

”دورة السلامة العامة والبيئة“

المخاطر الحيوية في المختبرات

**Biological Hazards in Laboratories**

إعداد

د. محمد القيسي      د. مهند أبوعجمية

الجامعة الأردنية - كلية الزراعة



# Introduction:

- Biological hazards include potential exposures to allergens, infectious zoonotic (animal diseases transmissible to humans), and experimental agents such as viral vectors.
- Allergens, ubiquitous in animal research facilities, are one of the most important health hazards, yet they are frequently overlooked.
- Safe work practices are the most critical part of preventing exposure when working with biohazardous materials.
- The best laboratory and safety equipment available cannot provide protection unless personnel use good work practices and have adequate training.



# Laboratory Biosafety Level Criteria

- There are four biosafety levels (BSL) that provide guidelines to ensure appropriate protection for laboratory users and the environment based on biological risk.



# BSLs

- **BSL-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-2** is suitable for work involving agents associated with human disease that pose moderate hazards to personnel and the environment
- **BSL-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 diseases through the inhalation route of exposure.



# BSLs

- ➔ **BSL- 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.


Biology ● ● ●

# BIOSAFETY LEVELS (BSL)

## Biosafety level (BSL)

Indicate biocontainment precautions required to isolate dangerous biological substances





- 
- Labs at our school mostly involves working with agents not known to consistently cause disease in immunocompetent adult humans. Thus, only BSL-1 will be covered here


# Biosafety Level 1 (BSL-1) Minimum Criteria


- Personnel are trained in analytical methods, standard operating procedures (SOPs), spill response, potential hazards, and applicable safety training.
- Access to the laboratory is limited or restricted at the discretion of the laboratory director when work with cultures or specimens is in progress
- Laboratory walls, floors, and ceilings are designed to be easily cleaned.





- 
- Bench tops are impervious to water and resistant to heat, acids, bases, and solvents
  - Laboratory furniture is appropriate for use and easily accessible for cleaning
  - Laboratory windows are fitted with insect screens
  - Laboratory lighting must be adequate for all activities
  - The laboratory is outfitted with a hand-washing sink with soap and towels

- 
- Appropriate PPE is used in the laboratory (for example lab coats, safety glasses/goggles, closed-toe shoes, and gloves).
  - Where research animals are present in the laboratory the risk assessment considers appropriate eye, face, and respiratory protection, as well as potential animal allergens.

- 
- Only animals or plants associated with current studies are present in the laboratory.
  - Personnel wash their hands after handling biohazardous agents or animals, after removing gloves, or before leaving the room
  - Eating, drinking, smoking, applying cosmetics, handling contact lenses, and storing food for human consumption are prohibited in the laboratory.

- 
- Mouth pipetting is prohibited. Mechanical devices are used for pipetting
  - First aid kit is accessible and suitably stocked.
  - Procedures are in place to minimize splashes and the creation of aerosols
  - Work surfaces are decontaminated with an appropriate disinfectant at least once a day and after a spill of viable material
  - Laboratory equipment is decontaminated routinely and after contamination; and before repair, maintenance, or removal from the laboratory.

- 
- Cultures, stocks, and regulated waste are decontaminated by an effective method before disposal
  - Proper biological waste labeling is in place for off-site decontamination
  - Appropriate biohazard containers are used for containment of biohazardous waste
  - The Laboratory has a rodent and pest control program in place.

- 
- A biohazard sign indicating the required biosafety level, required PPE, exit procedures, required immunizations, and the PI's name and phone number are posted at the laboratory entrance during work with human pathogens.

# Signs and Labeling

## ➤ Red door signs

Indicating “human biohazards” must be posted at the entrance of rooms where microorganisms or biological toxins known to cause disease in humans are used.

This includes microorganisms, human blood, tissues, and cells.

**RESTRICTED AREA**  
**NO UNAUTHORIZED PERSONNEL**

**HUMAN BIOHAZARD**

Exposure Date: \_\_\_\_\_  
(For Animal Rooms Only)

Biosafety Level:  BSL1  BSL2  BSL3  BSL4

Building: \_\_\_\_\_ Room# \_\_\_\_\_

INCUB Log # \_\_\_\_\_  
(For Animal Rooms Only)

Room Entrance Protocol (Check all items **NEED** to order)

Boots  Gown Coversalls  Head Cover  
 Gloves  Face Shield  Hair Net  
 Shoe Wash (spray type) \_\_\_\_\_

Other (specify) \_\_\_\_\_

Additional Instructions \_\_\_\_\_

Emergency Contacts:

NAME	Work Phone	Home Phone	Office Location
Dr. _____			
Mr. _____			

- Red biohazard labels must be placed on containers and storage units (refrigerators, freezers, incubators, waste containers, etc.) used for microorganisms or biological toxins causing disease in humans, or human blood, tissues, and cells

**RESTRICTED AREA**  
**NO UNAUTHORIZED PERSONNEL**



**HUMAN BIOHAZARD**

Exposure Date: \_\_\_\_\_  
(For Animal Rooms Only)

Biosafety Level:  BSL1  BSL2  BSL3  BSL4

Building: \_\_\_\_\_ Room# \_\_\_\_\_

IN/CDC Log # \_\_\_\_\_  
(For Animal Rooms Only)

Room Entrance Protocol (checked items must be worn to enter):

<input type="checkbox"/> Boots	<input type="checkbox"/> Clean Coveralls	<input type="checkbox"/> Head Cover
<input type="checkbox"/> Gloves	<input type="checkbox"/> Face Shield	<input type="checkbox"/> Rain Suit

Face Wash (specify type): \_\_\_\_\_

Other (specify): \_\_\_\_\_

Additional Instructions: \_\_\_\_\_

\_\_\_\_\_


Emergency Contacts:

Name	Work Phone	Home Phone	Office Location



- ➔ Yellow door signs indicating “animal biohazards” must be posted at the entrance of rooms where strict animal pathogens are used.

**RESTRICTED AREA**  
**NO UNAUTHORIZED PERSONNEL**



**ANIMAL BIOHAZARD**

Exposure Date: \_\_\_\_\_  
(For Animal Rooms Only)

Building: \_\_\_\_\_ Room# \_\_\_\_\_  
MCUC Log #: \_\_\_\_\_  
(For Animal Rooms Only)

Room Entrance Protocol (Checked items **must** be worn to enter):

- Boots      Clear Coveralls      Head Cover
- Gloves      Face Shield      Rain Suit
- Face Mask (specify type) \_\_\_\_\_
- Other (specify) \_\_\_\_\_

Additional Instructions: \_\_\_\_\_  
\_\_\_\_\_

Emergency Contacts:

Name	Work Phone	Home Phone	Office Location
1 <sup>st</sup> :			
2 <sup>nd</sup> :			
Department of Public Safety	294-4428 <small>(24-hour number)</small>	N/A	N/A

Documents and Instructions Center/One/One/One/Animal/Personnel

- **Dark green door signs** indicating “plant biohazards” must be posted at the entrance of rooms where strict plant pathogens or pests are used, or where certain Genetically Modified (GM) plants are grown or processed
- The emergency contact information contains the minimum requirements for working in each space and shall be displayed at the laboratory entrance.

**RESTRICTED AREA  
NO UNAUTHORIZED PERSONNEL**



**PLANT BIOHAZARDS/PESTS**

USCA Permit # \_\_\_\_\_

Biocontainment Level:  BSL-1-P  BSL2-P  BSL3-P  BSL4-IF

Building: \_\_\_\_\_ Room#: \_\_\_\_\_

IDC Log # \_\_\_\_\_

Room Entrance Protocol (Checked items **must** be worn to enter):

Gloves  Face Mask  Goggles  Lab Coat

Face Shield  Rain Suit

Face Mask (specify wear) \_\_\_\_\_

Other (specify) \_\_\_\_\_

Additional Instructions: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

**Emergency Contacts:**

Name	Work Phone	Home Phone	Office Location
Department of Public Safety	298-4420 (24 hours on-call)	N/A	N/A



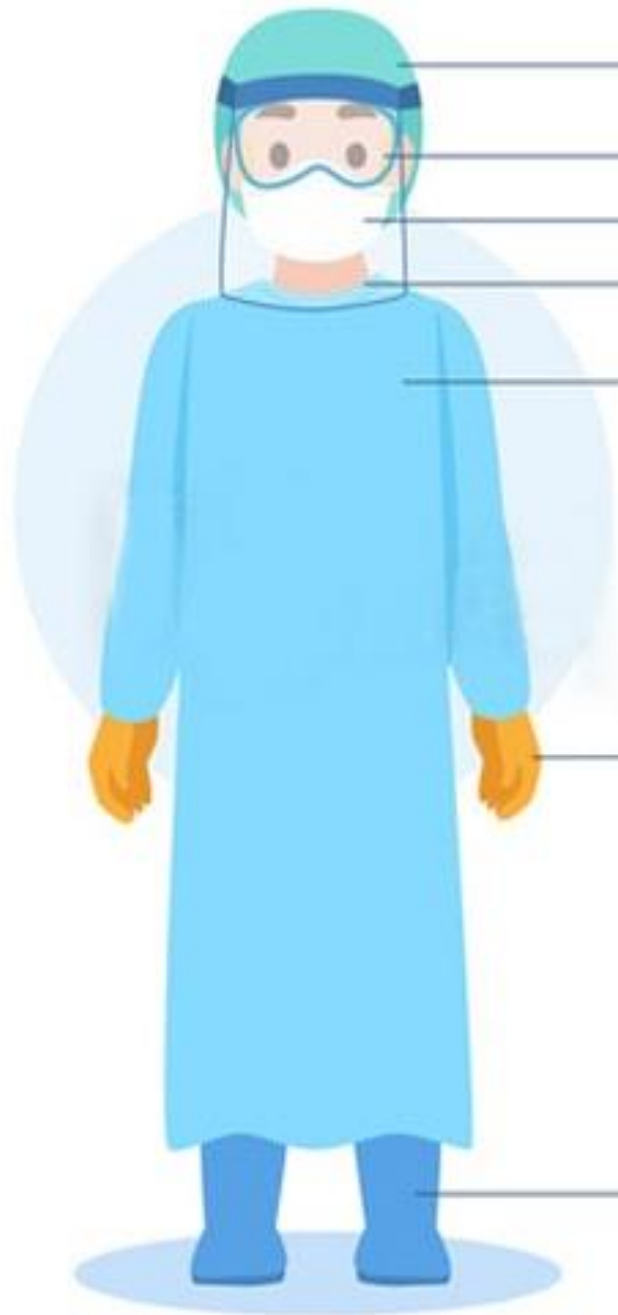
# Security

- Each laboratory should conduct a risk assessment to determine appropriate security measures
- Examples:
  - Locked buildings
  - locked laboratories
  - locked storage units
  - key codes
  - personnel background

# Personal Protective Equipment (PPE)

- Appropriate PPE is chosen by considering the potential routes of exposure that need to be protected to prevent exposure and infection.
- It is essential that PPE be removed before leaving the room where biohazardous materials are used.
- PPE must never be taken home.
- PPE is removed in a manner that minimizes personal contamination.
- PPE should be disposed of or decontaminated in the work area where it is used.

## Full PPE



Hair Net

Goggles / Eyes - Visors

N95 Mask

Face Shield

Isolation Gown

Disposable Gloves



Shoes Cover

# Laboratory Coats and Uniforms

- At minimum, a long-sleeved lab coat worn over clothing and closed-toe shoes must be worn in any laboratory.
- Lab coats must remain in the laboratory when personnel leave the laboratory.
- Elastic-cuffed lab coats help prevent spills that can be caused by catching a loose cuff on laboratory equipment.
- When working with biohazardous materials inside a biosafety cabinet, elastic cuffs or double gloving (second pair over cuff) prevent contaminated air from being blown up the lab coat sleeve onto clothing.

# Gloves

- Both latex and nitrile disposable gloves will prevent exposure to microorganisms. However, nitrile gloves must be worn when handling chemicals, since latex provides little to no protection from chemical exposure
- For best protection, the cuffs of the gloves should overlap the lower sleeves of the lab coat. Disposable gloves must not be reused.
- Utility gloves, such as rubber dish washing gloves, may be disinfected for re-use if they do not show signs of wear or degradation.
- Change gloves when contaminated and when glove integrity is compromised,



<b>TYPE</b>	<b>DEFINITION</b>	<b>ADVANTAGE</b>	<b>PROTECTION</b>
Latex	Natural rubber	Comfort, flexibility, fit and tactile sensitivity	Bacteria, viruses
Nitrile	Synthetic material	Stretchy, durable	Chemicals, viruses
Vinyl	Synthetic material	Cost-efficient, comfortable	Chemicals





# Eye and Face Protection

- Goggles or safety glasses must be worn when working with laboratory hazards
- Face shields should be used for full face protection.
- N-95 masks provide splash protection for the mouth and nose.





# Respirators


- ➔ Respirators prevent the inhalation of aerosolized microorganisms (inhalation exposure) when safety equipment designed to contain infectious aerosols, such as a biosafety cabinet, is not available.


# Laboratory Practice and Technique


- ▶ Personnel can be infected with organisms they come in contact with in the workplace. In order for infection to occur, there must be an adequate number of organisms to cause disease (infectious dose) and a route of entry into the body. Knowing how infectious organisms are transmitted and the infectious doses can help in evaluating risk and avoiding infection.

- 
- Infectious agents are transmitted through one or more routes of exposure:
  - Sharps injuries (needlesticks, cuts with contaminated broken glass, etc).
  - Inhalation of aerosols (microscopic solid or liquid particles (5 micrometers or less) dispersed or suspended in air).
  - Ingestion (oral-fecal routes of contamination are a common source of infection).
  - Mucous membrane exposure (including the eyes, inside of the mouth and nose).


- 
- How to protect yourself.
  - Wear appropriate PPE .
  - Eating, drinking, smoking, chewing tobacco, applying cosmetics, or storing food in laboratories is strictly prohibited.
  - Hands must be washed frequently, even after wearing gloves, and scrubbed vigorously with soap and water for a full 30 seconds.
  - Work surfaces and equipment must be decontaminated.

- 
- Suggestions help prevent biohazardous materials from entering the body.
  - Pipetting.
  - Mouth pipetting is prohibited .
  - All biohazardous materials must be pipetted in a biosafety cabinet.
  - After use, pipettes should be placed horizontally in a pan filled with enough liquid disinfectant to completely cover them.
  - Plastic micropipette tips and pipettes are sharp and should be disposed of in a puncture-resistant container.

- 
- Suggestions help prevent biohazardous materials from entering the body.
  - Centrifugation.
  - Leaks can be prevented by not overfilling centrifuge tubes.
  - Ensure that rotors are balanced before centrifugation.
  - Centrifuges and accessories must be cleaned and disinfected regularly or when contaminated.

- 
- Suggestions help prevent biohazardous materials from entering the body.
  - Using Needles, Syringes, and Other Sharps
    - Needles and syringes may only be used when there is no reasonable alternative.
    - Substitute plastic ware for glassware when possible.
    - Sharps must be kept away from fingers as much as possible. Sharps must never be bent, sheared, or recapped.
    - An appropriate sharps container must be kept close to the work area.



- 
- Suggestions help prevent biohazardous materials from entering the body.
  - Blending and Grinding.
    - Must be operated in a biosafety cabinet whenever possible.
    - Safety blenders should be used.
    - Avoiding glass blender jars prevents breakage


- Suggestions help prevent biohazardous materials from entering the body
  - Open flames.
  - Must be operated in a biosafety cabinet whenever possible.
  - Safety blenders should be used.
  - Avoiding glass blender jars prevents breakage





# Animal Handling

- Where available use footbaths when entering and exiting animal rooms.
- All animal room doors must remain closed at all times.
- Disposable gloves must be worn when dealing with animals.
- Disposable or washable outer cloths (such as lab coats, gowns, coveralls) protect personal clothing from contamination when working with animals.

- 
- Eating, drinking, smoking, applying cosmetics, and handling contact lenses in animal rooms or procedure rooms are prohibited.
  - Hand contact with the nose, eyes, or mouth is strongly discouraged when working with animals.
  - Hands must be washed with soap and water immediately after handling any animals.
  - Extra caution must be taken with needles or other sharp equipment used with animals.
  - Any bites or other wounds must be washed immediately with soap and water and appropriate medical attention sought.
  - Unauthorized persons are prohibited from entering animal rooms.



# Safety Equipment


- Primary containment equipment is designed to reduce or eliminate exposure to biohazardous materials.
- Biosafety cabinets (BSC) serve as the primary containment for biohazardous materials in the lab.





# What is a Biosafety cabinet??


- BSCs are designed to protect personnel, the products being handled, and the environment from particulate hazards.
- BSCs use uniform vertical laminar airflow to create a barrier to airborne particulates.
- BSCs utilize High Efficiency Particulate Air (HEPA) filters to clean both the air entering the work area and the air exhausted to the environment.



- A biosafety cabinet is not a chemical fume hood. Chemical fume hoods are designed to protect personnel by removing chemical vapors and aerosols from the work area.

- The HEPA filter removes airborne particles from the air but does not remove chemical fumes.



- 
- BSCs are designed to protect personnel, the products being handled, and the environment from particulate hazards.
  - BSCs use uniform vertical laminar airflow to create a barrier to airborne particulates.
  - BSCs utilize High-Efficiency Particulate Air (HEPA) filters to clean both the air entering the work area and the air exhausted to the environment.

## When Must I Use a BSC?

- Biosafety cabinets should be used whenever you are conducting lab procedures with biohazardous materials that may produce aerosols or anytime you are working with large amounts of infectious materials

# Disposal and Disinfection of Biohazardous Materials

## Autoclaves

- Autoclaves must be properly used to effectively sterilize their contents.
- Autoclave use for microbiological media preparation requires various time and temperature settings for sterilization.
- Autoclaving biohazardous waste must take into account the volume of waste and the ability of steam to penetrate the load.
- The minimum autoclave cycle time for biohazardous waste is 45 minutes at 121°C.



## **The following elements all contribute to autoclave effectiveness:**

- Temperature - unless specifically instructed by media manufacturers' directions, autoclave chamber temperature should be at least 121°C (250°F).
- Time - Autoclave cycle time will vary according to the contents of the autoclave. Adequate autoclaving time for biohazardous waste is a minimum of 45 min.
- Contact - Steam saturation of the load is essential for effective decontamination.



# Disposal and Disinfection of Biohazardous Materials

## Chemical disinfectants

- Items that cannot be autoclaved can generally be decontaminated using a chemical disinfectant.
- Choosing the appropriate chemical disinfectant depends on the surface or item needing decontamination, as well as the particular organism requiring inactivation.



# Considerations for choosing a chemical disinfectant!!

- What is the organism requiring inactivation? (Different disinfectants are more effective against different types of organisms).
- How many of the organisms are present?
- What needs decontamination?
- Work surfaces (metals, plastic, wood, concrete).
- Glassware.
- Equipment (surgical tools, BSC).

# Types of Chemical Disinfectants

## ➤ Alcohols

- These are most effective against lipophilic viruses
- Optimal disinfection is attained by using 70% ethanol for 15 minutes.
- These types of disinfectants evaporate quickly, so sufficient contact time may be difficult to achieve. Concentrations above 70% are less effective because of increased evaporation rate.
- Chlorine compounds
- Formalin
- Effective against vegetative bacteria, spores, and viruses
- Effective concentration at 5-8%
- Equipment (surgical tools, BSC)



**BIOLOGICAL  
HAZARD**



**NO ENTRY**  
AUTHORISED  
PERSONS ONLY