The Islamic University

Department of Medical Laboratory Techniques

practical microbiology

Second Class

Lab.1-4

M.S.C. Dhuha Haider

M.S.C.Akram fuad

Lab.1 : Instructions in the lab

1) Lab coat should be worn before the entry to the lab over street clothes and remove before leaving

2) In typical way, laboratory workers should wear goggles, mask and gloves.

3) Do not eat, drink or smoke inside the lab, these should be done in isolated room or rest room.

4) Do not bite the nail or put the pen into the mouth.

5) Do not mouth pipette, rubber teat or mechanical pipette should be used.

6) Assume that patients are infectious for HIV or blood borne disease.

7) Wash the hands thoroughly after removing the gloves or if there is any contamination

Essential requirement of microbiological laboratory

Equipment used for sterilization

Autoclave

The autoclave is an example of **moist heat sterilization**. The **primary purpose** of the autoclave is sterilizing culture media and laboratory supplies. Saturated steam under pressure above 100°C is used for sterilization in autoclaves.

Hot Air Oven

A hot air oven **applies dry heat for sterilization**. **Its main application** is sterilizing glassware like pipettes, flasks, metallic instruments, and scissors.

Incinerator

An incinerator is the best way to discard those dangerous waste. Incinerators use heat to eliminate solids; powder, pastes, pills, sludges, liquids, boxes, and tubes.

Instruments for Culture and Identification

Analytical Balance

An analytical balance **measures** the precision in determining the mass of solid objects, powders, and granular substances.

Biological Safety Cabinets

A Biological Safety Cabinet (BSC), also known as a Biosafety Cabinet is **mainly used for** handling pathogenic biological samples or for applications that require a sterile work zone.

Bunsen Burner

The Bunsen burner is a gas burner that **uses dry heat to sterilize materials**. The materials are heated by holding them almost vertically in the flame until red hot.

Centrifuge

A centrifuge is a laboratory device **that helps separate** fluids, or liquid-based on their density. The spinning of a vessel containing material at high speed helps achieve the separation.

VITEK 2 Compact system

uses disposable cards. One card is required for identification of one bacteria. So enabling the identification of several bacteria at a time.

Colony Counter

A colony counter is a machine that **automatically counts colonie**s in the agar plate in the microbiology laboratory.

Gel Electrophoresis Apparatus

Electrophoresis is a process that **separates nucleic acid and protein**-based on their size and charge.

Hot Plate

A hot plate **provides heat to solutions and materials uniformly**. It is much safer than a Bunsen burner because an open flame is not present in the hot plate.

Incubator

An incubator is an instrument that provides the desired temperature for in vitro culture of microorganisms.

Laboratory Freezer

A Laboratory freezer is used **to store samples**, specimens, and other materials at -10°C to -30°C. whereas ultra low-temperature freezer can freeze samples to almost -80°C.

Microscope

Microscope **used to examine** objects that are too small to be seen by the naked eye.

Micropipette

Micropipettes are semi-automatic instruments that **use disposable pipette tips to withdraw and dispense the liquid sample.**

PCR Thermocycler

Thermocyclers are the instrument that helps in amplifying DNA and RNA samples by the process of polymerase chain reaction (PCR).

pH Meter

A pH meter **measures** the acidity/alkalinity of the solution.

Refrigerator

The refrigerator provides a low-temperature environment. The use of refrigerators is to store cultures, media prepared, blood, serum, antibiotics, and other chemicals.

Vortex Mixers

A vortex mixer mixes laboratory samples in test tubes, well plates, or flasks.

Water Bath

A water bath is an instrument that provides constant temperature to a sample.

Instruments for Antibiotic Susceptibility Testing

Antibiotic Disc Dispenser

The antibiotic disc dispenser is the laboratory equipment that helps accurately place the antibiotic disc in the media of choice.

Antibiotic Zone Reader

An antibiotic zone reader is a laboratory instrument that helps to obtain an accurate reading of the diameter of the zone of inhibition of the used antibiotic.

Glassware

Beaker

A beaker is a glass container that has a flat bottom. The use of beakers in a microbiology laboratory is holding or storing liquids.

Conical flask

A conical flask is a cone-shaped flask with a flat round bottom and a cylindrical neck. Its purpose in the microbiology laboratory is to prepare media.

Glass Rods

A glass rod is a piece of laboratory equipment used to mix chemicals and liquids.

Measuring Cylinder

A measuring cylinder is an instrument made of glass or plastic used to measure the volume of different liquids.

Microscopic Slide

It is used in staining and observing under a microscope.

Petri Dish

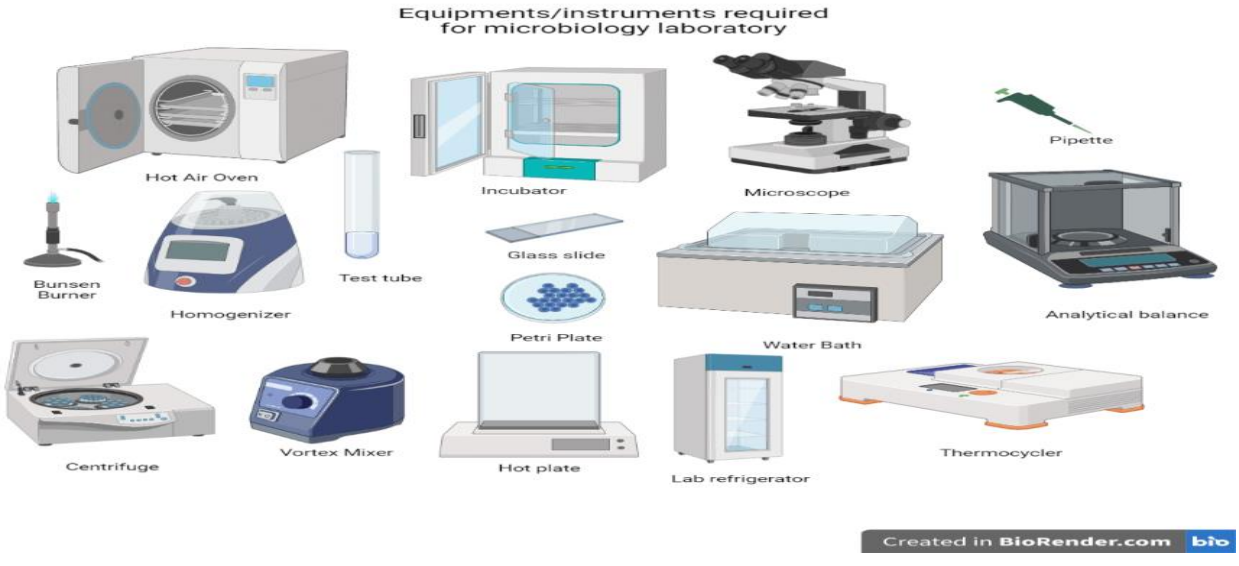
A petri dish is made up of glass/plastic. It is an apparatus in which culture media is poured, providing a suitable environment for the growth of microorganisms.

Test Tube

A standard test tube has high utility in the microbiology laboratory. It helps grow microorganisms by providing an artificial environment, preparing stock cultures, and performing various biochemical tests.

Inoculating Loop and Needle

An inoculating loop and needle transfer microbial growth from one container to another without introducing any unwanted organisms.



Lab.2 Sterilization & Disinfection

Sterilization: The processes by which **all forms of microbial life**, including vegetative and spore forming bacteria are **destroyed or killed**.

Disinfection: The processes that result in the destruction of only the vegetative forms of microbial life (pathogenic organisms) **but not spores** .

Antisepsis: Destruction or inhibition of microorganisms on living tissues by chemicals (non-toxic and non-irritating) known as **Antiseptics .**

Bacteriostatic : is a condition where the multiplication of the bacteria is inhibited without killing them.

Bactericidal : is that chemical that can kill or inactivate bacteria. Such chemicals may be called variously depending on the spectrum of activity, such as bactericidal, virucidal, fungicidal, microbicidal, sporicidal, tuberculocidal or germicidal.

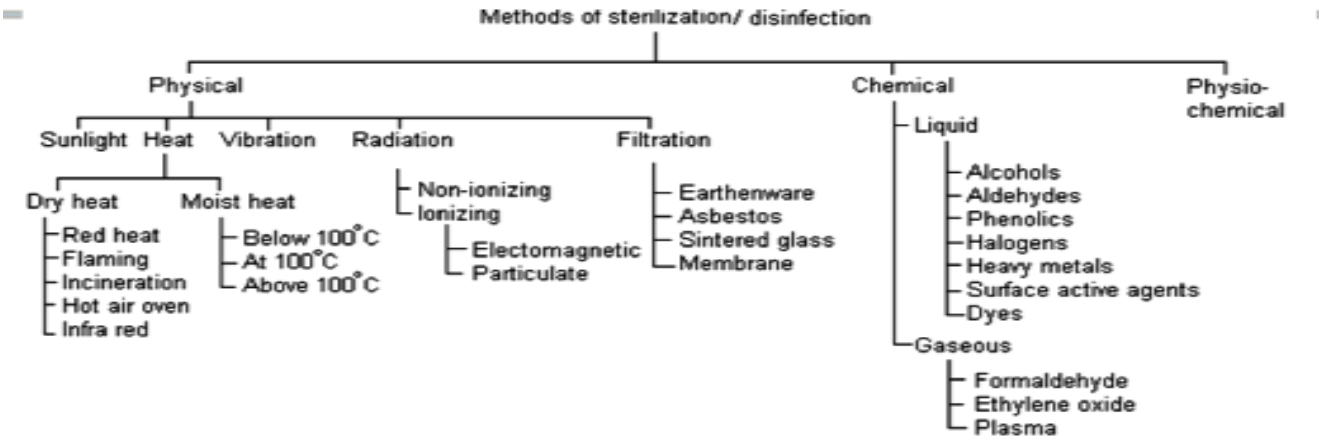
Antibiotics : are substances produced by one microbe that inhibits or kills another microbe. Often the term is used more generally to include synthetic and semi-synthetic antimicrobial agents.

Importance of sterilization and disinfection:

1. Safety of laboratory workers and patients.
2. Accuracy and validity of microbiological tests.

Methods of sterilization and disinfection:

There are several methods of sterilization and disinfection. They are very broadly classified into physical and chemical methods .Figure ( )



Methods of sterilization:

1. **Physical Sterilization (by sun light , heat, filtration or radiation):**

A.Sunlight:

The microbicidal activity of sunlight is mainly due to the presence of ultra violet rays in it. It is responsible for spontaneous sterilization in natural conditions.

B. Heat:

The most effective and a rapid method of sterilization and disinfection is Heat. Excessive heat acts by thickening or clotting of cell proteins. Less heat interferes metabolic reactions. The two most common methods for sterilization

i. Sterilization by Dry Heat

Dry heat : Temperature of 160 degree for 2 h. or 170 degree for 1 h. (Oven, flaming and Incineration) .

•Red heat : Articles such as bacteriological loops, straight **wires, tips of forceps and searing spatulas** are sterilized by holding them in Bunsen flame till they become red hot.

• Flaming: The object is passed through flame without allowing it to become red hot, e.g. scalpel. Temperature is not high to cause sterilization. **mouth of test tubes, flasks, glass slides and cover slips** are passed through the flame a few times. Even though most vegetative cells are killed.

• Incineration : Incineration is the process of sterilization along with a significant reduction in the volume of the wastes. It is usually conducted during the **final disposal of the hospital** or other residues.

• Infrared radiation : Infrared radiation (IR) is a method of thermal sterilization in which the radiation is absorbed and then converted into heat energy. IR is applicable for mass **sterilization of packaged items like syringes and catheters.**

ii. Sterilization by Moist Heat

Moist heat sterilization describes sterilization techniques that utilize saturated steam. Moist heat can denature and coagulate the protein. It can cause breakage of DNA strands and loss of functional integrity of cell membrane. Methods include:

• Boiling: Boiling **at 100°C** is done in a **water bath** for 30 minutes. By this method **Syringes, rubber goods and surgical instruments** can be sterilized. The method is effective against all bacteria and some spores.

• Steaming: Moist heat sterilization usually involves the use of steam at temperatures in the range **121–134°C**. An **autoclave** is a device that works on the principle of moist heat sterilization through the generation of steam under pressure.

The 2 common sterilization temp.

a) 121c for 15 min for sterilizing items such as media, liquid and medical instruments.

b) Infectious waste such as unused portion of patients' specimens, patients' culture, sharp disposable sharp instruments such as scalpels, needles sterilized at 132 C for 30-60 Min.

• **Steam Sterilizer At temperatures below 100°:**

Pasteurization- In this process, all non-spore forming microbes are killed in milk by subjecting the milk to a temperature of 63°C for 30 minutes (the holder method) or 73°C for 20 seconds (the flash method).

C. Filtration :

Sterilization by filtration is done mainly for thermolabile solutions. These may be sterilized by passage through sterile bacteria-retaining filters, for e.g. membrane filters (cellulose derivatives, etc.), plastic, porous ceramic, or suitable sintered glass filters, or combinations of these. Filters containing Asbestos should not be used. Normally, the membranes of not greater than 0.22 μm having, nominal pore size is used.

D. Radiation:

Two types of radiation, ionizing and non-ionizing.

- **Non-ionizing rays:** Rays having the wavelength longer than the visible light are non-ionizing. A high-pressure mercury vapor lamp is used to generate UV rays. UV rays induce the formation of thymine-thymine dimers and eventually inhibit the replication of DNA. UV radiation induces mutations in cells of bacteria, viruses, yeast, etc. When exposed to the effective UV radiation is inactivated within seconds.
- **Ionizing rays:** ionizing rays are high-energy rays which have good penetrative power. It is termed as "cold sterilization", as the radiation does not generate heat There are two types of ionizing rays; particulate and electromagnetic rays. Electron beams are particulate in nature while gamma rays are electromagnetic in nature

2. Chemical sterilization:

Disinfectants are those chemicals that destroy pathogenic bacteria from inanimate surfaces.

ALCOHOLS

A 70% aqueous solution is more effective at killing microbes than absolute alcohols. they are used as antiseptic on skin. Alcohols dehydrate cells, disrupt membranes and cause coagulation of
protein

ALDEHYDES:

It kills all microorganisms, including spores. Formaldehyde, Gluteraldehyde

HALOGENS:

widely used antiseptics and disinfectants; iodine acts by oxidizing cell constituents and iodinating cell proteins; chlorine acts primarily by oxidizing cell constituents

PHENOL:
Act by disruption of membranes, precipitation of proteins and inactivation of enzymes .

3. Physiochemical Methods of Sterilization:

A physiochemical method includes both physical and chemical method. Use of steam-formaldehyde is an example of physiochemical method of sterilization.

Lab.3 Routine microbiology specimen collection procedures

General considerations

All specimens must be properly marked with the following:

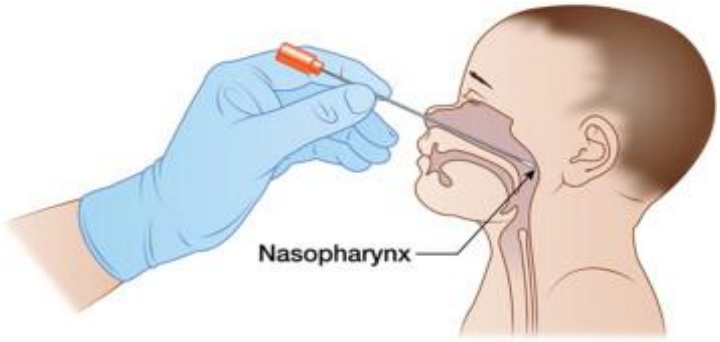
- Patient name
- Patient identification number
- Source of specimen
- Date and time of collection

NOTE\Samples should be collected **before the start of any treatment**

1-**Respiratory tract specimens**

A-Nasopharyngeal swab

• Insert a sterile swab into the nose until resistance is met at the level of the turbinate.
• Rotate the swab against the nasal mucosa.
• Place into swab transport system, and deliver promptly to the lab. **Rayon / Dacron Swab** to collect samples



B-Sputum

Have patient rinse mouth and throat with fresh water, cough deeply and expectorate into Sputum Collector. An early morning specimen is usually more productive. 5-10 cc of specimen is desired.

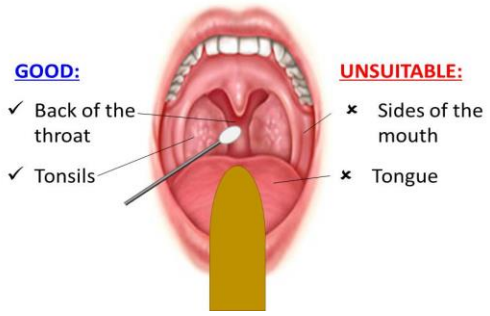
Label specimen and send to laboratory immediately.

If there is a delay in transport to the lab, specimen should be refrigerated.

C-Throat

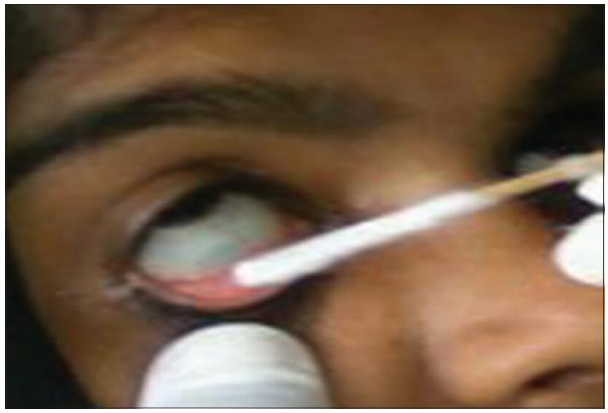
Do not obtain throat samples if epiglottis is inflamed (sampling may cause serious respiratory obstruction)

1. Depress tongue gently with tongue depressor.
2. Extend sterile swab between the tonsillar pillars (Avoid touching cheeks, tongue or lips).
3. Sweep the swab back and forth across the posterior pharynx, tonsillar areas and any inflamed or ulcerated areas to obtain sample.
4. Return swab to swab transport system.
5. Transport as soon as possible.



2-**Eye specimens**

- Where possible ask the patient to look upwards and gently pull the lower lid down or gently part the eyelids. Use a sterile cotton wool swab and gently role the swab
- over the conjunctival sac inside the lower lid or using gauze. Hold the swab parallel to the cornea to avoid injury if the patient moves.
- Place the swab in the transport medium.



3-Ear swabs

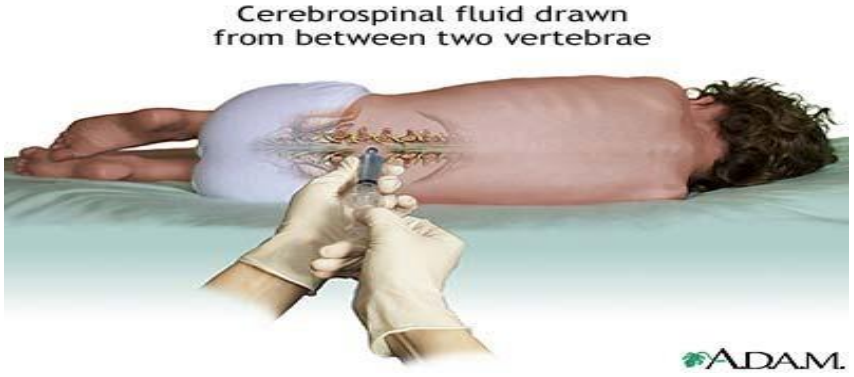
- If there is purulent discharge this should be sampled.
- Place a sterile swab into the outer ear and gently rotate to collect the secretions.
- Place swab in transport medium.
- For deeper ear swabbing a speculum may be used

4-wound and miscellaneous specimens

Use a sterile swab and gently rotate on the area to collect exudate from the wound and place into transport medium. Where there is pus collect as much as possible in a sterile syringe or sterile container (do not use a swab) and send to the laboratory.

5-Cerebrospinal fluid

Sampling of cerebrospinal fluid is essential for the accurate diagnosis of infective meningitis and may aid in the diagnosis of encephalitis.



6-Urinary tract specimens (Midstream or 'clean catch' specimen)

- Ask the patient to void a small amount of urine into the toilet first.
- Then ask the patient to urinate 10-20 ml directly into the specimen container.
- Instruct the patient that the remaining urine can passed into the toilet.
- Place the lid securely on the specimen container

7-fecal specimens

Collect feces from patients as soon after onset of illness as possible, and before the start of treatment. For liquid stool specimens, no more than 10 ml should be added to the **Cary Blair medium** and mixed.

8-Genital tract specimens

Use swab to obtain a sample of end cervical, vaginal, or urethral discharge. Return the swab to the transport tube and break the media ampule at the base of the tube to moisten the swab.

9-Fungal samples of hair, nail and skin

Special containers (**Dermapak**® : folded dark paper squares, secured with a paper clip) may be obtained from the microbiology department:

- Samples of infected hair should be removed by plucking the hair with forceps or gloves. The root of the hair is infected not the shaft.
- Samples of the whole thickness of the nail or deep scrapings should be obtained.
- The skin should be cleaned with an alcohol swab. Epidermal scales scraped from the active edge of a lesion or the roof of any vesicle should be obtained.

Rejection

Samples will be rejected if they are:

- Unlabeled - All specimens must have a unique patient identifier.
- Insufficient in Quantity - No specimen received, no specimen in container, or insufficient specimen to perform testing.
- Improperly Preserved - Specimens must be received in the transport media as defined by the laboratory.
- Damaged - Specimen leaked or broken in transit.
- Too Old - Aged specimens are diagnostically unreliable .

Lab.4 Culture media

Bacterial Growth: Bacterial Growth: refers to an increase in the numbers of individuals.

Culture media : Is a liquid (broth) or solid (agar) to support the growth of microorganisms or cells. A culture medium is composed of different nutrients (Carbohydrate , lipids, amino acids, vitamins as well as inorganic compounds) .

Types of Culture Media

Culture media can be classified based on the :

A. Based on their consistency(physical state):

1- **Solid medium:** contains 2% agar, Colony morphology, pigmentation, hemolysis can be appreciated ex. Blood agar, nutrient agar ect…. .

2- Liquid medium sometimes referred as "broth": no agar for inoculum preparation, Blood culture, for the isolation of pathogens from a mixture. The bacteria grow uniformly producing turbidity

3- Semi solid medium: 0.5% agar used to check for motility and also used as a transport media **.**

B .Based on the purpose of use (Types of culture media):

1-Simple media The most common growth media for microorganisms are nutrient broths and agar plates; specialized media are sometimes required for microorganism and cell culture growth. Nutrient agar, and Plate count agar,

2- Selective Media They are the media containing a specific composition of ingredients, which supports the growth of some target bacteria and inhibits the growth of the others. They are used to isolate specific groups of bacteria. e.g : MacConkey agar for Gram-negative bacteria, it's contain on crystal violet and bile salt which is inhibit Gram positive bacteria and some fastidious gram-negative bacteria. Eosin methylene blue (EMB) enteric isolation media.

3-Differential media Distinguish one microorganism type from another growing on the same media. E.g: MacConkey agar (MCK), which is differential for lactose fermentation. Mannitol Salt Agar (MSA): Mannitol fermentation by pathogenic staphylococci is indicated by a yellow halo surrounding the colonies. Sodium chloride is the inhibitor agent .

4-Enriched media fortified with blood, yeast extract or brain or heart infusion are useful in growing of fastidious organisms. For example sheep blood agar contain protein sources, sodium chloride and 5% sheep blood and supports the growth of most gram-positive and gram-negative bacteria. However some bacteria are highly fastidious organisms. They required Chocolate agar which contain red blood cells (RBCs) that have been lysed. Enriched media are useful for culturing normally sterile body fluids such as blood and CSF

5-Assay media Support the growth of most microorganism. Ex.: Mueller Hinton Agar is commonly used for antibiotic susceptibility testing: disk diffusion antibiotic susceptibility

6-Indicator media They provide additional information for the identification of the bacterium Ex: Triple sugar iron agar (TSI)

7-Transport media Transport media should fulfill the following criteria: Temporary storage of specimens being transported to the laboratory for cultivation and maintain the viability of all organisms in the specimen without altering their concentration. e.x: Thioglycolate broth for strict anaerobes, Stuart transport medium and Cary Blair medium .

How to prepare the culture medium:

1- The weight of the culture medium.
2- Melt the medium using heat with stirring Then Autoclave sterilization
3- Cool the medium after sterilization.
4- The nozzle of the beaker is inflamed before pouring.
5- Pour the medium into a Petri dish
6- Flaming the petri cap after casting.
7- Let the agar harden Then placed in the incubator upside down