AL-Mustaqbal University Collage.
Department of Pathological Analysis Technique.
Subject: - Advanced laboratory techniques.
Lecture-No. 2.
Safety and Sterilization.

Safe working practices: -

Health and safety in district laboratories including full coverage of microbial hazards, safe working practices and the decontamination of infectious material and disposal of laboratory waste.

The following are some important points which apply when working with infectious materials: -

1-Never mouth-pipette. Use safe measuring and dispensing devices.

2-Do not eat, drink, smoke, store food, or apply cosmetics in the working area of the laboratory.

3-Use an aseptic technique when handling specimens and culture.

4-Allways wash the hands after handling infectious materials, when leaving the laboratory and before attending patients. Cover any open wound with a water proof dressing.

5-Wear appropriate protective clothing when working in the laboratory.

6-Wear protective gloves, and when indicated a face mask, for all procedures involving direct contact with infectious materials.

7-Avoid practices which could result in needle-stick injury.

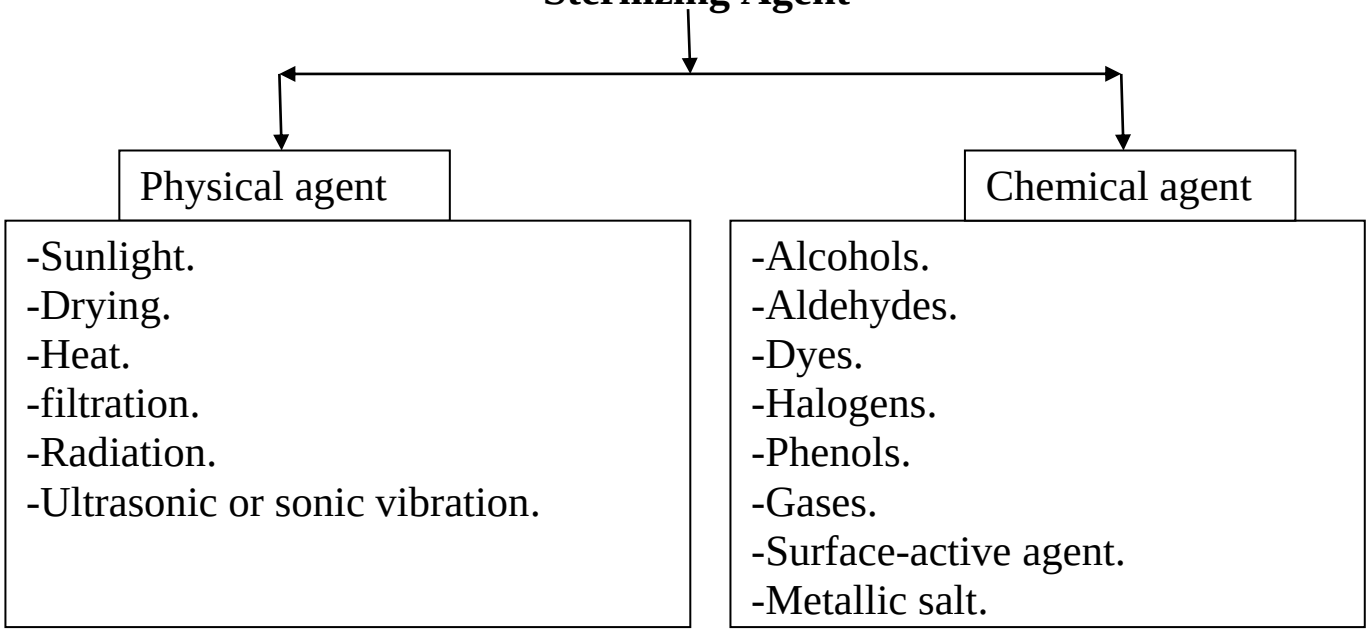
8-Do not use chipped or cracked glassware and always deal with breakage immediately and safely.

9-Do not allow unauthorized persons to enter the working area of the lab.

10-Report immediately to the laboratory officer in charge any spillage or the other accident involving exposure to infectious material.

Sterilization and disinfection

Sterilizing Agent



INTRODUCTION

-**Sterilization: -** is define as the process by which an article, surface or medium is freed of all living microorganisms either in the vegetative or spore state.

-**Disinfection**: - is the destruction or removal all pathogenic organisms, or organisms capable of giving rise to infection.

-**Asepsis**: - is the term used to indicate the prevention of infection, usually by inhibiting the growth of bacteria in wound or tissues.

-**Antiseptics: -** are chemical disinfectants that can safely applied on the skin or mucous membrane and are used to prevent infection by inhibiting the growth of bacteria.

-**Bactericidal agents (or germicides) are those that can kill bacteria.**

-**Bacteriostatic agent: -** is prevent multiplication of bacteria which may be however remain alive. A chemical which is bactericidal at a particular concentration may only be bacteriostatic at a higher dilution.

-**Cleaning: -** plays an important preparatory role before sterilization or disinfection, by removing soil and other dirt and reducing the microbial burden, making sterilization more effective.

Sterilization Agent

The agent used in sterilization can be classified as following: -

A-Physical agents: -

-Sunlight

-Drying

-Dry heat: - flaming incineration, hot air.

-Moist heat: - pasteurization, boiling, steam under normal pressure, steam under pressure.

-Filtration: -candles, asbestos pads,

-Radiation.

-Ultrasonic and sonic vibration.

B-Chemical agent: -

-Alcohols: - ethyl, isopropyl, tri-chloro-butanol.

-Aldehydes: -formaldehyde, glutaraldehyde.

-Dyes.

-Halogens.

-Phenols.

-Surface: - active agent

-Metallic salts.

-Gases: - ethylene oxide, formaldehyde, beta propiolactne.

Advantages of sterilization

1- Prevent transmission of diseases

2- Prevent contamination and growth of undesirable bacteria

3- Prevent spoilage of material by microorganisms.

The processes of sterilization primarily use moist or dry heat and those of disinfection are often restricted to the use of chemicals. There are 3 methods of

A. Physical:

(1)- Heat: the most practical and dependable methods of sterilization, these methods may divide into:

1. Dry heat:

a. Red heat or flaming (Incineration): - points of forceps, spatulas, wire loop, needles, mouth of culture tubes, glass slide. The temperature employed is about 160°C for 1 hour.

b. Hot air oven: (glass pet radishes, glass pipettes, glass flasks, dry glass ware, measuring cylinders, all glass syringes, metal instruments oils and grease can also sterilize by this method. The temp (160°C -180°C). Conditions of sterilization by hot air oven: - 160°C for (2hrs) 120 minutes. 170°C for (1hr) 60 minutes. 180 °C for (1/2hr) 30 minutes**.**

2.Infra-radiation: It is used for sterilizing metal instruments and glass wares.

3. Moist heat: The most effective method, means the use moist heat in the form of steam under pressure. Heat transfer is rapid when moisture is present. The autoclave uses steam under pressure

A. Single exposure at 100 °C for 90 min (Spore for some thermophilic and rare mesophilic bacteria) can be survive.

B. Intermittent exposure at 100 °C for several times(Tyndallization): This method is applied in the microbiologic laboratory for the sterilization of those.

a. At temperature below 100 °C (Pasteurization): is not a method of sterilization; it is a process used principally in food industries for the preservation of milk and other dairy products. This process consists of exposing the product to temperature of 62.8°C for 30 min. or 71.6°C for 15 min and then cooling it immediately.

b. At temperature of 100 °C either in boiling water or in free steam (all glass ware, metal instruments, needles and syringes) by 2 ways, media and solutions adversely affected by high temp of autoclave (containing sugars that may be decomposed at high temp.)

c. At temp. above 100 °C (saturated steam under increased pressure) as sterilization by autoclaves This procedure is suitable for culture media, lab coating, surgical dressing and aqueous solution. Since the atmosphere of steam prevent the loss of water by evaporation during heating.

Note: *50%of safety period is usually added to these minimum holding time.* * 121 °C for 15 min at 15 psi(pound/inch) *126C° for 10 min* 134°C 3 min

(2)-Radiation: The process of transmission of energy through space is done by UV light, X- ray and Gamma rays (X-ray and Gamma rays are very expensive, so it not available to use except in certain condition.

Ultra- Violet radiation: - One component of sun light is (ultra- violet rays). It has a powerful bactericidal effect.

This wave is not markedly bactericidal until (250nm), increased activity by decreased wave length. The shortened wave in sun light reached the earth in length(**290nm)** but even more effective radiation (240- 280nm) can

produced by vapor lamp are used to lower the bacterial count in rooms and screens where aseptic handling of materials is undertaken.
It has a little penetration power; they are only effective for surfaces where bacteria are not protected by other materials.
Precaution should be taken to protect the skin and cornea from highly irritant rays.
UV light causes the following changes in cell:
1- Denaturation of protein.
2- Damage of DNA.
3- Inhibition of DNA replication.
B. Mechanical methods (Filtration): Microorganisms can be removed from solutions and fluids by filtration. These solutions are then sterile. This method is used for solutions and fluids that cannot be exposed to heat or chemicals without being chemically changed. Microorganisms are not killed by filtration but are physically separated from the fluid as it passes through the filter. Filters with pore size of 0.2 µm in diameter will remove bacteria and Microorganisms other than viruses. Filters of porosity of 10 nm are recommended for the removed of viruses. Filtration is used to remove the Microorganisms from biologic fluid such as (normal sera, antisera, microbial toxins, enzymes containing solution, various sugar solution, and other materials put in solution that cannot be sterilized by other methods.
C. Chemical methods: Chemical agent that kills pathogenic and nonpathogenic microorganisms but not spores. Disinfection and antiseptic are generally applied to different types of substance, it cannot kill all types of microorganisms but reduce no., to be not effect or produce disease**.**
Disinfection: is reducing the number of bacteria to a level low enough that disease is unlikely to occur. Spores and some bacteria will survive. e.g. ethyl alcohol is used in concentration (50-70) %, phenol group (2- 5%), Chlorine compounds 5% (added to water), Formaldehyde (gas), 37% Dettol or alcohol 70% are effective in vegetative cells (denaturation of protein and nucleic acids, solvents of lipid in cell membrane and active in reducing normal flora from the skin and clinical thermometers).