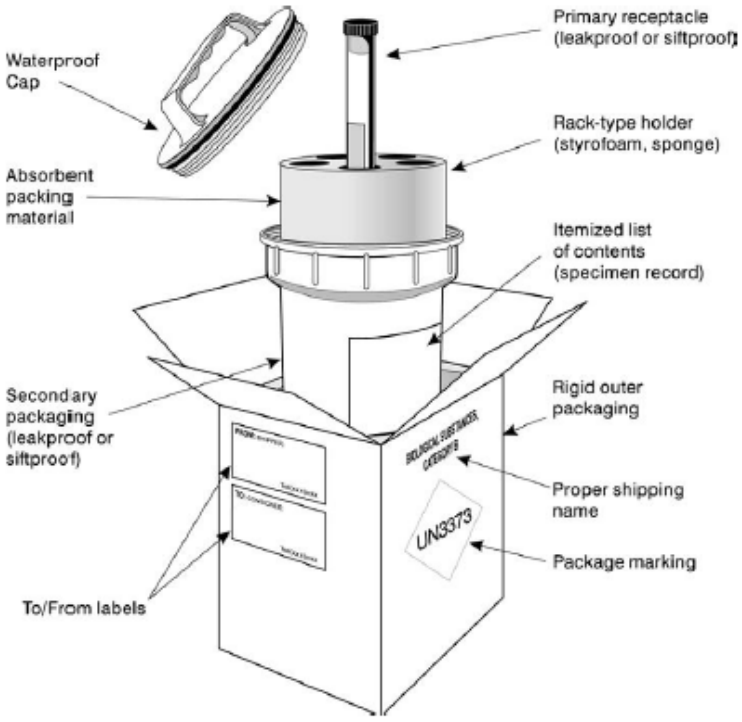
Packaging for Category B Infectious Substances

The shipping of a Category B substance will need to comply with the UN 650 packing instructions.

Packaging's meeting the UN 650 instructions are available from many suppliers. The packaging follows the triple packaging standard. The maximum for a single container is 1L of liquid or 1 Kg of solid material. An over pack can contain a maximum of 4L of liquid and 4 Kg of solid materials.

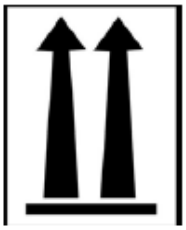
These quantities don not include the addition of ice, dry ice, or liquid nitrogen as refrigerant for the materials.

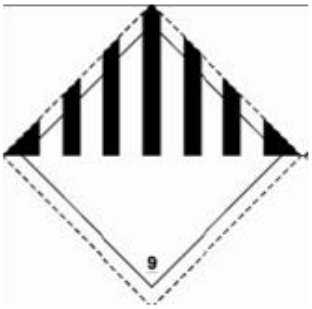
Below is an example of a Category B package (P650):



The outside markings for a Category B shipment need to include the following:

1. UN 3733 diamond
2. Proper shipping name, *Biological substance, Category B*
3. To and from labels, include responsible person
4. Package orientation label, if volume is over 50ml
5. If using dry ice, use the Class 9 Miscellaneous Dangerous goods diamond. Include the weight of the dry ice on the outside of the package.





Shipping Documentation


SHIPPER'S DECLARATION FOR DANGEROUS GOODS

Shipper

Air Waybill No.

Page of Pages

Shipper's Reference Number (optional)

Consignee

Two completed and signed copies of this Declaration must be handed to the operator.

TRANSPORT DETAILS

This shipment is within the limitations prescribed for: (Delete non-applicable)

PASSENGER AND CARGO AIRCRAFT

CARGO AIRCRAFT ONLY

Airport of Departure:

Airport of Destination

WARNING

Failure to comply in all aspects with the applicable Dangerous Goods Regulations may be in breach of the applicable law, subject to legal penalties.

Shipment Type (Delete non-applicable)

NON-RADIOACTIVE

RADIOACTIVE

NATURE AND QUANTITY OF DANGEROUS GOODS

Dangerous Goods Identification

UN or ID No. | Proper Shipping Name | Class or Division (subsidiary risk) | Packing Group | Quantity and Type of Packing | Packing Inst. | Authorization

Additional Handling Information

I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name, and are classified, packaged, marked and labelled/placarded, and are in all respects in proper condition for transport according to applicable international and national governmental regulations.

Name/Title of Signatory

Place and Date:

Signature (see warning above)

A Declaration of Dangerous goods form must be completed for any shipment of infectious substances. The finished printed copy must be in color. At least four copies must be with the package when shipped. One copy is retained for the shipper's documentation and archiving. The remaining copies are usually attached to the outside of the packaging and are accessible for inspection. The following areas of the Dangerous Form must be completed for the package to be shipped properly. Incomplete and or incorrect forms are the most common reason for refusal of the shipment.

- Shipper, name, address, and phone number of the person shipping the package
- Consignee, Full name, and address of the person who will be receiving the package
- Transport details, mark if the package is restricted to cargo aircraft only or both passenger and cargo aircraft.
- Shipment types: mark out the non-applicable radiological category.
- UN or ID number: enter the appropriate UN or ID number, for example UN 2814.
- Proper shipping name: fill in the proper shipping name as determined by the UN number.
- Class or Division, enter the appropriate hazard class (6.2 infectious substances, 9 dry ice or GMOs)
- Packaging group, Biological materials do not have a packaging group, dry ice will be III

- Quantity and Type of Packaging: enter the net amount of the materials, use metric units. Towards the bottom of this column indicate "All packed in one fiberboard box" or "Overpack used".
- Packaging instructions: enter the correct packaging instructions (E.g. 602 for Category A, 650 for Category B and 904 for dry ice).
- Authorization: used for special quantity limitations, special packaging, any other relevant information, or documents
- Additional handling instructions: this can be a good place to include any emergency contact information for the package.
- Signature box: sign, and date the document. Signing the document verifies that the signee has met all applicable national and international requirements to ship that package.

Shipping with Dry Ice

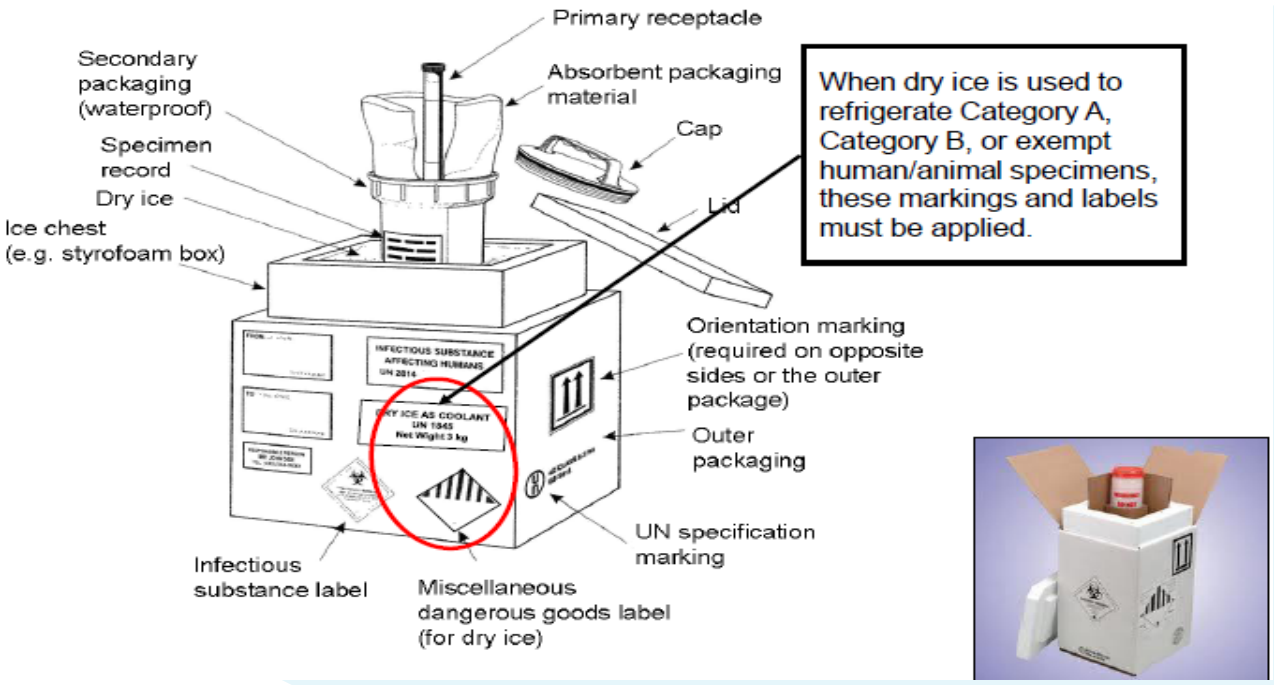
Many shipments of infectious materials require that the materials being shipped be kept cold. Dry ice is a common material used to keep shipments cold. Dry ice is considered a hazardous material based on the suffocation hazard from the CO_2 that is given off, the possibility of a contact hazard because of the extreme cold and the possibility of explosion if kept in a sealed container with no venting. Because of these hazards' authorities and the International Air Transport Association considers dry ice a hazardous material. As with any hazardous material being shipped training must be met with associated records.

The UN identification number for dry ice is 1845. Packaging instruction 904

To ship a package with dry ice one should meet the following:

The package must be able to vent the carbon dioxide that is released as the dry ice sublimates. The packaging must no seal tightly this may develop an explosive situation as pressure will build up inside a sealed container. Because of the extreme low temperature of dry ice the containers and packaging should be chosen carefully. Many plastics become brittle at low temperature. Use only approved containers for shipments using dry ice. Approved containers are part of approved shipping packages for Category A & B infectious substances. The package that is being shipped must be labeled with a miscellaneous hazard class 9 label. The net quantity in Kilograms of the dry ice must be marked on the outside of the package. Information for the Dangerous goods declaration are Un or ID number, UN 1845; Proper shipping name, Dry ice or Carbon dioxide,

solid; Class or Division, 9: Quantity, Enter the amount of dry ice used in Kg; Packing instructions, 904. Remember when shipping with dry ice not to seal the package completely to prevent venting of the evolved CO_2. Do not completely tape every seam fully on the package.



➢ Shipping Companies' Acceptance

United Parcel service (UPS)

The UPS will mail packages falling under the UN3373 class and patient specimens. No category A class shipments will be accepted.

DHL

DHL will accept all shipments that are compliant with the IATA and DOT regulations.

Federal Express

FedEx will not accept shipments involving Risk Group 4 organisms. FedEx Express and Ground will accept shipments that are compliant with the IATA and DOT regulations.

Be sure to check with the specific carrier for any restrictions. The policies can and will change over time.

➢ Training Requirements

The training frequency is every two years for IATA.

1. General awareness with aspects of shipping dangerous goods
2. IATA and national regulations
3. Specific training to duties
4. Safety training
5. Test of material
6. Successful completion, certificate issuance.

Occupational Health and Medical Surveillance

A health and medical surveillance program (including pre-employment and then periodic testing) needs to be appropriate to the agents in use and the programs in place in the laboratory. As such, the details of the health and medical surveillance program would be determined and defined by a risk assessment process based upon national and International practices that clearly demonstrates the reasons, indications and advantages for such a program to be in place.

This health and medical surveillance program may include but is not limited to the following: a medical examination; serum screening, testing and sometimes storage; immunizations; and possibly other tests as determined by the risk assessment process.
Risk assessment should be carried out by a multidisciplinary group including management, safety, and occupational health professionals. The health and medical surveillance program risk assessment would include consideration of those people working with high risk organisms, because knowledge of immune status is critical for decisions concerning immunizations, prophylaxis, etc. Only people meeting these identified medical entry requirements (e.g., immunizations) may enter the laboratory unless the facility has been appropriately decontaminated.

The risks of working with particular agents should be fully discussed with individual researchers. The local availability and utility of possible vaccines and/ or therapeutic drugs (e.g. antibiotic treatments) in case of exposure should be evaluated before work with such agents is started. Some workers may have acquired immunity from prior vaccination or infection. If a particular vaccine is available, it should be offered after a risk assessment of possible exposure and a clinical health assessment of the individual have been carried out. First aid should be defined, widely promulgated, and immediately available to an injured worker. Facilities for specific clinical case management following accidental infections should also be available.

Each institution should have an Emergency Response Guide flipchart posted in each laboratory. The guide should contain procedures for spills, exposure incidents, reporting instructions, contact numbers, and the location of emergency equipment. The PI or lab safety coordinator must review the guide with new personnel.

- Vaccines

Commercial vaccines should be made available to workers to provide protection against infectious agents to which they may be occupationally exposed. The US Advisory Committee on Immunization Practices (ACIP) provides expert advice on the most effective means to prevent vaccine-preventable diseases and to increase the safe usage of vaccines and related biological products

If the potential consequences of infection are substantial and the protective benefit from immunization is proven, acceptance of such immunization may be a condition for employment. Current, applicable vaccine information statements must be provided whenever a vaccine is administered. Each worker's immunization history should be evaluated for completeness and currency at the time of employment and re-evaluated when the individual is assigned job responsibilities with a new biohazard.

When occupational exposure to highly pathogenic agents is possible and no commercial vaccine is available, it may be appropriate to immunize workers using vaccines or immune serum preparations that are investigational, or for which the specific indication constitutes an off-label use. Use of investigational products, or of licensed products for off-label indications must be accompanied by adequate informed consent outlining the limited availability of information on safety and efficacy. Use of investigational products should obey the country drug regulations.

➢ Instructions for Injuries, Accidents, Animal Bites, and Exposures

Report all injuries, accidents, animal bites, and exposures to your supervisor and complete the Accident Report Form.

The medical provider's description of the injury should include:

- The potential infectious agent.
- The mechanism and route of exposure (percutaneous, splash to mucous membranes or skin, aerosol, etc.).
- Time and place of the incident.
- Personal Protective Equipment (PPE) used at the time of the injury.
- Prior first aid provided (e.g., nature and duration of cleaning and other aid, time that lapsed from exposure to treatment).
- Aspects of the worker's personal medical history relevant to risk of infection or complications of treatment.

OBTAINING MEDICAL ATTENTION

- For serious medical emergencies, call emergency department

HAZARDOUS MATERIAL ON SKIN OR SPLASHED IN EYE

- Remove contaminated clothing, shoes, jewelry, etc.
- Immediately flood exposed areas with lukewarm water from safety shower, eyewash, or faucet for at least 15 minutes (use soap on skin for biological/blood exposure). Hold eyes open to ensure effective rinsing behind both eyelids.
- Immediately after rinsing, obtain medical attention.
- Review MSDS(s) for hazards and report the incident

NEEDLESTICK OR CUT WITH CONTAMINATED SHARP ITEM

- Immediately wash the area with soap and water for at least 15 minutes.
- Immediately after rinsing, obtain medical attention.
- Report the incident.

MONKEY-RELATED INJURIES/BITES

- Immediately stop what you are doing and secure the animal in its cage.
- EYE SPLASH: immediately rinse eye for 15 minutes.

- BITE/SCRATCH/CUT: go to nearest B Virus Bite Kit
- Wash the wound with the Betadine scrub brush for 15 minutes.
- Rinse the wound with sterile saline solution and bandage with sterile gauze.
- Immediately obtain medical attention for ANY exposure or possible exposure. Report the incident.

INJURY INVOLVING RESEARCH ANIMAL (If monkey-related, see above.)

- BITE/SCRATCH/CUT: wash the area with soap and water for at least 15 minutes.
- Obtain medical attention and report incident to the animal facility.

ASSISTING IN MEDICAL EMERGENCY OR PERSONAL INJURY

- Do not move injured person unless there is a danger of further harm from remaining in the location. If the area is unsafe, then evacuate, close doors to area, and prevent access. Provide information to emergency responders.
- Remain with the injured person until medical assistance arrives.

Initiate life-saving measures if necessary and you are trained

Bloodborne Pathogens Program and Exposure Control Plan

Countries have created enforced regulations for the protection of workers who may have exposures to blood and other potentially infectious materials (OPIM). OPIM are defined as: semen, vaginal secretions, cerebrospinal fluid, peritoneal fluid, amniotic fluid, synovial fluid, pleural fluid, pericardial fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood and all body fluids in situations where it is difficult or impossible to differentiate between blood and body fluids.

Occupational exposure to human blood, tissues and body fluids poses a significant health risk because these may contain bloodborne pathogens such as:

- Babesia species
- Human Immunodeficiency virus (HIV)
- Borrelia species
- Colorado Tick Fever viruses
- Hepatitis B virus (HBV)
- Hepatitis C virus (HBC)
- Hepatitis D virus
- Brucella species
- Arboviruses
- Leptospira species
- Spirillum minus
- Francisella species
- Creutzfeldt-Jakob virus
- Plasmodium species
- Treponema species
- Streptobacillus moniliformis
- Human T-lymphotropic Virus Type I
- Hemorrhagic Fever viruses

The work practices covered include procedures with needles, medical equipment or devices, research with HIV or Hepatitis B and C and many other procedures were there would be a reasonable expectation of coming into contact with blood or body fluids.

An employer is responsible to provide to their employees training, personal protective equipment, access to Hepatitis B vaccination at no cost, safer devices, access to an exposure control plan and appropriate follow up care after an exposure. Each year the employer must review the exposure control plan, provide refresher training, and review many other aspects of the Blood borne pathogen program.

All institutions are committed to protecting its employees from risks associated with exposure to bloodborne pathogens. The following principles must be followed when employees are potentially exposed to bloodborne pathogens:

- Minimize all exposure to bloodborne pathogens.
- Institute as many engineering and work practice controls as possible to eliminate or minimize employee exposure to bloodborne pathogens.
- Routinely employ "Universal Precautions" when exposure to blood or potentially infectious materials is anticipated.

All employees working with Bloodborne Pathogens need to attend an initial training class on bloodborne pathogens as well as an annual refresher course.

➢ Standard Universal Precautions

1. Blood and OPIM must be handled in a manner that minimizes splashing, spraying, splattering, and generation of droplets. The use of personal protective equipment (PPE) i.e. gloves, gown, eye shields, masks etc., is required to reduce the risk of occupational exposure. In addition to the use of PPE hand hygiene is required at the start of the work shift, and between dirty and clean procedures on the same patient. Hand hygiene is also required after: contact with equipment or environment that may be contaminated, using the restroom, eating, drinking, smoking, and applying cosmetics. It is required as well before and after: contact with patients and specimens, wearing gloves or other PPE, contact with mucous membranes, and preparing food. Hand hygiene may be accomplished with either an antimicrobial gel or soap and water washing.
2. Gloves are required when anticipated hand exposure to blood or OPIM is anticipated. Examples include: venous access procedures (e.g. phlebotomy), specimen collection, open wound contact and when handling or touching contaminated items or surfaces.
3. PPE such as gowns and disposable plastic aprons are required during procedures when splashing with blood or OPIM is anticipated.

4. PPE such as masks, face shields, ventilation devices and protective eye wear are required during procedures when splashing, spraying, splatter, or droplets of blood and OPIM to the eyes, nose or mouth is anticipated. Eyeglasses are NOT PPE.
5. Used syringes and disposable sharps must be disposed of immediately at point of use in puncture resistant containers. Do not overfill the containers. Needles should not be recapped or manipulated in any way. If needles must be recapped, a one-hand scooping technique or recapping device must be used.
6. Laboratory specimens must be processed and handled in a safe manner with gloves and placed into leak proof containers labeled with biohazard symbol when required.
7. Hepatitis B vaccination is strongly recommended for all employees who have the potential for occupational exposure to blood and OPIM. This is administered in a series of three injections. It is highly effective and safe and is offered free of charge to all employees.

➢ Response to Blood and OPIM Exposure

Exposure to blood or OPIM via needles, other sharps injury, mucous membranes or non-intact skin requires:

- immediate and thorough washing of the affected area,
- contacting your supervisor,
- seeking immediate medical evaluation, and
- documentation of the exposure incident on the Sharps Log.

Bioethics and Dual Use Research of Concern

I. Bioethics

➢ Introduction

The term `ethics" broadly describes the way in which we look at and understand life, in terms of good and bad or right and wrong. Moral theories are the frameworks we use to justify or clarify our position when we ask ourselves "what should I do in this situation?" or "what is right or wrong for me?" There are many moral theories and there is no one right theory. They converge and often borrow from one another. Three theories will be described here. Consequentialism and Deontology have dominated moral reasoning over the last 300 years; Bioethics, a common morality theory, is a recent theory that dominates current thinking in health care settings.

1. Consequentialism

"The End Justifies the Means"

In consequentialism, the consequence of an action justifies the moral acceptability of the means taken to reach that end. The results of actions outweigh any other consideration; in other words, "the end justifies the means". Jeremy Bentham was an early and influential advocate of utilitarianism, the dominant consequentialist position. A utilitarian believes in "the greatest happiness for the greatest number". The more people who benefit from a particular action, the greater its good.

Scenario

Consider the following scenario: A doctor is working in the Accident and Emergency Department of a hospital. A homeless man is brought in with brain damage sustained in a road traffic accident. The doctor recognizes him; the man has no family and is in reasonable physical, if not mental, health. The doctor knows there is still time to save the man's life. He also knows that if he does not start treatment, the man will suffer brain death and his organs could possibly be used to improve the quality of or even save several other people's lives. Assuming there is no penalty associated with either choice, what would a strict utilitarian do?

- Save the man's life
- Contact the transplant team to ready them to harvest any available organs

Answer: The doctor would allow the man to die and try to use his organs to save as many people as possible. This would bring the greatest happiness to the greatest number of people.

2. Deontology

Deontology or Kantianism is an obligation-based theory whose chief author was Immanuel Kant, who lived in the 18th century. This theory emphasizes the type of action rather than the consequences of that action. Deontologists believe that moral decisions should be made based on one's duties and the rights of others. According to Kant, morality is based on pure reason. As people have the innate ability to act rationally, they therefore must act morally, irrespective of personal desires. Another way of stating Kant's theory is "Act morally regardless of the consequences".

- In the case of the doctor and the homeless man, again assuming there is no penalty for either decision, which would a deontologist do?

Answer: The doctor would save the man's life, as it is his duty to treat a patient. The fact that several people's lives might be improved by allowing the man to die would not justify making that decision.

3. Bioethics

Common morality theories are usually based on principles that are used to guide ethical thinking, based on a shared moral belief. One of these theories is Bioethics, the ethics of biology, biological research and the applications of that research. It is an ethical theory that brings together medicine, the law, social sciences, philosophy, theology, politics and other disciplines to address questions related to clinical decision making and medical research.

Principles of Bioethics

Some of the early founders of bioethics put forth four principles that form this framework for moral reasoning. These four principles are:

- Autonomy – one should respect the right of individuals to make their own decisions
- Nonmaleficence – one should avoid causing harm
- Beneficence – one should take positive steps to help others
- Justice – benefits and risks should be fairly distributed

One commentator has said, "…the four principles should…be thought of as the four moral nucleotides that constitute moral DNA – capable, alone or in combination, of explaining and justifying all the substantive and universalisable moral norms of health care ethics…"

Scenario

Consider this scenario: A woman goes to her doctor to receive the results of a genetic test. The results show she does have the condition for which she was tested and this allows her doctor to prescribe a treatment strategy that will reduce her symptoms and delay (and perhaps prevent) the progression of the condition. Her doctor knows from their discussions that the woman has an identical twin sister and he asks if she is going to tell her sister of the test results. The sister could then undergo genetic testing herself and perhaps enter treatment. The woman says she does not want her family to know about her condition; as well she will not talk to her sister as they are not on speaking terms. The doctor knows the woman's twin should be notified so that she can be helped also, but he has a duty of care to his patient. Which principles should be considered when trying to decide how to proceed?

- Autonomy
- Nonmaleficence
- Beneficence
- Justice

➢ Summary

In summary, ethics is how we look at and understand life, while moral theories are frameworks we use to decide how to act. Consequentialism is a theory that emphasizes the results of actions, while Deontology stresses the requirement to act morally, irrespective of the outcome. Bioethics is a principle-based theory that brings together the ethics of biology, biological research and their applications. The four principles of Bioethics are autonomy, the right of individuals to make their own decisions; nonmaleficence, one should avoid causing harm; beneficence, positive steps should taken to help others; and justice, the benefits and risks should be fairly distributed. While no one moral theory is correct, and there are many more to be considered, they provide a useful tool to guide ethical decision-making.

II. Dual Use Research of Concern

➢ Introduction

In the field of life science and infectious diseases, acceleration in science has outpaced our legal and ethical frameworks. Dual-Use Research of Concern (DURC) is a term applied to life sciences research, which is conducted with the intent of benefitting human health, but could also be misused to cause harm([1];[2]). DURC is now a publicly available reality ([3];[4]). This includes creation of synthetic viruses, and since the 2011 controversy over H5N1 avian influenza DURC, genetic engineering of pathogens that are not infectious to humans, to make them unnaturally infectious. This new era in DURC raises questions about potential population-level harm to human beings, which have not traditionally been considered by human research ethics committees or Institutional Review Boards (IRBs), and may require us to revise systems and processes for conduct of such research. Whilst some individual scientific bodies, journals and institutions have created policies around DURC, there is no standardized approach to consideration of DURC when such research is proposed, nor to considering the population health impacts on a global level. In the USA, the 2004 Fink Report (National Research Council 2004) recommended the creation of a National Science Advisory Board for Biosecurity (NSABB). The NSABB was created and faced its first major challenge in 2011, over the controversy about publication of methods for engineered transmissible H5N1 influenza virus[5]. The concern is that if the methods for engineering potentially pandemic strains of influenza were made public, terrorists may use these methods to cause harm to populations. The NSABB initially recommended censorship of publication of full research methods, but later, after an outcry from scientists, reversed the decision in 2012[6]. The governance of DURC has been questioned after this decision, with questions raised about process, conflicts of interest, lack of transparency, and decision-making in the NSABB ([7];[8]). DURC could potentially be self-regulated by scientists, or

1 Selgelid MJ (2009) Dual-use research codes of conduct: lessons from the life sciences. NanoEthics 3(3): 175-183

2 WHO (2014) Dual use research of concern (DURC), World Health Organization. Retrieved 12 Dec 2014, from http://www.who.int/ csr/durc/e

3 Herfst S, Schrauwen EJA, Linster M, Chutinimitkul S, de Wit E, Munster VJ et al (2012) Airborne transmission of influenza A/H5N1 virus between ferrets. Science 336(6088): 1534-1541

4 Imai M, Watanabe T, Hatta M, Das SC, Ozawa M, Shinya K *et al* (2012) Experimental adaptation of an influenza H5 HA confers respiratory droplet transmissionto a reassortant H5 HA/H1N1 virus in ferrets. Nature 486(7403): 420-428

5 Keim PS (2012) The NSABB recommendations: rationale, impact, and implications. mBio 3(1): e00021-12

6 Fedson DS, Opal SM (2013) The controversy over H5N1 transmissibility research. Human Vaccines & Immunotherapeutics

9 (5): 977-986

7 Brown T (2012) H5N1 Flu expert takes issue with NSABB approach. Medscape Medical News. http://www.medscape.com/viewarti cle/762183. Accessed Dec 2014

by government, or by a combination[9]. It has been argued that self-regulation is flawed because of obvious vested interests and conflicts of interest which would heavily favour publication of DURC ([10];[11]). A combination of government and scientist regulation is preferable given the vested interests of medical researchers in regulating their own activities.

➢ Experiments of Concern

In relation to the dual-use dilemma in the biological sciences, the approach of the US National Research Council (NRC) in its influential 2004 report, Biotechnology Research in an Age of Terrorism[12], is to map the range of these dual-use dilemmas by identifying and taxonomising a set of salient ``experiments of concern''.

According to the NRC report ``experiments of concern'' are those that would:

1. demonstrate how to render a vaccine ineffective.
2. confer resistance to therapeutically useful antibiotics or antiviral agents.
3. enhance the virulence of a pathogen or render a non-pathogen virulent.
4. increase the transmissibility of a pathogen.
5. alter the host range of a pathogen.
6. enable the evasion of diagnosis and/or detection by established methods; or
7. enable the weaponization of a biological agent or toxin.

Other possible categories are:

8. genetic sequencing of pathogens.
9. synthesis of pathogenic micro-organisms.
10. any experiment with variola virus (smallpox).
11. attempts to recover/revive past pathogens.

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8 Roos R (2014) Wholesale roster change coming for US biosecurity board. CIDRAP News. http://www.cidrap.umn.edu/news-per spective/2014/2007/wholesale-roster-change-coming-us-biosecurity-board

9 Resnik D (2010) Can scientists regulate the publication of dual use research? Studies in Ethics, Law, and Technology 4(1): Article 6

10Selgelid MJ (2007) A tale of two studies: ethics, bioterrorism and the censorship of science. The Hastings Center Report 37(3): 35

11 van Aken J (2006) When risk outweighs benefit: dual-use research needs a scientifically sound risk–benefit analysis and legally binding biosecurity measures. EMBO Rep 7:S10–S13

12 National Research Council (2004). Biotechnology research in an age of terrorism. Washington, DC: National Academies of Sciences.

➢ The Ethics of Dual-Use Research

By definition, dual-use research is morally problematic. On the one hand, such research provides benefits (at least potentially); on the other hand, there is the risk of misuse by rogue states, terrorists groups, and the like.

Broadly speaking, the most obvious benefits of research in the biological sciences of the kind in question are; the protection of human life and physical health against diseases (including novel ones), the protection of existing, and (more controversially) the provision of novel, food sources; and the protection of human populations against biological weapons. By contrast, the potential burdens of such research are death and sickness caused by the use of biological agents as weapons in the hands of malevolent state actors, terrorist groups, criminal organizations, and individuals.

More fine-grained analyses of the benefits and burdens of such research would elaborate on the additional kinds of benefit/burden and recipients/bearers thereof, e.g., the economic wealth accrued by large pharmaceutical corporations and their shareholders, the economic costs of expensive, unsuccessful (or only marginally beneficial) research programs in the biological sciences and, more generally, the dis/ utility and in/justice of specific allocations of resources to, and the distribution of benefits and burdens from, different research programs in the biological sciences, e.g., the evident disutility of the large 1946–1992 Soviet biological weapons program.

Fine-grained ethical analyses of dual-use research in the biological sciences would seek to quantify actual and potential benefits and burdens, and actual and potential recipients/ bearers of these benefits and burdens. These analyses would also identify a range of salient policy options. Each option would embody a set of trade- offs between present and future benefits and burdens, and recipients and bearers thereof. The construction of these options and the process of selection between them would consist in large part in the application of various ethical principles, including human rights principles—e.g., right to life, freedom of inquiry, and free speech— and principles of utility and of justice. Here we note that there is no simple inverse relationship between specific benefits and burdens such that, for example, any increase in security requires a reduction in scientific freedom. Rather an increase in security might simply involve greater safety precautions and, therefore, a financial cost without any commensurate reduction in scientific freedom. At any rate, relevant benefits and burdens need to be disaggregated and subjected to individual analysis in the context

of any process of determining trade-offs and selecting options.Providing such fine-grained ethical analysis is not easy. Thus, the focus will be on a single ethical consideration (to simplify the analysis), namely, human health (including human life) that gives rise to the dilemma; and do so without exploring questions of which human populations or how many individual humans have benefited/been burdened or are likely to benefit/be burdened, and so on. Viewed from this perspective the dual-use dilemma concerns human health (as a simple, unquantified human good), and the dilemma consists in the fact that research undertaken to promote human health might instead be used to destroy human health. As such, the dilemma gives rise to questions of security; what are reasonable and ethically justified forms and degrees of security in this context?

The security in question is a complex notion. It consists in part in the physical security of, for example, samples of biological agents against theft. Relatedly, security consists in part in the processes in place to ensure, for example, that the researchers themselves cannot, or will not, conduct research for malevolent purposes. As we will see in the section following this one, security in this sense also consists in part in restrictions that might be placed on the dissemination of research findings.

Thus far, we have offered a somewhat static mode of analysis of the dual-use dilemma consisting of the quantification of harms and benefits, the identification of salient options, and the selection of an option on the basis of ethical principles. However, a more dynamic, indeed creative, mode of analysis is called for.

➢ Dissemination of Dual-Use Research Results

A primary area of contention in the context of dual-use science surrounds questions about whether or not, or the extent to which, restriction on dissemination of information gleaned from dangerous discoveries is warranted. This is an important issue in the life sciences in particular, because the tradition of information sharing in the life sciences has historically been almost completely open, especially in comparison with nuclear science, where discoveries with implications for weapons-making are automatically ``born classified''. Ironically, because biological weapons are so much easier to make than nuclear weapons—with regard to the expertise, expense, equipment, and materials required—one might think that secrecy and restriction on information dissemination would have been more important in the former context than the latter. However, it might be that because biological weapons

are easier to make secrecy and restriction on information dissemination is less possible than in the case of nuclear weapons. Besides, secrecy might interfere with the development of counter measures.

A reason there has not been more control over dissemination of information in the life sciences is that the dual-use phenomena is the clearest and strongest in this area of science. In biology, it is more often the very same discovery that has both a direct beneficial purpose (with regard to medicine, for example) and a potentially harmful purpose (with regard to weapons making). Nuclear discoveries related to weapons making do not so often have such a direct and obvious link to human benefit (aside from the potential of nuclear weapons to be used for deterrent and/or defensive purposes).

While the protection of security and public health may arguably provide grounds for limiting dissemination of information related to dual-use discoveries, at least in certain instances, the issue of censorship should not be taken lightly. Governmental control over dissemination of information poses threats to widely cherished goods such as academic freedom (of inquiry), scientific autonomy, and freedom of speech itself. It is commonly held that these things are not only good in themselves but also essential to the progress of science. Governmental control over science has an unfortunate history illustrated by examples ranging from Galileo in renaissance Europe to Lyshenko in the former Soviet Union. Other examples include the Biopreparat program in the USSR and the history of research on biological weapons in general.

Scientific openness and the free sharing of information are important to the methodology of the scientific enterprise as a whole. In response to claims that the mousepox and polio studies and others like them should not have been published, or to claims that the materials and methods sections of such articles should have been altered or omitted, for example, defenders of publication point out the importance of recognizing the extent to which a discovery in one area of science may have profound implications for progress in other areas. Because limiting description of materials and methods would interfere with processes considered essential to science—i.e., replication and verification—it was objected that such a practice would be at odds with the way that science actually works.

For these and other reasons—including the commonly held belief among scientists that knowledge is good in-and-of itself[13]—many in the scientific community strongly believe that things like secrecy and/or governmental control over science is contrary to what science

is about. According to Robert Oppenheimer, for example, who provided the scientific leadership of the Manhattan project which produced the first atomic weapons in the United States, ``Secrecy strikes at the very root of what science is, and what science is for''[14]. From this perspective, even self-censorship is problematic from a scientific standpoint.

➢ Censorship/Constraint of Dissemination

Freedom of speech and freedom of dissemination of knowledge are human rights that find institutional expression in universities in the form of academic freedom. In the context of a liberal democracy there is a presumption against governmental restriction of human rights, including in the name of protecting other human rights. Moreover, arguably progress in science is importantly dependent on scientific—and, therefore, academic—freedom. On the other hand, restrictions on the dissemination of new scientific research that is likely to facilitate the malevolent purposes of bioterrorists is warranted.

Given the status attached to numbers and quality of publications by scientists—and the corresponding connection between status seeking behavior and scientific advancement—censoring or otherwise restricting the dissemination of scientific work may to a corresponding extent undermine scientific advancement, unless alternative reward structures for the scientists in question are developed, e.g., monetary payments, medals.

➢ Decision-Making for Dual-Use Dilemmas in the Biological Sciences

To facilitate the decision-making, the following options should be considered:

Option 1-<u>Complete Autonomy of the Individual Scientist</u>

The least intrusive/restrictive option is, of course, to do nothing about the dual-use dilemma. This laissez-faire option would allow the scientific status quo to run its own course. Individual researchers would be left to make their own decisions whether to engage in particular

13 Kitcher, P. (2001). Science, truth, and democracy. New York: Oxford University Press.

14 Schweber, S. S. (2000). In the shadow of the bomb: Bethe, oppenheimer, and the moral responsibility of the scientist. Princeton, New Jersey: Princeton University Press.

experiments of concern, and individual researchers and editors would be left to make their own decisions about whether or not to disseminate the findings of that research. Moreover, it would be up to individual researchers, editors and/or laboratories to determine safety and security protocols and to educate themselves about the dual-use dilemma and the potential dangers of publication and other forms of information dissemination. Scientists would be free to present whatever they consider to be appropriate at conferences, and free to discuss whatever they consider to be appropriate with colleagues.

Option 2-Institutional Control

Recognizing the inadequacy of Option 1 to address legitimate security concerns but acknowledging, nevertheless, the importance of freedom of intellectual inquiry and dissemination—and especially academic freedom—and of progress in science, Option 2 opts for: (i) government regulation (applied within institutions, i.e., universities, corporations and government research centers) in relation to physical safety and security (roughly speaking, the conditions under which dual-use research is conducted) and (ii) collegial decision-making in relation to the permissibility of dual-use research (at least in the case of university based research). Unregulated dual-use research—e.g., by non-institutionally based researchers—would be outlawed. However, there would be no licensing of dual-use technologies or mandatory personnel security provisions. Moreover, dissemination of dual-use findings of university-based researchers would be a matter for individual researchers and editors to decide. The dissemination of the dual-use findings of researchers hosted by corporations and government research centers would be a matter for determination by those institutions.

The principal institutional mechanism for determining the permissibility of dual-use research would be Institutional Biosafety Committees (IBCs). For example, university-based IBCs would be expected to apply government regulations in university-based research centers. Under this arrangement, researchers would be required to submit any research proposals falling within categories of concern to IBCs for review. In cases where sufficient dangers of experimentation are foreseen, the IBCs would make an adjudication that the research project in question not go ahead. In relation to dual-use research, these institutional committees would be the ultimate decision-makers (at least in universities, though presumably not in corporations and government research centers). Other things being

equal governments, for example, would not be able to override the determinations of these IBCs. Naturally, other things might not be equal. For example, the decisions of the IBCs would have to comply with regulations enacted by government; to this extent, they would be accountable to government.

Moreover, under Option 2 there would be some emphasis on education and training, albeit education and training would not be mandatory. For example, governments would be encouraged to become actively engaged in the promotion of education of scientists with regard to the dual-use dilemma and thus potential dangers of publication and other forms of information dissemination. This option would involve a campaign aimed at increasing awareness. Various means would be used to alert scientists to the general and to the specific dangers associated with particular areas of research. Reports would be published and disseminated by relevant research councils and governmental departments, government-sponsored seminars and/or short courses would be offered at relevant venues; scientist training programs, research institutes, and private companies would be advised about the dangers of dual-use, the importance of educating scientists about it, and resources (of various forms) would be offered to facilitate provision of appropriate education.

Option 3-A Dual System: Institutional and Governmental Control

Acknowledging that Option 2 does not do enough to address security concerns, Option 3 provides for a greatly enhanced regulatory system. Specifically, Option 3 provides for mandatory personnel security, licensing of dual-use technologies and mandatory education and training (in addition to mandatory physical safety and security). This regulatory system would apply to both public and private sector research centers.

Notwithstanding this enhanced regulatory system, academic freedom and progress in science remain. As with Option 2, Option 3 would involve collegial decision-making by means of IBCs in relation to the permissibility of dual-use research (at least in the case of university-based research).

Moreover, dissemination of dual-use findings of university-based researchers would be a matter for individual researchers and editors. Dual-use research in, and the dissemination of the dual-use findings of researchers hosted by, corporations and government research centers would be a matter for determination by those institutions.

As with Option 2, in Option 3 the principal institutional mechanism for determining not only the permissibility of dual-use research, but also for applying the regulatory system in respect of physical safety and security would be the IBCs. However, on Option 3 the IBCs

would have additional duties in relation to mandatory education and training, and the compliance of laboratories with the requirements of a licensing authority (including those pertaining to personnel security). Option 3 involves the establishment of such an authority, albeit one independent of government.

As indicated above, Option 3 provides an alternative to merely promoting education about the dual-use dilemma, namely, the formal requirement of this kind of training for those working in relevant fields of study (and relevant industries). Research ethics education is increasingly being required for those involved with research involving human or animal subjects worldwide (especially those receiving government funding). An educational requirement regarding the dual-use dilemma would fit in with this already existing trend. Specifications would be made about content to be covered in such training and heavy emphasis would be placed on experiments of concern in particular. Specific content most relevant to particular areas of research could be part of the educational requirement of those working in such areas. Given the grey area that exists between offensive and defensive biological weapons research, for example, it is especially important that those working in defence become especially familiar with the Biological Weapons Convention (BWC) and related issues. Those working with pox viruses would study the history of smallpox (which is important for understanding weapons threat of smallpox), and so on.

Option 4-An Independent Authority

Option 4 involves the establishment of an authority that is independent of both the research institutions (universities, corporations, and government research centers) and government. This independent authority would be comprised of scientists, security experts (including those with the highest feasible level security clearance) and ethicists.

This independent authority would have ultimate decision-making powers in relation to both the conduct of dual-use research and the dissemination of dual-use research findings. Moreover, it might also constitute the above-mentioned independent authority issuing licenses to laboratories in relation to dual-use technologies (and providing for personnel security). Other things being equal, decisions of this independent authority would not be able to be overridden by government. Naturally, other things might not be equal. For example, the decisions of the independent authority would have to comply with regulations enacted by government; to this extent, it would be accountable to government.

In addition, this independent authority might have an accountability role on behalf of government in relation to the application of government regulations in respect of physical safety and security, and dual-use education and training. Its determinations in these respects would not be final; rather these determinations would have the status of advice to government.

Under this arrangement researchers might be required to submit any research proposals falling within categories of concern to IBCs for review; and they would be required to submit any research findings which end up falling within categories of concern to IBCs after the fact (i.e., if a relevant dual-use discovery is unexpectedly made). In cases where sufficient dangers of experimentation or of information dissemination are foreseen, the IBCs will issue prohibitions or refer the studies to the independent authority for determination.
It is important to note that even under this form of meta-regulation the independent authority would have the power to intervene at any lower level, including overturning decisions at the lower level and auditing the work of the IBCs. In effect, this independent body would have the ultimate authority to determine what was permissible or impermissible dual-use research, and to determine whether and in what form dual-use research findings could be disseminated.

In addition to the research screening process described above, a national code of scientific conduct including statements analogous to the American Medical Association's (AMA's) Guidelines to Prevent the Malevolent Use of Biomedical Research would be developed by the independent authority. The code would include the requirement that scientists refer any research or research findings that falls within the eleven categories of experiments of concern to IBCs or other institutional ethics committees for determination. The code of conduct would be legally binding and apply to those working in industry as well as academia.

Option 5-Governmental Control

Option 5 would include a regulatory system authorized by, and accountable to, government that comprised mandatory physical safety and security, mandatory personnel security, and mandatory education and training. However, unlike Options 1, 2, 3, and 4 government would have ultimate and overriding decision-making authority in relation to both dual-use

research and dual-use publication, and in relation to the licensing of laboratories.
Option 5 is consistent with government receiving advice from individual researchers/editors, non-government institutions, e.g., scientific associations, universities and corporations, and independent authorities.

Moreover, Option 5 is consistent with various forms of meta-regulation, including a two-tiered system. For example, university-based biosafety committees (IBCs) might be expected to apply government regulations in university-based research centers. Under this arrangement, researchers might be required to submit any research proposals falling within categories of concern to IBCs for review; and they would be required to submit any research findings, which end up falling within categories of concern to IBCs after the fact. In cases where sufficient dangers of experimentation or of information dissemination are foreseen, the IBCs will issue prohibitions or refer the studies to the relevant government committee for determination.

It is important to note that even under this form of meta-regulation the government committee would have the power to intervene at any lower level, including overturning decisions at the lower level and auditing the work of the IBCs. In effect, government would have the ultimate authority to determine what was permissible or impermissible dual-use research, and to determine whether and in what form dual-use research findings could be disseminated.

Decision-Making for Dual-use Dilemmas in the Biological Sciences: Conclusion
Options 1 and 5, namely the Complete Autonomy of the Individual Scientist and the Governmental Control options, exist at the two extremes of the spectrum of possibilities, and both have very significant, albeit contrasting, disadvantages. Option 1 is largely oblivious to the security threat; Option 5 gives insufficient weight, among other things, to the liberal democratic values of freedom of intellectual inquiry, freedom of communication and, relatedly, academic freedom. Moreover, the main advantages of Options 1 and 5 (autonomy and security, respectively) are in large part made available in one form or another by each of Options 2, 3 and 4. Accordingly, we have recommended against Options 1 and 5. What of the choice between Options 2, 3 and 4? Are there good and decisive reasons for preferring one of these over the others?

Unlike Options 3 and 4, Option 2 (Institutional Control) does not involve mandatory licensing of technology, mandatory education/training or mandatory personnel security regulation. These omissions are a weakness of Option 2. Moreover, Option 2 does not have any compensating

advantages, particularly in comparison with Option 3. Accordingly, we recommend against Option 2.

Thus, we are left with Option 3 (Institutional and Governmental Control) and Option 4 (An Independent Authority). Both options seem to us to be both feasible and ethically justifiable, depending on the precise institutional form that each might take[15]. Moreover, as things stand, we do not believe that there are good and decisive reasons in favor of one option over the other. Rather what is needed now is the development of a more detailed description of each of these two competing institutional models being proposed under Options 3 and 4 (respectively). Such a process of institutional design would enable a meaningful calibration of the advantages and disadvantages of the two competing models and, as a consequence, allow for the possibility of an informed decision to be made as to which to adopt.

➢ Governance of Dual-Use Research of Concern

Governance structures and guidelines are not uniform internationally, and only some institutions and countries have specific DURC policies. The table below lists some of major organizations that are involved in dual-use policy and addressing dual-use research concerns.

15 Beck, V. (2003). Advances in life sciences and bioterrorism. EMBO Reports, 4(Special Issue), S53-S56.

Name	Aim/thrust	Web link	Relation to dual use
European Commission	Policy-making, regulation, research funding.	http://ec.europa.eu/trade/import-and-export-rules/export- from-eu/dual-use- controls/	Includes dual use as an ethical issue to be addressed in funding applications.
Wassenaar Arrangement on Export Controls for Conventional Arms and Dual-Use Goods and Technologies Secretariat	The Wassenaar Arrangement has been established in order to contribute to regional and international security and stability, by promoting transparency and greater responsibility in transfers of conventional arms and dual-use goods and technologies.	http://www.wassenaar.org/	Participating States seek, through their national policies, to ensure that transfers of these items do not contribute to the development or enhancement of military capabilities that undermine these goals and are not diverted to support such capabilities.
National Science Advisory Board for Biosecurity (NSABB)	An US federal advisory committee	http://osp.od.nih.gov/office-biotechnology-activities/biosecurity/nsabb	Addresses issues related to biosecurity and dual-use research at the request of the United States Government. See the NSABB Proposed Framework for the Oversight of Dual Use Life Sciences Research[16].
The Royal Netherlands Academy of Arts and Sciences (KNAW)	Advisory body to the Dutch Government/ responsible for sixteen research institutes.	http://www.bureaubiosecurity.nl/en/Policy/Dual_use_research	In 2007, KNAW, commissioned by the Ministry of Education, Culture and Science drew up a Code of Conduct for Biosecurity aimed at raising awareness of the risks of working with high-risk pathogens[17]. In 2013, KNAW published the advisory report 'Improving biosecurity – Assessment of dual-use research.'
InterAcademy Panel (IAP)	Global network of science academies	http://www.interacademies.net/File.aspx?i d=5401	IAP Statement on Biosecurity 2005 suggests that "scientists have a special responsibility when it comes to problems of "dual use" and the misuse of science and technology".

16 http://osp.od.nih.gov/office-biotechnology-activities/nsabb-reports-and-recommendations/proposed-framework-oversight-dual-use-life-sciences-research

16 http://osp.od.nih.gov/office-biotechnology-activities/nsabb-reports-and-recommendations/proposed-framework-oversight-dual-use-life-sciences-research

17 The Code of Conduct for Biosecurity was drawn up by the Royal Netherlands Academy of Arts and Sciences, 2007. http://www.bureaubiosecurity.nl/en/Policy/Dual_use_research/Code_of_Conduct_for_Biosecurity

Biosecurity

Today, facilities handling infectious agents need not only a biosafety program but also a biosecurity plan in place to prevent the theft, misuse, or intentional release of pathogens. There is unfortunately a dual use potential that takes place with these agents. There are many international recommendations and position papers which can provide further assistance with the management of biological threats.

Laboratory Security Is Related to But Different Than Laboratory Safety

The planning and implementation of a biosecurity plan needs to be specific to the nature of each facility, the type of research and diagnostics conducted, and the local environment. A diverse working group needs to be involved. It should include scientific directors, safety officers, principal investigators, laboratory workers, administrators, security staff, maintenance staff, and law enforcement agencies where appropriate. A designated Biosafety Official (BO) is typically responsible for the development, training, and implementation of safety, security, and emergency response plans. As such, the BO is contacted with timely notice of any theft, loss, or release of agents. The BO can assist with maintaining detailed records of information necessary to give a complete accounting of all activities related to pathogens and is involved in the transfer and transportation of agents from the facility. The BO is involved in allowing only approved individuals to have access to agents.

A primary component to a biosecurity plan must be a detailed risk assessment. The biosecurity risk assessment should review and list the relevant assets, define the threats, outline the vulnerabilities, and determine the countermeasures or mitigation strategies specific for each facility. The biosecurity plan should then address the following factors: physical protection; personnel suitability/reliability; pathogen accountability; and related incident and emergency response. Management should regularly review policies and procedures to ensure that they are adequate for current conditions and consistent with other facility-wide policies and procedures. Laboratory supervisors should ensure that all laboratory workers and visitors understand security requirements and are trained and equipped to follow established procedures. Review safety policies and procedures whenever an incident occurs, or a new threat is identified.

Physical Protection

The physical protection risk assessment should include all levels of biosecurity review: perimeter security, facility security, laboratory security and agent specific security, and outline procedures for securing the area, e.g., card access, keypads, locks etc. All laboratories should adopt biosecurity practices to minimize opportunities for unauthorized entry into laboratories, animal and storage areas, as well as the unauthorized removal of infectious materials from their facility. Similarly, information security for data and electronic technology need to be addressed, including protection against external hacking of critical systems.

Personnel Suitability/Reliability

Background checks and security clearances may be required before employees are granted access to containment facilities. These factors should be considered as part of the local risk assessment process when developing a biosecurity plan. Photo identification badges for employees and temporary badges for escorted visitors can also be used to identify individuals with clearance to enter restricted areas. Procedures are needed for approving and granting visitors access to controlled areas. In this capacity the access to agents and storage facilities is limited to legitimate use/individuals only. Biosecurity training needs to be provided to all personnel who are given access.

Pathogen Accountability

Pathogen accountability procedures should include inventory requirements for proper labeling, tracking of internal possession, inactivation, and disposal of cultures after use, and transfers within and outside the facility. These inventory controls also assist in keeping track of pathogen storage locations and under whose responsibility the pathogens lie. Inventories must be updated regularly to include new additions as a result of diagnosis, verification of proficiency testing, or receipt from other locations as well as to remove agents after transfers or appropriate inactivation and disposal mechanisms have been used. The record keeping should include pathogen inventories, who has access to agents, who has access to areas where agents are stored or used, as well as transfer documents. A notification process for identifying, reporting, and remediating security problems, i.e., inventory discrepancy, equipment failure, breach of security, release of agents, etc., should be in place.

Information Security

Information security establishes prudent policies for handling "Sensitive information", which is related to the security of pathogens and toxins, or other critical infrastructure information. Sensitive information may also include personnel names, identifying information, and any personally identifiable information on patients or the origin of samples held by the laboratory. Examples of sensitive information may include facility security plans, access control codes, agent inventories and storage locations.

Information security should ensure that the required and appropriate level of confidentiality is preserved by the system that is used to acquire, store, manipulate and manage information.

Computer Drives Must Be Effectively Protected.

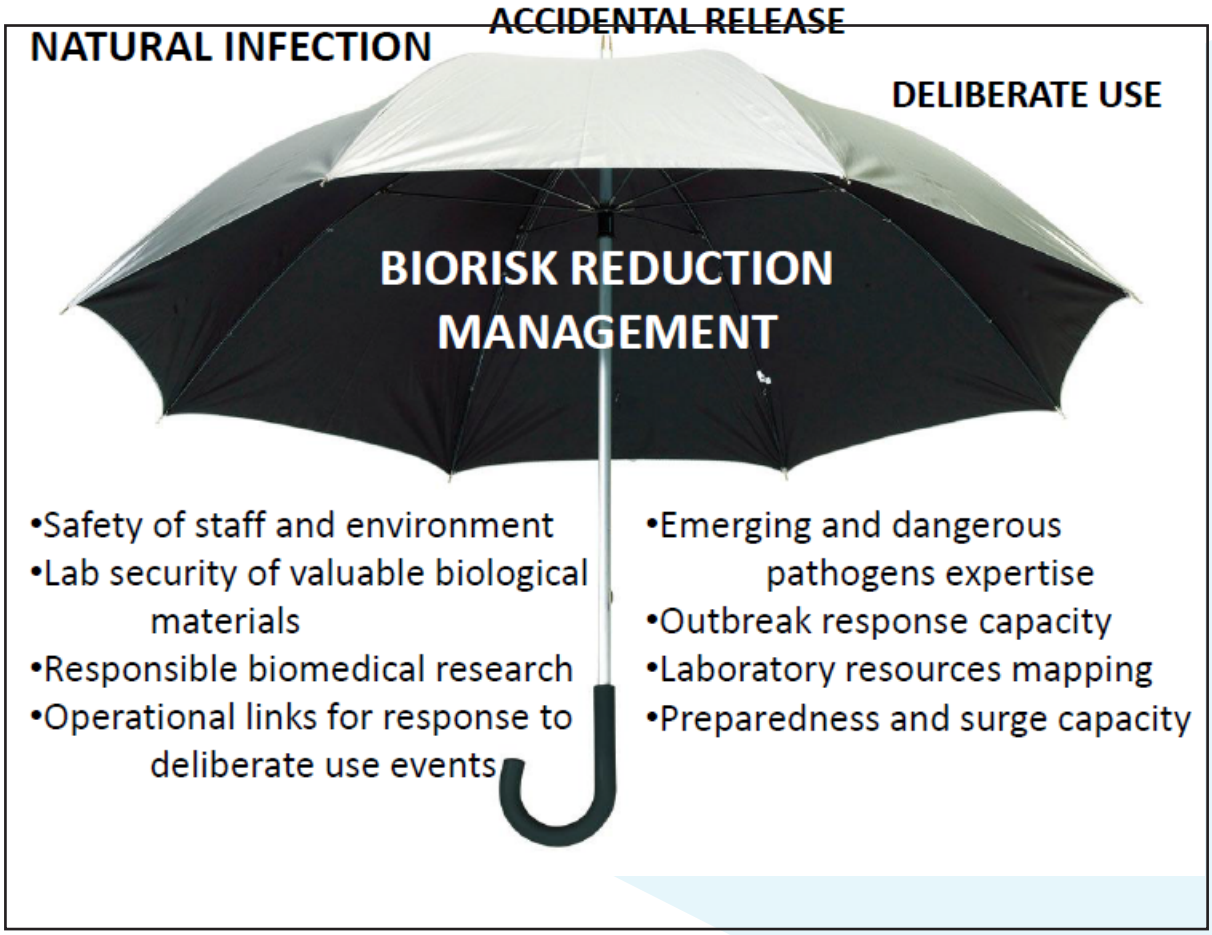
In practice, the facilities will store sensitive information on computer drives. Protecting this type of information will require a system designed by a reliable company that specializes in IT security. Websites, emails, and other IT-based media can also contain sensitive information. The facility should carefully consider which parts of its website should be available to the public. It should also consider which of its application forms should be available for downloading to "outside" computers. In this regard, protection of information should be consistent with the level of risk it poses in terms of potentially compromising a VBM. The higher the level of risk associated with the VBM the institution holds, the greater protection the information associated with the security system will require.

Biosecurity Incident and Emergency Response

A protocol for reporting and investigating security incidents e.g., missing infectious substances, unauthorized entry, should be addressed. A mechanism needs to be in place for the reporting and removal of unauthorized persons. Biosecurity incident and emergency plans should include response to intentional (bomb threats etc.), unintentional (accidental release) and natural events (power outages, severe weather). Training needs to be provided to all relevant personnel.

Expert advice from security and/or law enforcement experts should be sought in the development of threat assessments and security protocols specific to each facility. The threat assessment and

security practices should be regularly reviewed and updated to reflect new threats that may be identified.



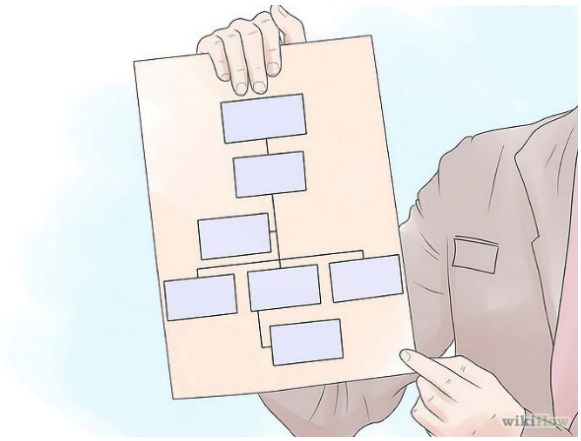
Appendix 1

How to Write a Standard Operating Procedure

A Standard Operating Procedure (SOP) is a document consisting of step-by-step information on how to execute a task. An existing SOP may need to just be modified and updated, or you may be in a scenario where you have to write one from scratch. It sounds daunting, but it is really just a very thorough checklist.

Part 1: Planning Your SOP

Step 1: Decide on a Format for the SOP



There is no right or wrong way to write an SOP. However, your organization or company probably has a number of SOPs you can refer to for formatting guidelines, outlining how they prefer it done. If that is the case, use the pre-existing SOPs as a template. If not, you have a few options:

- A simple steps format. This is for routine procedures that are short, have few possible outcomes, and are fairly to the point. Apart from the necessary documentation and safety guidelines, it is really just a bullet list of simple sentences telling the reader what to do.
- A hierarchical steps format. This is usually for long procedures -- ones with more than ten steps, involving a few decisions to make, clarification and terminology. This is usually a list of main steps all with sub-steps in a very particular order.
- A flowchart format. If the procedure is more like a map with an almost infinite

numberof possible outcomes, a flowchart may be your best bet. This is the format you should opt for when results are not always predictable.

Step 2: Consider your audience

There are three main factors to take into account before writing your SOP:

- Your audience's prior knowledge. Are they familiar with your organization and its procedures? Do they know the terminology? Your language needs to match the knowledge and investment of the reader.
- Your audience's language abilities. Are there any chance people who do not speak your language will be "reading" your SOP? If this is an issue, it is a good idea to include lots of annotated pictures and diagrams.
- The size of your audience. If multiple people at once are reading your SOP (those in different roles), you should format the document more like a conversation in a play: user 1 completes an action, followed by user 2, and so on and so forth. That way, each reader can see how he or she is an integral cog in the well-oiled machine.

Step 3: Consider your knowledge

What it boils down to is this: Are you the best person to be writing this? Do you know what the process entails? How it could go wrong? How to make it safe? If not, you may be better off handing it over to someone else. A poorly-written -- or, what's more, inaccurate -- SOP will not only reduce productivity and lead to organizational failures, but it can also be unsafe and have adverse impacts on anything from your team to the environment. In short, it is not a risk you should take.

If this is a project you have been assigned that you feel compelled (or obligated) to complete, do not shy away from asking those who complete the procedure on a daily basis for help. Conducting interviews is a normal part of any SOP-creating process.

Step 4: Decide between a short or long-form SOP

If you were writing or updating an SOP for a group of individuals that are familiar with protocol, terminology, etc., and just would benefit from a short and snappy SOP that is more like a checklist, you could just write it in short-form.
Apart from basic purpose and relevant information (date, author, ID#, etc.), it is really just a short list of steps. When no details or clarification are needed, this is the way to go.

Step 5: Keep your SOP's purpose in mind

What is obvious is that you have a procedure within your organization that keeps on getting repeated over and over and over. But is there a specific reason why this SOP is particularly useful? Does it need to stress safety? Compliance measures? Is it used for training or on a day-to-day basis? Here are a few reasons why your SOP is necessary to the success of your team:

- To ensure compliance standards are met
- To maximize production requirements
- To ensure the procedure has no adverse impact on environment
- To ensure safety
- To ensure everything goes according to schedule
- To prevent failures in manufacturing
- To be used as training document

If you know what your SOP should emphasize, it will be easier to structure your writing around those points. It is also easier to see just how important your SOP is.

Part 2: Writing Your SOP

Step 6: Cover the necessary material

In general, technical SOPs will consist of four elements apart from the procedure itself:

- Title page. This includes 1) the title of the procedure, 2) an SOP identification number, 3) date of issue or revision, 4) the name of the agency/division/branch the SOP applies to, and 5) the signatures of those who prepared and approved of the SOP. This can be formatted however you like, as long as the information is clear.
- Table of Contents. This is only necessary if your SOP is quite long, allowing for ease of reference. A simple standard outline is what you would find here.
- Quality Assurance/Quality Control. A procedure is not a good procedure if it cannot be checked. Have the necessary materials and details provided so the reader can make sure they have obtained the desired results. This may or may not include other documents, like performance evaluation samples.
- Reference. Be sure to list all cited or significant references. If you reference other SOPs, be sure to attach the necessary information in the appendix.

Your organization may have different protocol than this. If there are already pre-existing SOPs you can refer to, abandon this structure, and adhere to what is already in place.

For the procedure itself, make sure you cover the following:

- Scope and applicability. In other words, describe the purpose of the process, its limits, and how it is used. Include standards, regulatory requirements, roles and responsibilities, and inputs and outputs.
- Methodology and procedures. The meat of the issue -- list all the steps with necessary details, including what equipment needed. Cover sequential procedures and decision factors. Address the "what ifs" and the possible interferences or safety considerations.
- Clarification of terminology. Identify acronyms, abbreviations, and all phrases that aren't in common parlance.
- Health and safety warnings. To be listed in its own section and alongside the steps where it is an issue. Do not gloss over this section.
- Equipment and supplies. Complete list of what is needed and when, where to find equipment, standards of equipment, etc.
- Cautions and interferences. Basically, a troubleshooting section. Cover what could go wrong, what to look out for, and what may interfere with the final, ideal product.

Give each of these topics their own section (usually denoted by numbers or letters) to keep your SOP from being wordy and confusing and to allow for easy reference.

This is by no means an exhaustive list; this is just the tip of the procedural iceberg. Your organization may specify other aspects that require attention.

Step 7: Make your writing concise and easy to read

Odds are your audience is not choosing to read this for fun. You want to keep it short and clear -- otherwise their attention will stray, or they will find the document formidable and hard to grasp. In general, keep your sentences as short as possible.

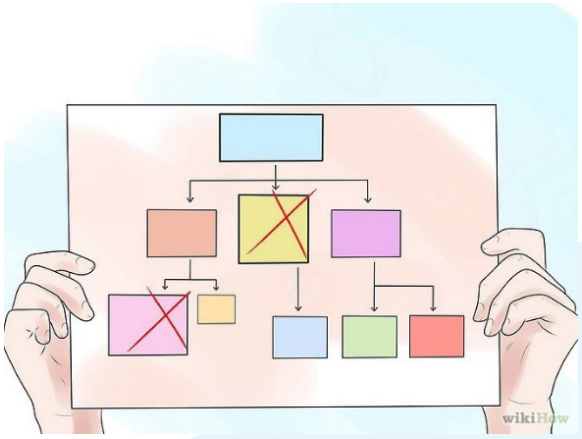
- **Here is a bad example:** Make sure that you clean out all of the dust from the airshafts before you begin using them.
- **Here is a good example:** Remove all dust from airshafts before use.

In general, do not use "you". It should be implied. Speak in the active voice and start your sentences with command verbs.

Step 8: Interview the personnel involved in the process on how they execute the task

The last thing you want to do is write an SOP that is just plain inaccurate. You are compromising the safety of your team, their efficacy, their time, and you are taking an established process and not paying it any mind -- something your teammates may find a little offensive. If you

need to, ask questions! You want to get this right.
Of course, if you do not know, ask multiple sources, covering all roles and responsibilities. One team member may not follow standard operating procedure, or another may only be involved in a portion of the deed.



Step 9: Break up large chunks of text with diagrams and flowcharts

If you have a step or two that are particularly intimidating, make it easy on your readers with some sort of chart or diagram. It makes it easier to read and gives the mind a brief hiatus from trying to make sense of it all. And it will be appearing more complete and well written for you.
Do not include these just to bulk up your SOP; only do this if necessary or if trying to bridge a language gap.

Step 10: Make sure each page has control document notation

Your SOP is probably one of many SOPS -- because of this, hopefully your organization has some type of larger database cataloguing everything within a certain reference system. Your SOP is part of this reference system, and therefore needs some type of code in order to be found. That is where the notation comes in.

Each page should have a short title or ID #, a revision number, date, and "page # of #" in the upper right-hand corner (for most formats). You may or may not need a footnote (or have these in the footnote), depending on your organization's preferences.

Part 3: Ensuring Success and Accuracy

Step 11: Test the procedure

If you do not want to test your procedure, you probably have not written it well enough. Have someone with a limited knowledge of the process (or a person representative of the normal reader) use your SOP to guide them. What issues did they run across? If any, address them and make the necessary improvements.

It is best to have a handful of people test your SOP. Different individuals will have different issues, allowing for a wide variety of (hopefully useful) responses. Be sure to test the procedure on someone who is never done it before. Anyone with prior knowledge will be relying on their knowledge to get them through and not your work, thus defeating the purpose.

Step 12: Have the SOP reviewed by those who actually do the procedure

At the end of the day, it does not really matter what your bosses think of the SOP. It is those who actually do the work that it matters to. So, before you submit your work to the higher ups, show your stuff to those that will be doing (or that do) the job. What do they think? Allowing them to get involved and feel like they are part of the process will make them more likely to accept this SOP you are working on. And they will inevitably have some great ideas!

Step 13: Have the SOP reviewed by your advisors and the Quality Assurance team

Once the team gives you the go ahead, send it to your advisors. They will probably have less input on the actual content itself, but they'll let you know if it meets formatting requirements, if there's anything you missed, and the protocol for making it all official and inputted into the system.

Route the SOP for approvals using document management systems to ensure audit trails of the approvals. This will vary from organization to organization. Basically, you want everything to meet guidelines and regulations.
Signatures will be necessary, and most organizations nowadays have no problem accepting electronicsignatures.

Step 14: Once approved, start implementing your SOP
This may involve executing a formal training for the affect personnel (e.g. classroom training, computer-based training, etc.) or it may mean your paper is hung up in the bathroom. Whatever it is, get your work out there! You worked for it. Time for recognition!
Be sure your SOP remains current. If it ever gets out-dated, update it, get the updates reapproved and documented, and redistribute the SOP, as necessary. Your team's safety, productivity, and success matter on it.

Tips to Remember

- Remember to involve the stakeholders whenever possible, so that the documented process is the actual process.
- Check for clarity. Make sure there are not multiple interpretations. Show the procedure to someone unfamiliar with the process and have them tell you what they think it says, you may be surprised.
- Use flowcharts and pictorial representations so that the reader is clear about the process.
- Get people to review your document before getting approval.
- Use simple language to explain the steps.
- Ensure document history is documented for every version change.

Appendix 2: Proper Regulated Medical Waste Packing Procedures.

Medical Waste Packaging Procedures

1- Regulations are the same regardless of whether you are using cardboard medical waste boxes or reusable plastic medical waste tubs.

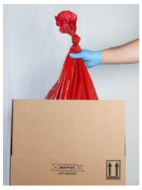
2- All regulated medical waste must be placed into a DOT approved red bag prior to sealing or closing the medical waste box or reusable medical waste tub.

3- Labeling Sharps Containers
All full sharps containers must be labeled with the generator's name and address prior to being placed into the medical waste box or reusable medical waste tub for final disposal.


4- Sharps containers that are full should be closed and locked and then placed into the red bag that is lining the medical waste box or reusable medical waste tub.

Final Closure of the Red Bag

1- The red bags must be tied in a single knot before the medical waste box or reusable medical waste tub is closed and sealed.



2- Labeling the Bag

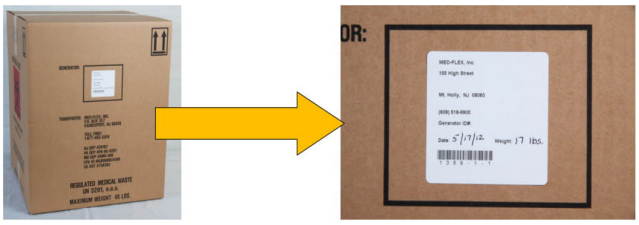
The inner red bag must also be labeled with the generator's name and address.

3- Closing and Taping the Box

All Boxes must be properly sealed using 2" poly tape. Both flaps should be secured using a single strip of poly tape down the middle seem.

4- Labeling the Box

The medical waste box and reusable medical waste tub must be labeled with the generator's name, address and date of shipment. The box and tub should also have the transporter's name and identify the contents as regulated medical waste.

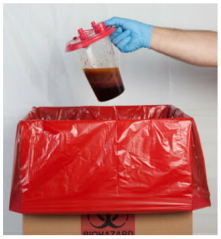


Common Packaging Mistakes

1- Loose needles may never be placed directly into the red bag. They must be first placed into a sharps container. The sharps container then must be closed and locked prior to being placed into the medical waste box or reusable medical waste tub.

2- Full sharps containers must always be closed and locked prior to being placed into the medical waste box or reusable medical waste tub.

3- Un-solidified suction canisters should never be placed into the medical waste box or reusable medical waste tub without first being placed into a leak proof container or a solidifying agent added to the canister and closing all the caps so that the container is secure and leak proof.



4- In order to meet DOT standards for transportation, medical waste boxes and reusable medical waste tubs must be properly sealed.

Appendix 3: Proper Regulated Medical Waste Disposal.

Categry	Type of waste	Type of bag or container to be used	Treatment and disposal option
Yellow	Human tissues, organs, body parts Animal Anatomical Waste: Experimental animal carcasses, body parts, organs, tissues, including the waste generated from animals used in experiments or testing in veterinary hospitals or colleges or animal houses.	Yellow colored non-chlorinated plastic bags	Incineration or deep burial *
	Soiled Waste: Items contaminated with blood, body fluids like dressings, plaster casts, cotton swabs and bags containing residual or discarded blood and blood components.		Incineration or deep burial * In absence of above facilities, autoclaving or microwaving followed by shredding or mutilation or combination of sterilization and shredding.
	Expired or Discarded Medicines: Pharmaceutical waste like antibiotics, cytotoxic drugs including all items contaminated	Yellow colored non-chlorinated	Expired cytotoxic drugs and items contaminated with cytotoxic drugs to be returned back to the

Categry	Type of waste	Type of bag or container to be used	Treatment and disposal option
	with cytotoxic drugs along with glass or plastic ampoules, vials etc.	plastic bags or containers	manufacturer or supplier for incineration at temperature >1200 º C or to common bio – medical waste treatment facility or hazardous waste treatment, storage and disposal facility for incineration at >1200 º C. All other discarded medicines shall be either sent back to manufacturer or disposed by incineration.
	Chemical Waste: Chemicals used in production of biological and used or discarded disinfectants.	Yellow colored containers or non-chlorinated plastic bags	Disposed of by incineration or Encapsulation in hazardous waste treatment, storage and disposal facility.
	Chemical Liquid Waste : Liquid waste generated due to use of chemicals in production of biological and used or discarded disinfectants, Silver X – ray film developing liquid, discarded	Separate collection system leading to effluent treatment	After resource recovery, the chemical liquid waste shall be pretreated before mixing with other wastewater.

Categry	Type of waste	Type of bag or container to be used	Treatment and disposal option
	Formalin, infected secretions, aspirated body fluids , liquid from laboratories an d floor washings, cleaning, house – keeping and disinfecting activities etc.	system	
	Discarded linen, mattresses, beddings contaminated with blood or body fluid.	Non-chlorinated yellow plastic bags or suitable packing material	Non – chlorinated chemical disinfection followed by incineration. In absence of above facilities, shredding or mutilation or combination of sterilization and shredding. Treated waste to be sent for energy recovery or incineration.
	Microbiology, Biotechnology and other clinical laboratory waste: Blood bags, Laboratory cultures, stocks or specimens of microorganisms, live or attenuated vaccines, human and animal cell cultures used in research, industrial laboratories, production of biological, residual toxins, dishes and devices used for cultures.	Autoclave safe plastic bags or containers	Pretreat to sterilize with non – chlorinated chemicals on – site as World Health Organization guidelines thereafter for Incineration.

Categry	Type of waste	Type of bag or container to be used	Treatment and disposal option
Red	Contaminated Waste (Recyclable) , Wastes generated from disposable items such as tubing, bottles, intravenous tubes and sets, catheters, urine bags, syringes (without needles and fixed needle syringes) and vaccutainers with their needles cut) and gloves.	Red colored non-chlorinated plastic bags or containers	Autoclaving or micro waving/ hydroclaving followed by shredding or mutilation or combination of sterilization and shredding. Plastic waste should not be sent to landfill sites.
White (Translucent)	Waste sharps including Metals: Needles, syringes with fixed needles, needles from needle tip cutter or burner, scalpels, blades, or any other contaminated sharp object that may cause puncture and cuts. This includes both used, discarded and contaminated metal sharps	Puncture proof, Leak proof, tamper proof containers	Autoclaving or Dry Heat Sterilization followed by shredding or mutilation or encapsulation in metal container or cement concrete; combination of shredding cum autoclaving; and sent for final disposal to iron foundries or sanitary landfill or designated concrete waste sharp pit.
Blue	Glassware: Broken or discarded and contaminated glass including medicine vials and ampoules except those contaminated with cytotoxic wastes	Cardboard boxes with blue colored marking	Disinfection (by soaking the washed glass waste after cleaning with detergent and Sodium Hypochlorite treatment)
	Metallic Body Implants		or through autoclaving or microwaving or hydroclaving and then sent for recycling.

Appendix 4: Decontamination of Used Instruments, Equipment and Surfaces.

Decontamination is the first step in handling used instruments, equipment, and contaminated surfaces.

a. Decontamination Solution

The recommended decontamination agent is a 0.5 percent chlorine solution.

Make a fresh solution every morning, or after 8 hours, or more often if the solution becomes visibly dirty. A 0.5 percent chlorine solution can be made from readily available liquid chlorine or chlorine tablets.

The formula for making a dilute solution from concentrated solutions is as follows:

Total Parts (TP) water = (percentage chlorine in manufacturers concentration % desired chlorine concentration) - 1

Mix 1-part concentrated bleach solution with the total parts water required.

Example:

To make a 0.5 percent chlorine solution from 5 percent concentrated chlorine solution:

TP water: (5.0% 0.5%) - 1= 10 - 1 = 9

Add 1-part concentrated solution to 9 parts water.

Cover containers containing 0.5 percent chlorine solution and protect them from light.

Note: Do not mix chlorine solutions with ammonia-based solutions, because toxic gas might be produced.

b. Decontaminating Equipment

Decontaminate large surfaces that might have come in contact with blood and body fluid, Wipe them with a cloth soaked in the 0.5 percent chlorine solution.

c. Decontaminating Used Instruments and Other Items

Keep surgical or examination gloves on after completing the procedure. Decontaminate the instruments while wearing the gloves:

i. Immediately after use, place all instruments in an approved disinfectant, such as 0.5 percent chlorine solution, for 10 minutes to inactivate most organisms, including HBV and HIV.
ii. Use plastic, noncorrosive containers for decontamination to prevent sharp instruments from getting dull (due to contact with metal containers) and to prevent instruments from getting rusted (due to electrolysis between two different metals when placed in water).
iii. Remove instruments from 0.5 percent chlorine solution after 10 minutes and immediately rinse them with cool water to remove residual chlorine before thoroughly cleaning them.
iv. Remove gloves and dispose of them appropriately.

Appendix 5: Susceptibility of Microorganisms to Chemical Disinfectants.

Chemical Disinfectant	Commonly Available Form	Effective Against							Contact Time
		Bacteria			Viruses		Fungi		
		Vegetative	Mycobacteria	Spores	Enveloped	Non-enveloped	Fungi	Fungal Spores	
Chlorine	Liquid, powder, and tablet	+	+	+	+	+	+	+	Generally short; longer for bacterial spores (≥ 30 min)
Iodine	Aqueous solutions, tinctures and iodophors	+	L	L	+	L	+	L	Generally short for vegetative bacteria and enveloped viruses; contact time for other organisms is product-specific
Alcohol	Ethyl or isopropyl alcohol; 70% in water is most effective	+	+	-	+	L	+	L	Generally short for vegetative bacteria and enveloped viruses; longer for fungi and mycobacteria
Phenolics	Wide variety. generally used as substituted phenols in combination with detergents	+	V	-	+	-	V	V	
Quaternary ammonium compounds	Wide variety available with built-in detergent action	+	-	-	+	-	+	-	

Chemical Disinfectant	Commonly Available Form	Effective Against							Contact Time
		Bacteria			Viruses		Fungi		
		Vegetative	Mycobacteria	Spores	Enveloped	Non-enveloped	Fungi	Fungal Spores	
Glutaraldehyde	2% acidic solution supplied with a bicarbonate compound	+	+	+	+	+	+	+	≥ 20 min required for non-enveloped viruses and mycobacteria; >3 hours required for bacterial spores
Formaldehyde	Available as solid paraformaldehyde and liquid formalin	+	+	+	+	+	+	+	
Hydrogen peroxide (H2O2)	Accelerated formulations and 30% solutions in water	+	+	+	+	+	+	+	Short contact time with 6% H2O2, for all viruses, vegetative bacteria, fungi, mycobacteria, and some bacterial spores; higher concentrations and longer contact times required for sporicidal activity
Chlorhexidine	4% solution of chlorhexidine gluconate in a detergent base and concentrated alcohol –based solutions	+/L*	-	-	+	-	L	-	

+: effective; L: limited activity; V: variable activity; –: no activity

* Effective against Gram-positive bacteria; limited activity against Gram-negative bacteria

Appendix 6: Disadvantages of Chemical Disinfectants.

Chemical Disinfectant	Chlorine	Alcohol	Iodine	Quaternary ammonium compounds	Glutaraldehyde	Formaldehyde	Phenolics	Chlorhexidine	Hydrogen peroxide
Disadvantages	solutions are light sensitive and should be prepared fresh and stored in light-protected containers • highly corrosive to metals • neutralized by organic material • concentrated solutions may be toxic to humans • reaction of chlorine with some organic molecules may lead to the production of carcinogens • not suitable for autoclaving	• alcohol should generally not be used to disinfect large areas of the laboratory as it may be a fire hazard • longer contact times are difficult to achieve due to evaporation • variable compatibility with certain materials (e.g., may harden rubber and deteriorate glues and some plastics)	• staining of treated objects • corrosive • neutralized by organic material	• decreased activity in hard water • reduced effectiveness in the presence of organic matter • due to detergent-like properties, QACs may make surfaces (including floors) slippery, which can be a hazard to both personnel and animals	• limited shelf-life • highly irritating and toxic to skin and mucous membranes	• more susceptible to inactivation by organic material than glutaraldehyde • pungent odour • extremely toxic • a known carcinogen	• toxicity • pungent unpleasant smell • neutralization by hard water	• incompatible with anionic detergents	• may be unstable when exposed to heat and light (some stabilized products are now commercially available) • high concentrations can cause skin burns, irritation or damage to the mucous membranes (with direct exposure), and can pose a risk of explosion • equipment used in H2O2 disinfection may be expensive when compared with other methods

Appendix 7: Recommended Procedures for the Use of Autoclaves and for Efficacy Monitoring of Autoclaves.

Before Loading the Autoclave

1. Before opening the door of a double-door barrier autoclave, confirm that the door on the opposite side of the autoclave is closed (i.e., through visual and audible alarms).
2. Check inside the autoclave for any items left by the previous user that could pose a hazard (e.g., sharps).
3. Clean the drain strainer.

4. Confirm that any plastic materials used, including bags, containers, and trays, are compatible with autoclaving. Some bags can impede steam penetration while others may melt during the cycle.
5. Avoid overloading containers and bags (they should never be more than 3/4 full).
6. Autoclave bags should be closed loosely to allow adequate steam penetration.
7. Loosen the caps of liquid containers to prevent bottles from shattering during pressurization. This should be done immediately prior to loading in order to minimize the risk of exposure or contamination if the container is tipped. Vented caps may be a suitable alternative.

Loading the Autoclave

1. Load autoclave according to the manufacturer's recommendations.
2. Arrange containers, bags, and trays in a manner that allows steam to circulate freely around all items. Avoid stacking or crowding items.
3. Consider placing containers and bags in trays with a solid bottom and walls to contain spills.
4. Avoid placing individual containers on the floor of the autoclave.
5. Make sure the door of the autoclave is fully closed (i.e., latched) and that the correct cycle has been selected.

Unloading the Autoclave

1. Verify the autoclave cycle log to ensure decontamination parameters have been achieved.

2. Visually check the pressure gauge to ensure that the pressure has decreased inside the chamber.
3. Don PPE, including eye protection, heat resistant long cuff gloves, rubber apron, rubber sleeve protectors, and, when handling sharps, cut resistant gloves.
4. Materials removed from the autoclave after effective (i.e., verified) decontamination should be placed in disposal bags that clearly indicate that the waste has been decontaminated, and any biohazard symbols removed or defaced.

Verifying the Autoclave Run

1. After decontaminated material has been removed from the autoclave, and prior to disposal, it is important to verify that the run has been effective (i.e., that all validated parameters have been reached). Parametric monitoring devices, chemical indicators and integrators, and biological indicators can be used for routine monitoring of the decontamination process.
2. Remove the indicator or integrator from the autoclaved material and visually inspect. Chemical indicators and integrators provide immediate information on the parameters to which they react. If it was required to also include biological indicator, the material cannot be released for disposal or reuse until the results of the biological indicator are known.
3. Biological indicators require incubation for a pre-determined period of time before reading.

Appendix 8: Steps to put on and take off personal protective equipment (PPE) including gown.

Steps to **put on** personal protective equipment (PPE) including **gown**

1 Remove all personal items (jewelry, watches, cell phones, pens, etc.)

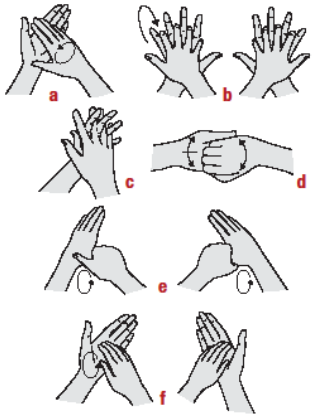
2 Put on scrub suit and rubber boots[1] in the changing room.

3 Move to the clean area at the entrance of the isolation unit.

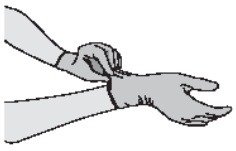
4 By visual inspection, ensure that all sizes of the PPE set are correct and the quality is appropriate.

5 Undertake the procedure of putting on PPE under the guidance and supervision of a trained observer (colleague).

6 Perform hand hygiene.



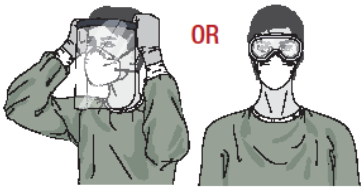
7 Put on gloves (examination, nitrile gloves).



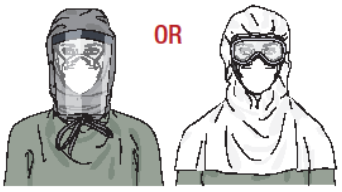
8 Put on disposable gown made of fabric that is tested for resistance to penetration by blood or body fluids OR to blood-borne pathogens.

9 Put on face mask.

10 Put on face shield OR goggles.

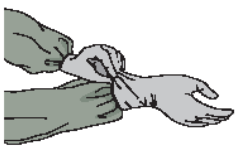


11 Put on head and neck covering surgical bonnet covering neck and sides of the head (preferable with face shield) OR hood.



12 Put on disposable waterproof apron (if not available, use heavy duty, reusable waterproof apron).

13 Put on second pair of (preferably long cuff) gloves over the cuff.



[1] If boots are not available, use closed shoes (slip-ons without shoelaces and fully covering the dorsum of the foot and ankles) and shoe covers (nonslip and preferably impermeable)

World Health Organization

Steps to **take off** personal protective equipment (PPE) including **gown**

1 Always remove PPE under the guidance and supervision of a trained observer (colleague). Ensure that infectious waste containers are available in the doffing area for safe disposal of PPE. Separate containers should be available for reusable items.

2 Perform hand hygiene on gloved hands.[1]

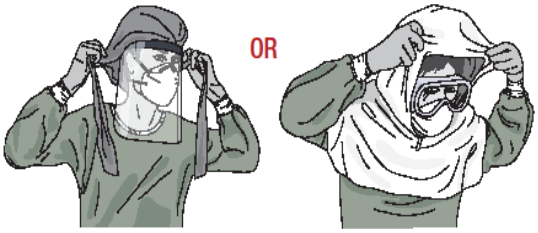
3 Remove apron leaning forward and taking care to avoid contaminating your hands. When removing disposable apron, tear it off at the neck and roll it down without touching the front area. Then untie the back and roll the apron forward.

4 Perform hand hygiene on gloved hands.

5 Remove outer pair of gloves and dispose of them safely.
Use the technique shown in Step 17

6 Perform hand hygiene on gloved hands.

7 Remove head and neck covering taking care to avoid contaminating your face by starting from the bottom of the hood in the back and rolling from back to front and from inside to outside, and dispose of it safely.

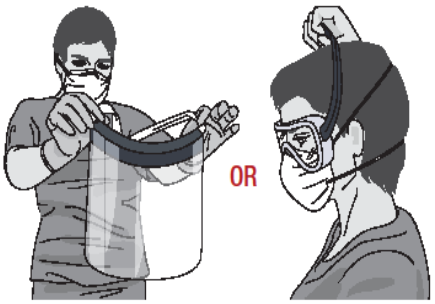


8 Perform hand hygiene on gloved hands.

9 Remove the gown by untying the knot first, then pulling from back to front rolling it from inside to outside and dispose of it safely.

10 Perform hand hygiene on gloved hands.

11 Remove eye protection by pulling the string from behind the head and dispose of it safely.



12 Perform hand hygiene on gloved hands.

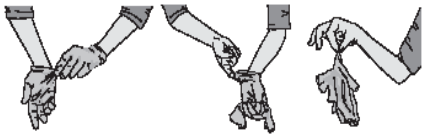
13 Remove the mask from behind the head by first untying the bottom string above the head and leaving it hanging in front; and then the top string next from behind head and dispose of it safely.

14 Perform hand hygiene on gloved hands.

15 Remove rubber boots without touching them (or overshoes if wearing shoes). If the same boots are to be used outside of the high-risk zone, keep them on but clean and decontaminate appropriately before leaving the doffing area.[2]

16 Perform hand hygiene on gloved hands.

17 Remove gloves carefully with appropriate technique and dispose of them safely.



18 Perform hand hygiene.

1 While working in the patient care area, outer gloves should be changed between patients and prior to exiting (change after seeing the last patient)

2 Appropriate decontamination of boots includes stepping into a footbath with 0.5% chlorine solution (and removing dirt with toilet brush if heavily soiled with mud and/or organic materials) and then wiping all sides with 0.5% chlorine solution. At least once a day boots should be disinfected by soaking in a 0.5% chlorine solution for 30 min, then rinsed and dried.

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Appendix 9: Related Training Materials

a. Biosecurity
   - ➢ Case Study: The 2007 Foot-and-Mouth Disease Outbreak in the UK

b. Bio-Risk Assessment and Management
   - ➢ Class Activity: Risk and Infrastructure - New Biological Agent in the Facility

c. Biosafety Cabinets (BSCs)
   - ➢ Demonstration on How to Use a BSC

d. Laboratory Emergencies
   - ➢ Class Activity: Emergency Response to a Natural Calamity Involving Biosafety and Biosecurity Issues

e. Preparing for a Power Failure in the Laboratory
   - ➢ Discussion Paper: Electrical Power in the Clinical Laboratory-Considerations for Ensuring Adequate Quality

f. Shipping of Infectious Substances
   - ➢ Class Activity: Transportation of Dangerous Goods

g. Occupational Health and Medical Surveillance
   - ➢ Class Activity: Health and Safety of Laboratory Workers Related to the Activities of BSL-2 Laboratory

h. Blood-borne Pathogens Program and Exposure Control Plan
   - ➢ Cases of Laboratory Safety Incidents

i. Dual use and Bioethics
   - ➢ Case Studies: Examples of Experiments of Concerns

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