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# BIOSAFETY

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# Introduction

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Bio-related research activities may involve manipulation of microbial, animal or plant cells.

The risks associated with these activities arise from the samples and /or the procedural requirements.

Adherence to standard microbiological techniques and using facilities suitable to the risk level of the pathogen helps to protect the researcher from laboratory-acquired infections.



# Bio hazards

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Hazards related to bio research can be classified into two categories.

- hazards related with the **pathogen** or **human/animal cells** being used in research.
- related with the **procedures and practices** followed in the lab.



# Pathogenic risks

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## Cell cultures

- Researchers who handle or manipulate human or animal cells are at risk of possible exposure to potentially infectious pathogens that may be present in those cells/ tissues.
- The human cell lines may contain blood borne pathogens, which can be transmitted due to improper handling.



# Routes of entry for pathogen

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The probable routes of entry are

- Inhalation of infectious aerosols.
- Contact of the agent with the skin, eyes or mucous membrane.
- Inoculation by contaminated sharps.
- Bites from infected animals or contact with their body fluids.
- Ingestion of infectious agent through mouth pipetting or contaminated hands.



# Aerosols

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Aerosols generated during research activities remain undetected and can spread easily and remain suspended in the laboratory atmosphere for a long time.

They possess a serious hazard to the person performing the task and also to others who are exposed to the air from the laboratory.



# Aerosols

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Aerosols can be generated during the following activities

- Pipetting
- Blending
- Centrifugation
- Use of sonicators and vortex mixers



These respirable size particles when inhaled are retained in the lungs and can cause infection to the person.



# Pathogenic risks

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The risk from the pathogen handled depends on the following factors.

- Capability to cause infection in the host and the severity of the same.
- Preventive measures and treatment available.
- Route of entry
- Infective dose level
- Stability in the environment
- The range of cells/strains that can act as a host.

Based on the above factors the microorganisms are classified into four risk groups.



# Classification of pathogenic microorganisms

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## Risk group I

A pathogen that is unlikely to cause any disease in humans or animals.

All bacterial, fungal and parasitic agents not included in higher groups.



# Classification of pathogenic microorganisms

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## Risk group II

A pathogen that can cause disease in humans or animals but is unlikely to be a serious hazard.

Effective treatment and preventive measures are available and the risk of spread of infection is limited.

- **Bacterial**- *Vibrio cholerae*
- **Fungal**- *Aspergillus fumigatus*, *Actinomyces*
- **Parasitic**- *P.falciparum*, *Plasmodium thcilera*
- **Viral and Rickettsial** - *Vole rickettsia*, *Mumps virus*



# Classification of pathogenic microorganisms

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## Risk group III

A pathogen that can cause serious human or animal disease , but does not ordinarily spread from one infected person to another.

Effective treatment and preventive measures are available.

- **Bacterial** - *Clostridium botulium*, *Francisella tularensis*
- **Fungal** - *Coccidioides immitis*, *Histoplasma capsulatum*
- **Parasitic**- *Schistosoma mansoni*
- **Viral and Rickettsial** - *Foot-and- Mouth disease virus*



# Classification of pathogenic microorganisms

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## Risk group IV

A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly.

Effective treatment and preventive measures are not usually available.

- *Korean hemorrhagic fever*
- *Omsk hemorrhagic fever and*
- *Central European Encephalitis viruses*



# Containment

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The term containment is used to describe the safe work practices in handling infectious agents to reduce exposure to laboratory personnel and others.

## Types of containment

- Biological containment
- Physical containment



## Biological containment (BC)

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Any combination of vector and host which is to provide biological containment must be chosen or constructed to limit the infectivity of vector to specific hosts and control the host-vector survival in the environment.



# Physical Containment (PC)

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Physical containment helps to confine the pathogenic organisms being handled and prevent exposure to personnel.

Physical containment is achieved by

- Laboratory practices
  - Containment equipment
  - Special laboratory design
- } Primary containment
- } Secondary containment

Primary containment offers protection to personnel and immediate laboratory environment whereas secondary containment offers protection to the environment outside the laboratory.



# Primary containment

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## Laboratory practices

Consists of adhering to standard microbiological practices and techniques and awareness of potential hazards.



# Primary containment

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## Containment equipment

This includes biological safety cabinets and enclosed containers (e.g. safety centrifuge cup).



## Secondary containment

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Proper design of the facility helps in protecting personnel inside the facility and also prevents the release of pathogenic organisms outside the facility.

Facility designs are of three types

**Basic Laboratory** (for Risk Group I and II)

**Containment Laboratory** (for Risk Group III)

**Maximum Containment Laboratory** (for Risk Group IV)



# Biosafety levels

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It consists of a combination of laboratory practices, equipment and facilities suitable to the procedures being performed and hazards of the pathogen.

The four biosafety levels corresponds to four risk groups.

A lower risk group can be assigned a higher biosafety level, if a biological risk assessment carried out requires so.



## Biosafety level I

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Suitable for teaching laboratories and for facilities in which work is done with defined and characterised strains of agents not known to cause any disease.

Good microbiological techniques(GMT) to be followed.



## Biosafety Level II

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Applicable to facilities in which work is done with indigenous moderate-risk agents present in the community and associated with human disease of varying severity.

**BSL II** is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines, in which presence of an infectious agent may be unknown

### BSL II requires

- Following GMT
- Use of personal protective equipment
- Use of BSC
- Use of autoclaves



## Biosafety level III

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Applicable to facilities in which work is done with indigenous or exotic agents where the potential for infection by aerosols is real and the disease may have serious or lethal consequences.

**BSL III** requires in addition to that of BSL II requirements

- Special clothing
- Directional airflow
- Controlled access
- Double door entry/Anteroom
- Supervision



## Biosafety level IV

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Applicable to work with dangerous and exotic agents which pose a high individual risk of life-threatening disease.

**BSL IV** requires in addition to BSL III requirements

- Positive pressure personnel suits
- Strictly limited access
- Double ended autoclave
- Class III BSC
- Airlock with shower
- Supervision



# Good microbiological techniques (GMT)

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- Specimen containers must be correctly labelled for easy identification.
- Use secondary containers (autoclavable) while transporting specimens to contain spill.



- Specimen containers received from external agencies must be opened in the biosafety cabinet.
- Use mechanical pipettes.



## Good microbiological techniques

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- Open flame must not be used in BSC as it can distort the air flow pattern and damage the filters.
- Always use disposable gloves. Do not touch mouth, eyes and face with contaminated hands.



# Good microbiological techniques

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- Food and drink must not be stored or consumed in the laboratory.
- Glassware must be replaced with plasticware wherever possible.



# Good microbiological techniques

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- Sharps(e.g., needle sticks, glass) must be avoided wherever possible as it can transmit blood borne pathogens in case of injury.



# Good microbiological techniques

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- Use engineered sharp-safety devices when syringes and needles are necessary.
- Needles must not be recapped, to prevent needle stick injury.
- Puncture-proof containers fitted with covers must be used for disposing sharps.



# Good microbiological techniques

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- Tubes and specimen containers must always be securely capped (screw-capped if possible) for centrifugation.
- Refer to manufacturer's instructions before operating equipments.
- Work area must be decontaminated with a suitable disinfectant at the end of the work.
- Hands must be thoroughly washed before leaving the lab.



# Personal protective equipment

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- Personal protective equipment act as a barrier to minimize the risk of exposure to aerosols, splashes and other injuries.
- Personal protective equipment must be selected on the basis of the risks involved in the task performed.
- Lab coat, safety glasses and toe covered footwear is a minimum requirement while working in the lab.
- Face shield must be used if there is any risk of splashing of infectious materials.



# Personal protective equipment

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- Gloves must be worn for all procedures that may involve direct contact with blood, infectious materials, or infected animals.
- Gloves must be removed aseptically and autoclaved with other laboratory wastes before disposal.
- If re-usable gloves are used, on removal they must be cleaned and disinfected before re-use.
- Lab coats and other personal protective equipment used must not be used outside the laboratory.



# Biosafety cabinets(BSC)

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Biological safety cabinets provide containment of infectious aerosols generated during the laboratory procedures.

Three types of BSCs are used in microbiological laboratories.

These are

Class I

Class II

Class III



# Biosafety Cabinets

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## Class I BSC

Offers protection to laboratory personnel and to the laboratory environment .

It doesn't protect the samples from external contamination.

## Class II BSC

Provides protection to the samples in the cabinet from external contamination in addition to personnel and laboratory environment protection.

## Class III BSC

Provides the maximum attainable level of protection to personnel and the environment.



## The following factors reduce the efficiency of the BSC

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- Poor location
- Room air currents
- Decreased airflow
- Leakage in HEPA filters
- Working with raised sashes
- Overcrowding the work surface
- Improper user methodology



# Emergency measures

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## In case of exposure to bio samples

- Remove the contaminated clothing.
- Wash the skin thoroughly with soap and water.
- In case of eye contact flush the eyes with water.
- Report the exposure to the Lab in charge.
- Get medical attention immediately.



# Decontamination

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- Decontamination renders an item (work bench, equipment, etc.) safe to handle by reducing the number of organisms to below the threshold infectious dose level such that transmission is unlikely to occur.
- Decontamination requirements will depend on the experimental work and the nature of the infectious agent handled.
- Decontamination is usually accomplished by steam sterilization or autoclaving.
- Sterilization and disinfection are different forms of decontamination.



# Decontamination

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## Sterilisation

- Sterilisation makes an item free from all living microorganisms and viruses.
- The process of sterilization can be accomplished by applying heat.



# Decontamination

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## Disinfection

- Is not as effective as sterilization, as some organisms such as bacterial endospores may survive.
- A disinfectant is a chemical or mixture of chemicals used to kill microorganisms, but not spores. They are usually applied to inanimate surfaces or objects.



# Decontamination

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## Disinfectants

- Sodium hypochlorite and formaldehyde are the disinfectants recommended for general laboratory use.
- For special purposes phenolic compounds, alcohols, iodine etc., can be used effectively.



# Biohazard waste disposal

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Biohazard waste generated in laboratories must be segregated into the following:

- Non-contaminated general waste
- “Sharps”-needles, glass pieces, etc
- Contaminated material for autoclaving and recycling
- Contaminated material for incineration



# Biohazard waste disposal

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- Biohazard waste for autoclaving must be collected in red plastic bags and those for incineration in yellow non chlorinated plastic bags.
- Biohazard waste of human and animal origin must be incinerated.



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