

جامعة الانبار

كلية: الصيدلة

قسم: العلوم المختبرية السريرية

اسم المادة باللغة العربية: احياء مجهرية طبية I

اسم المادة باللغة الإنكليزية: **Medical Microbiology I**

المرحلة: الثانية

التدريسي: م. د. رواء علي حسين

عنوان المحاضرة باللغة العربية: التعقيم

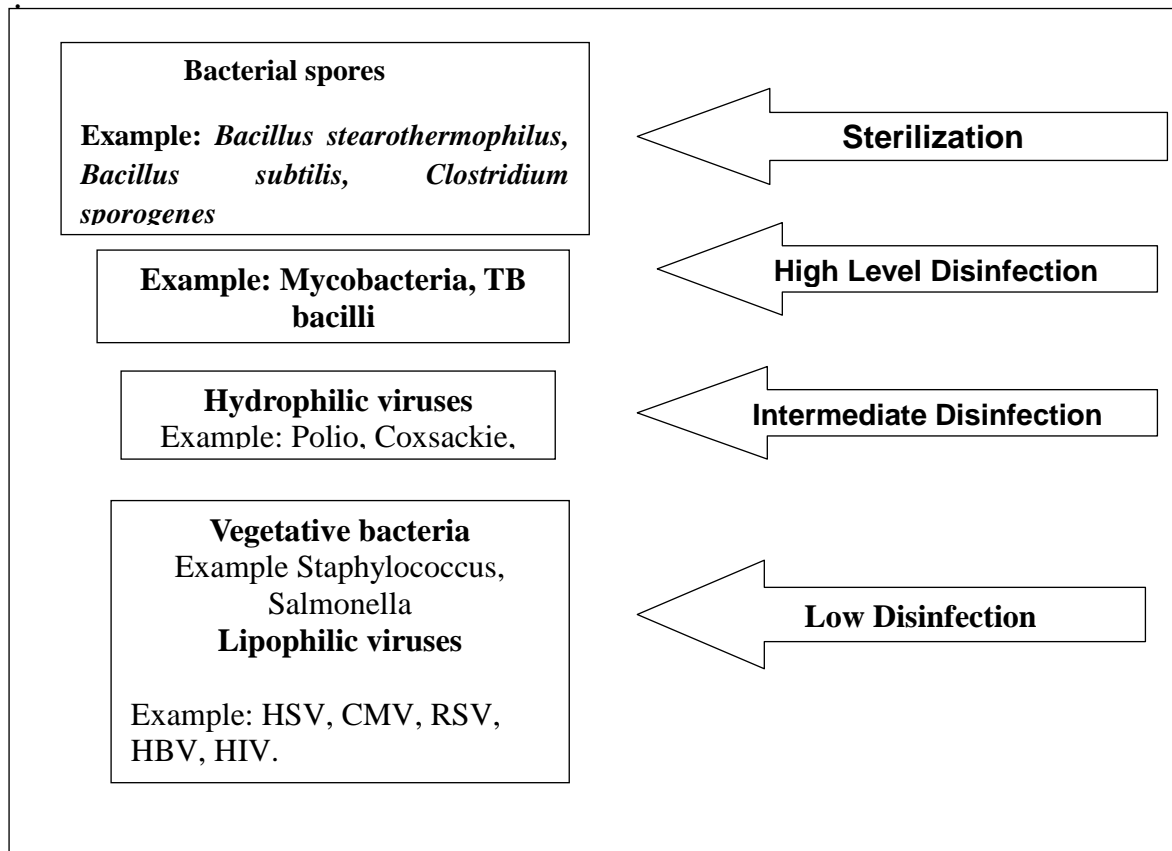
عنوان المحاضرة باللغة الإنكليزية: **STERILIZATION**

STERILIZATION

Sterilization is a term referring to any process that eliminates (removes) or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media. Sterilization can be achieved by applying heat, chemicals, irradiation, high pressure, or filtration.

DISINFECTION

Reducing the number of pathogenic microorganisms to the point where they no longer cause diseases or is the process of elimination of most pathogenic microorganisms (excluding bacterial spores) on inanimate objects such as tables, floors, etc. Examples chlorine, hypochlorites, lye, ect.



The level of disinfection effect on different organisms

Bacteriostatic Agent :An agent that inhibits the growth of bacteria, but does not necessarily kill them.

Bactericide:An agent that kills bacteria. Most do not kill Endospores.

Sporicide -: An agent that kills spores

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

Antiseptics: microbicidal agent harmless enough to be applied to the skin and mucous membrane should not be taken internally such as alcohols, iodine solution and detergents.

PHYSICAL METHODS:

1. Heat (Dry and moist)

Heat is considered to be most reliable method of sterilization of objects that can withstand heat.

A. Dry Heat: Techniques include:

- **Flaming** *Common uses:* bacterial loops, wires and spatula's.



Hot Air oven



Flaming

- **Hot Air oven** (160°C/2hrs or 170°C/1hrs) *common uses:* glassware and metallic instruments.

- **Incineration**

B. Moist Heat:

Moist heat is more efficient in contrast to dry heat; it causes *coagulation* and *denaturation* of proteins.

- **Pasteurization** (At temperature 63°C/30 minutes (Holder method) or 72°C/15 minutes (Flash method) followed by cooling quickly to 13°C is the use of mild heat to reduce number of microorganism in product or food. In the case of pasteurization of milk the time and temperature depend on killing potential pathogen that are transmitted in milk . such as *Staphylococci* , *streptococci*, *Brucella abortus* and *Mycobacterium tuberculosis*.

- **Boiling** (At temperature 100°C): 30 minutes of boiling kills microbial pathogens and vegetative forms of bacteria but may not kill bacterial endospores.

- **Intermittent boiling** (Tyndallisation):

- Named after John Tyndall.
- Exposure of 100 °C for 20 min for 3 successive day.
- Principle: It consists of heating the substance to boiling point (100 °C) and holding it there for 20 minutes, three days in succession. After each heating, the resting period will allow spores that have survived to germinate into bacterial cells; these cells will be killed by the next day's heating. During the resting periods the substance being sterilized is kept in a moist environment at a warm room temperature, conducive to germination of the spores.

- **Autoclave** (At temperature 121°C and 15 bar pressure for 15 minutes): kills all forms of life including bacterial endospores. Used to sterilize culture media, surgical instrument and cotton.



Autoclave

2. Radiation

There are 2 types of Radiation: usually destroys nucleic acid

A. Non-ionizing: .

- **UV Radiation** it has a germicidal effect on microorganisms.
- *Common uses:* Surface disinfection, in hospitals, operating theatre and laboratories.
- **Infrared light:** used to purify air, such as in the operating room.

B. Ionizing: 2 types: (Highly lethal to DNA and other vital constituents).

(**Electron beam**): *Common uses:* sterilization of instruments such as syringes, gloves, dressing packs, foods and pharmaceuticals.

(**Gamma rays**): *Common uses:* sterilization of disposable petri dishes, plastic syringes and fabrics.

CHEMICAL METHODS:

The mechanism of action of chemical agents are:-

- Cell membrane injury.
- Coagulation and Denaturation.
- Interactions with functional groups of proteins.

❖ Ethylene oxide gas:

- Colorless
- used to sterilize heat –sensitive objects
- Effective against all types of microorganisms including viruses and spores
- *Common uses:* medical and pharmaceutical products, plastic containers, oil, some foods and tobacco.

Formaldehyde gas: is a water-soluble gas. Formalin is a 35% solution of this gas in water. Formaldehyde is a broad-spectrum germicide for bacteria, fungi, and viruses. The mechanism of action of formaldehyde is based on protein denaturation. Kills endospores

- Common uses: Sterilization of instruments.
- ❖ **Alcohols.** Ethanol /Isopropyl alcohol are frequently used
 - Solutions of 70% ethanol are more effective than higher concentrations.
 - No action on spores
 - Alcohols are quite effective against bacteria and fungi, and viruses. They do not kill bacterial spores. denatures protein
 - Common uses: surgical and hygienic disinfection of the skin and hands.
 - Isopropyl alcohol is preferred as it is a better fat solvent, more bactericidal and less volatile. Used for disinfection of clinical thermometer.
- ❖ **Aldehydes**
 - Formaldehyde & Glutaraldehyde are frequently used
 - Formaldehyde is bactericidal, sporocidal & has a lethal effect on viruses. Destroying Anthrax spores in hair and wool.
- ❖ Glutaraldehyde is effective against Tubercle bacilli, fungi and viruses
Used to treat anesthetic tubes, Face masks, Plastic endotracheal tubes, Metal instruments and polythene tubing.
- ❖ **Halogens.** Chlorine, iodine, and derivatives of these halogens are suitable for use as disinfectants. halogens denatures proteins by binding to free amino groups. Actively bactericidal with moderate against spores.
 - **Common uses:** Chlorine is used to disinfect drinking water. Iodine used to disinfect skin and small wounds.
- ❖ **Oxidants.** This group includes ozone, hydrogen peroxide, potassium permanganate, and peracetic acid. Their relevant chemical activity is based on the splitting off of oxygen.
 - Common uses: Most are used as mild antiseptics to disinfect mucosa, skin, or wounds.
 - **Phenols:** Synthetic phenolic compounds are disinfectants with broad-spectrum disinfecting action including a tuberculosis kill. E. g: Lysol, cresol

- Common uses: phenols may be used for surface disinfection. Various combinations are used in the control of pyogenic cocci in surgical & neonatal units in hospitals. Aqueous solutions are used in treatment of wounds

❖ **DYES**

Two groups of dyes are used.

- **Aniline dyes**
 - Are Brilliant green, Malachite green & Crystal violet
 - Active against Gram positive bacteria
 - No activity against tubercle bacilli
- **Acridine dyes**
 - Acridine dyes in use are orange in color
 - Effective against Gram positive than Gram negative
 - Important dyes are Proflavine, Acriflavine, Euflavine

Dyes may be used topically as antiseptics to treat mild burns. The dyes are used as selective agents in certain selective media.

FILTRATION

Fluids that would be damaged by heat irradiation or chemical sterilization, can be only sterilized by Microfiltration using membrane filters

- commonly uses: for heat labile pharmaceuticals and protein solutions in medicinal drug processing (such as those containing proteins like large molecule drug products, serum, enzymes, sugars, toxins).



**Millipore filter or
Syringe Filter**



Membrane filters