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Practicals in Microbiology

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2 Sterilization and Disinfection

STERILIZATION

The process of freeing an article surface or media from all living organisms including bacteria and their spores is called sterilization.

DISINFECTION

It is the destruction or removal of all pathogenic microbes capable of giving rise to infection but not all microbial forms or spores.

ANTISEPSIS

It is the removal of all pathogens from a living surface, e.g. skin.
Sterilization is done by physical and chemical methods.

Physical agents

1. Sunlight
2. Drying
3. Dry heat
   - Flaming
   - Red heat
   - Incineration
   - Hot air oven
4. Moist heat
   - Temperature <100°C
     - Pasteurization
     - Inspissation
     - Vaccine bath
   - Temperature at 100°C
     - Steaming/Tyndallization
     - Boiling

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- Temperature >100°C
  - Autoclave

5. Filtration
6. Radiation
7. Ultrasonic and sonic vibrations.

Chemical agents

1. Alcohol
   - Ethyl alcohol
   - Isopropyl alcohol
   - Trichlorobutanol
2. Aldehyde
   - Formaldehyde
   - Glutaraldehyde
3. Dyes
4. Halogens
5. Phenols
6. Surface active agents
7. Metallic salts
8. Gases
   - Ethylene oxide
   - Beta propiolactone
   - Formaldehyde.

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STERILIZATION BY DRY HEAT

Mechanism

1. Protein denaturation
2. Oxidative damage
3. Toxic effects of elevated levels of electrolytes.

Heat to red heat

To hold an article in the flame till it is red hot, e.g. inoculating loop/wire, tips of forceps and searing spatulas.

Flaming

To pass an object rapidly through the flame several times without allowing it to become red hot, e.g. glass slide, mouth of test tube, scalpel and needle.

Incineration

For destroying contaminated cloth, animal carcasses and pathological materials.

Hot air oven

- Holding period—160°C for 1 hour.

Precautions

i. Overloading should be avoided
ii. Proper arrangement—To allow free flow of air circulation
iii. Glasswares should be completely dry
iv. Flasks and test tubes should be wrapped in paper
v. It should be allowed to cool down (2 hours).

Uses

- Glasswares, forceps, scissors, scalpels, glass syringes, swabs, liquid paraffin oil, dusting powder, fat and grease.

Disadvantages

i. Hot air—Bad conductor of heat, penetrating power is low.
ii. Fan—Needed for even distribution of air.
iii. Rubber materials and media cannot be sterilized.
iv. If door of oven is opened before room temperature is reached, glassware may crack by sudden or uneven cooling.

Sterilization control

i. Physical—Thermocouples
ii. Chemical—Browne's tube, Bowie Dick tape
iii. Biological—Spores of *Bacillus subtilis*, nontoxigenic *Clostridium tetani*.

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STERILIZATION BY MOIST HEAT

Mechanism

1. Denaturation
2. Coagulation of protein.

Pasteurization

- Holder's method—63°C for 30 minutes followed by cooling rapidly to 13°C.
- Flash method—72°C for 15–20 seconds followed by cooling rapidly to 13°C.
- Ultra High Temperature (UHT) Pasteurization—140°C for 3 seconds followed by cooling very quickly in a vacuum chamber.

Use

- Pasteurization of milk.

Inspissation

- Heating at 80–85°C for 30 minutes–1 hour on 3 successive days.

Use

- Sterilization of media containing egg or serum, e.g. Lowenstein Jensen media and Loeffler's serum slope.

Water bath

- Heating at 56°C for 1 hour.

Use

- Serum/body fluids.

Boiling

- Heating at 100°C for 10–30 minutes
- Mostly regarded as means of disinfection.

Precautions

- Materials should be completely immersed, the lid of the sterilizer should not be opened
- 2% sodium bicarbonate may be added to promote sterilization.

Tyndallization

- Arnold or Koch's steamer
- Heating at 100°C for 30 minutes on three consecutive days.

Principle

- Steam at atmospheric pressure—One exposure kills vegetative organisms. Between heatings the spores being kept in a favorable nutrient medium germinate to form vegetative forms which get killed during subsequent heating.

Steaming

- Single exposure at 100°C for 90 minutes may be done.

Use

- Gelatin media and media containing sugars.

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Autoclave

Principle

Steam under pressure—Water boils when its vapor pressure is equal to surrounding atmospheric pressure.

When pressure inside a closed vessel increases, the temperature at which water boils is also increased. Saturated steam has high penetrating power. When the steam comes in contact with cooler surface it condenses to water and gives up its latent heat to that surface (1600 ml of steam condenses into 1 ml of water at 100°C and releases 518 calories of heat).

Holding period

121°C for 15–20 minutes at 15 lb/inch^2 pressure.

Uses

Dressings, instruments, lab wares, media and pharmaceutical products.

Sterilization control

- Physical—Thermocouples
- Chemical—Bowie Dick tape
- Biological—Spores of *Bacillus stearothermophilus*.

Radiation

Nonionizing	***Ionizing***
Generates heat	No heat generated—Cold sterilization
Infrared radiation–Mass sterilization of syringes	Gamma and X-rays—Sterilizing plastic syringes, swabs and catheters
UV rays-damages DNA Disinfection of closed areas—Laboratories, operation theaters, biosafety cabinet	*Advantages*: • Effective against viruses, bacteria and bacterial spores • Good penetration of closed packs; items can be prepackaged before sterilization • Heat labile materials can be safely sterilized as no heat is involved
	Disadvantages: • Expensive process • Glass tends to go brown and may be damaged • Damages some types of rubber, PVC, nylon, paper, wood, cotton and metals if irradiated more than once

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Filtration

Use

- To remove bacteria from heat labile fluids like serum, sugar solution, urea solution, antibiotics for culture media.

Types

i. Candle filters—It is used for purification of water for industrial/drinking purposes
   a. Chamberland filters made of unglazed porcelain
   b. Berkefeld filters made of fossil diatomaceous earth.
ii. Asbestos filters—These are disposable and single use disks
   a. Seitz filters.
iii. Membrane filters—Cellulose esters
   - Water purification and analysis
   - Sterilization and sterility testing
   - 0.22 mm.
iv. Sintered glass filters
   Made of finely ground glass fused together.
v. High efficiency particle arresters (HEPA).

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Practicals in Microbiology

Practicals in Microbiology has been written primarily for Second Professional MBBS students in a hope that it would decrease the load of both the teachers and the students. The book will also help the DMLT and MSc medical microbiology students.



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