Practical Bacteriology lab. 1

Lab Equipment, Biosafety and Control of Microorganisms

By:

Lecturer Shiama'a Al-Salihy

Learning objectives:

After this lab, you must be able to:

1. Recognize equipment used in bacteriology lab with their functions.
2. Understand the principle of biosafety
3. Understand biosafety levels in lab.
4. Understand general disinfection principles.
5. Distinguish between methods of sterilization and disinfection.

Lab Equipment And Instrument

Lab. equipment and instrument:

1. **Anaerobic jar:** used for cultivation of anaerobic bacteria.
2. **Autoclave:** used for sterilization.
3. **Bunsen burner:** used for heating, sterilization of inoculating loop.
4. **Colony counter:** used for accurate counting of bacterial and mold colonies in petri dish.
5. **Hot plate with magnetic stirrer:** used for mixing and dissolving media or chemical solution.

Lab. equipment and instrument:

6. **Electrical sensitive balance**.
7. **Incubator:** used for incubating microbes at appropriate temperature for each type of microbes.
8. **Microscope:** used for studying very small organisms by magnifying them
9. **Inoculating loop and stabbing needle**
10. **Hood (working cabinet).**
11. **Petri dish**

Lab. equipment and instrument:

8. **Polymerase Chain Reaction (PCR):** amplification of nucleic acid
9. **UV lamp:** used for sterilization of lab environment.
10. **Vitek 2 system:** automated microbiology system for fast and accurate microbial identification, and antibiotic susceptibility testing.

Biosafety

Biosafety:

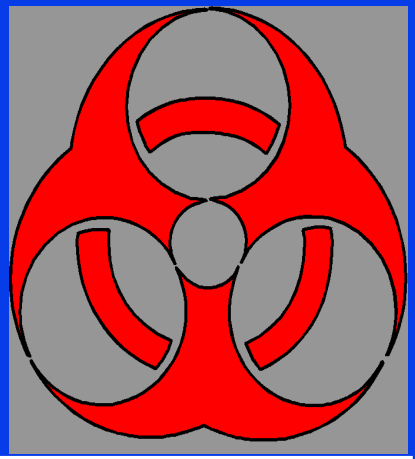
❖ Principle:

- Biosafety is the application of safety precautions that reduce a risk of exposure to a potentially infectious microbe and limit contamination of the work environment and, ultimately, the community.

- In medicine: it refers to the levels of lab containment protocols, measured as Bio safety level (BSL) 1,2,3,4 in rising order of danger.

Component of safety in all labs:

- **Safe handling, storage and disposal of:**
  - **Specimens**
  - **Chemicals**
  - **Instrument**
  - **Radioactive components**
- **Fire safety**
- **Electrical safety**



❖Determination of lab containment level:

The primary risks that determine levels of containment to handle biohazardous agent are:

- Virulence
- Pathogenicity
- Infectious dose
- Environment stability
- Route of spread
- Communicability
- Availability of vaccine or treatment.

Levels of Containments

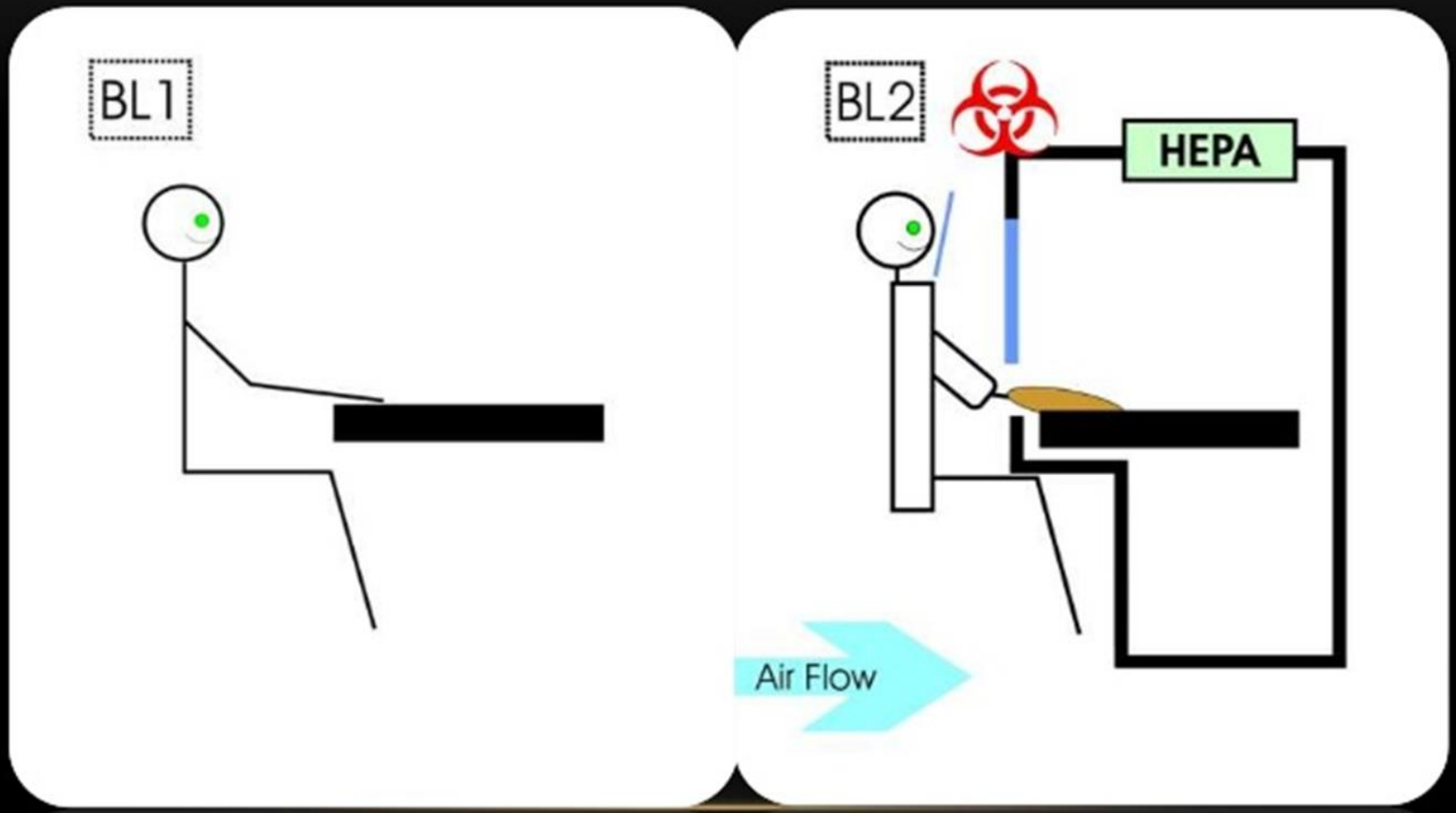
- **BSL1**: Microorganisms that don't consistently cause disease in healthy adults
  - *E. coli* K12, *S. cerevisiae*, Polyomavirus
  - Basic laboratory
  - Standard Microbiological Practices

- **Open bench top, sink for hand washing**

Levels of Containments

- **BSL2** - microorganisms of moderate potential hazard, transmitted by contact, ingestion, puncture
  - *Salmonella*, herpesvirus, human blood
  - Basic laboratory
  - Standard Microbiological Practices Plus:
  - Training in handling pathogens
  - Access to lab limited
  - Extreme sharps precautions
  - Use of BSC for aerosols

CDC/NIH: BIOSAFETY LEVEL 1 AND LEVEL 2.



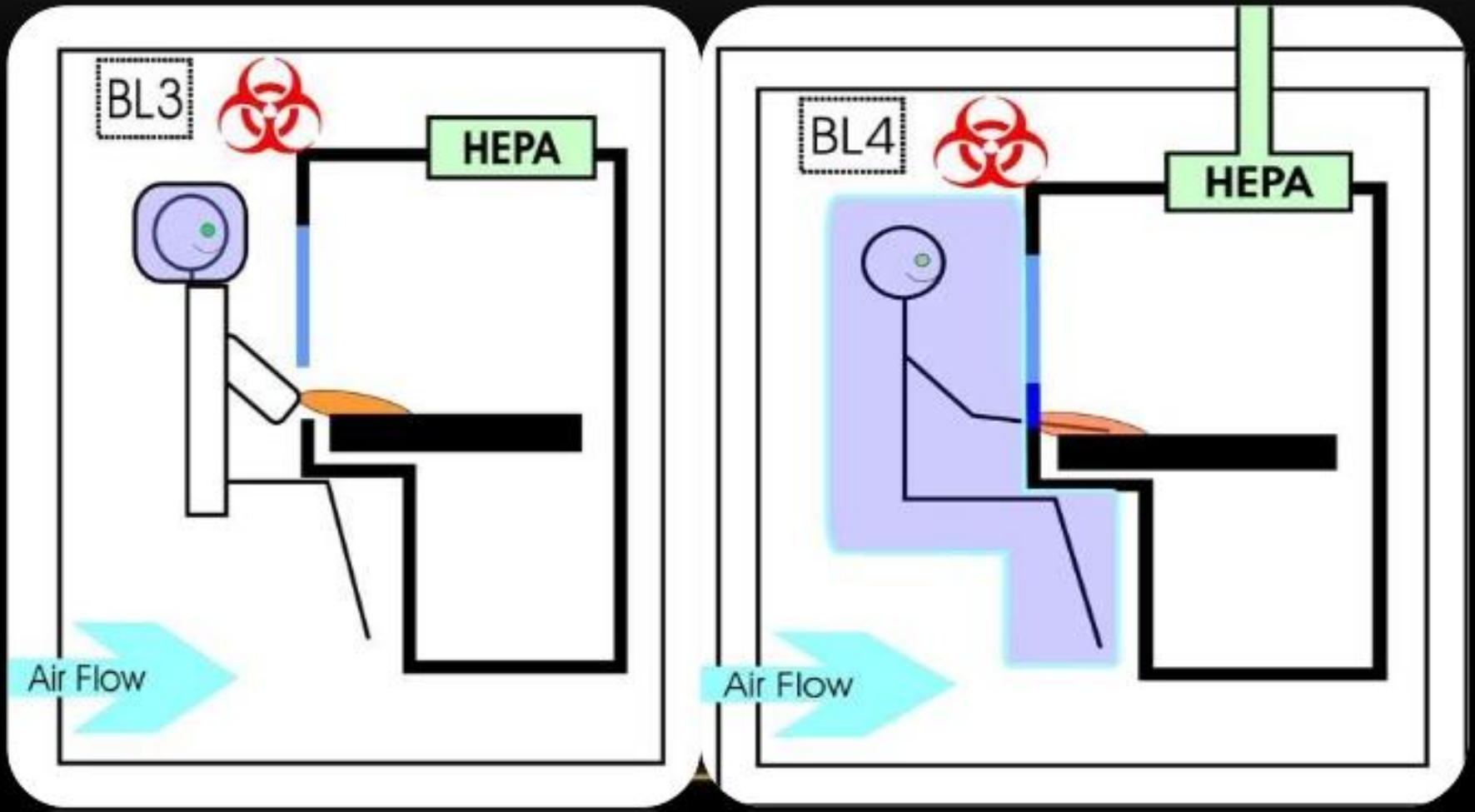
Levels of Containments

- **BL3** - microorganisms that cause serious disease, transmitted by inhalation
  - *M. tuberculosis*, yellow fever virus, hantavirus, *Y. pestis* (plague)
  - Containment lab: double door entry; directional airflow; all work in biosafety cabinet

Levels of Containments

- BL4 - microorganisms that cause lethal disease, with no known treatment or vaccine
  - Ebola virus, Marburg virus
  - Maximum containment lab; positive pressure ventilated suits (moon suits)

CDC/NIH: BIOSAFETY LEVEL 3 AND LEVEL 4.



Risk groups, biosafety levels, practices and equipment

BSL	Laboratory type	Laboratory practices	Safety equipment
1	Basic teaching, research	Good microbiological techniques	None Open bench work
2	Primary health services; diagnostic services, research	Good microbiological techniques, protective clothing, biohazard sign	Open bench PLUS biological safety cabinet for potential aerosols
3	Special diagnostic services, research	As BSL 2 PLUS special clothing, controlled access, directional airflow	Biological safety cabinet and/or other primary devices for all activities
4	Dangerous pathogen units	As BSL 3 PLUS airlock entry, shower exit, special waste	Class III biological safety cabinet, positive pressure suits, double ended autoclave (through the wall), filtered air

General Safety Rules

1. Listen to or read instructions carefully before attempting to do anything.
2. Wear safety goggles to protect your eyes from chemicals, heated materials, or things that might be able to shatter.
3. Notify your teacher if any spills or accidents occur.

General Safety Rules

4. After handling chemicals, always wash your hands with soap and water.

5. During lab work, keep your hands away from your face.
6. Tie back long hair.

General Safety Rules

7. Roll up loose sleeves.
8. Know the location of the fire extinguisher, fire blanket, eyewash station, and first aid kit.
9. Keep your work area uncluttered. Take to the lab station only what is necessary.

General Safety Rules

10. It is suggested that you wear glasses rather than contact lenses.
11. Never put anything into your mouth during a lab experiment.
12. Clean up your lab area at the conclusion of the laboratory period.
13. Never "horse around" or play practical jokes in the laboratory.

LAB SAFETY

What do you think is the most dangerous thing in any laboratory?

The most dangerous thing in any laboratory is some one who doesn't know what they are doing

Control Of Microorganism

Sterilization *vs.* Disinfection

- **Sterilization:** is the process by which all forms of microbial life including bacterial spores & vegetative pathogenic and non pathogenic are killed.

- **Disinfection:** is the process by which pathogenic organisms , but not necessarily all microorganisms or spores are destroyed (applied to inanimate surface)
- **Antisepsis:** is disinfection of animate objects e.g. disinfectants for the skin are called antiseptic agents.

Sterilization methods:

A. Physical methods:

1. **Incineration:** is the most common methods of treating infections waste (use in hospital).
2. **Moist heat:** is used to treat heat stable objects &culture media (old or new) or liquids (distilled water) e.g. autoclave which saturated steam under pressure (15 PSI) and 121C◦ for (15 – 20)minutes.
3. **Dry heat**: use for glassware, metal instruments. e.g. ovens (160 – 180 C◦) at time (1.5 – 2) hours. In other hand direct heat as **flaming** to sterilize loop, spreader, needle and orifice of tubes and flasks e.g. burner.

- **Filtration:** is the removing of microorganisms from solutions which heat sensitive like serum, antibiotic solution & carbohydrate. Filtration of liquids is accomplished by pulling the solution through a cellulose membrane with a vacuum, filtration of air by using high efficiency particulate air filters.

- **5.Ionizing radiation** : using in microwave and radiograph machines for sterilizing disposable material such as plastic syringes , catheters or gloves and plastic Petri dishes before use

B. **Chemical methods:** the most common chemical sterilizing are :

1. **Ethylene oxide** : used in gaseous form to sterilize heat sensitive object.
2. **Formaldehyde vapor.**
3. **Vapor phase of hydrogen** peroxide.
4. **Perocetic acid** : for surgical instruments.

Disinfection methods:

A. Physical methods :

1. **Boiling :** at 100C◦ for 15minutes which kills vegetative bacteria.
2. **Pasteurizing:** at 63C◦ for 30 minutes or 72C◦ for 15 minutes dairies & milk.
3. **Non ionizing (ultra violet radiation):** for biological safety cabinets.

B. Chemical methods:

1. **Alcohols** : like ethanol& isopropanol are used to disinfect hands in 70% solution.
2. **Aldehydes:** like formaldehyde and gluteraldehyde which sporocidal is used for medical equipment such as bronchoscope , incubators (5-10% solution in water)to preserve the corpses.
3. **Halogens chlorine & iodine** which is skin antiseptic before drawing blood or surgery.
4. **Quaternary ammonium compound** for bench & other surface in laboratories.
5. **Phenolic compounds:** used for benches & tables.

Questions?


Thank You
For
Attention