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STERILIZATION AND DISINFECTION

For Histology practical

STERILIZATION

It is the killing of all micro-organisms including their spores whether these are pathogenic or non-pathogenic.

DISINFECTION

It is the process of killing of vegetative form of micro-organism.

PHYSICAL METHODS

HEAT

- **Dry heat** can be applied in form of red heat. In red heat we hold objects in flame until it is red hot. It is used for scalpels, points of needle, forceps etc.
- **Flaming** is also used and objects are exposed for few seconds to flame.
- In **hot air oven**, we apply 160°C for 2 hours. Hot air oven is used for sterilization of surgical instruments like scissors, scalpel, dry glassware, knives, oils and fats.

Dry heat will cause damage to cells by oxidation and denaturation of proteins.

Moist heat is generally more effective than dry heat for killing microorganisms because of its ability to penetrate microbial cells. Moist heat kills microorganisms by denaturing their proteins (causes proteins and enzymes to lose their three-dimensional functional shape).

1. BOILING

Temperature is raised to 100°C. object is placed in boiling water. This will cause killing of all pathogenic micro-organisms but not affect spores. Purification of water for drinking purposes is also obtained by boiling.

2. PASTEURIZATION

Temperature is less than 100°C and there are three methods of pasteurization.

- **BATCH METHOD**: heat applied is 62°C for 30 mins.
- **FLASH METHOD**: heat applied is 72°C for 15 sec and then cooled rapidly. It is used for milk, baverages. Flash method is more effective because it doesnot affect flavor and maintain nutritional value.
- **ULTRA PASTEURIZATION**: exposure at 82°C for 3 seconds.

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3. AUTOCLAVING

Steam is applied at pressure of 15Ib/in^2 for 15 minutes and temperature is 121°C

Bacterial spores are resistant to boiling (100°C). As water boils at 100°C at normal atmospheric pressure (sea level), when the pressure is increased from normal level, water will boil at temperature higher than 100°C in a closed container. This high temperature of steam kills even highly resistant spores of *Clostridium botulinum.* Temperature of 121°C cause sterilization in 15-20 mins, 126°C cause sterilization in 10 min and 134°C for those that donot withstand autoclave temperature for a long time.

Characteristics of steam in autoclaving:

- Free from air
- Temperature is near condensation point
- No suspended water droplets

4. TYNDALIZATION

Tyndalization is intermittent steaming for 3 consecutive days at 100°C for 30 min. it is used for sterilization of sensitive culture medias that cannot withstand autoclave temperature for longer time.

FACTORS AFFECTING STERILIZATION BY HEAT

1. Time and temperature: At higher temperature, less time will be required.
2. Number of micro-organisms and spores: At higher degree of contamination, more time for sterilization is required. Organisms producing spores are heat resistant.
3. Species strain and spore forming ability: Different duration is required for different species e.g *Staphylococci* and *N-gonorrhea* are killed at 50-62°C in 10 min. Polio virus is killed at 60°C for 30 min. Hepatitis virus are killed at 100°C after 10 min, other strains may require more time at the same temperature.
4. Organic material: Organisms heated in high organic media require more time for being killed.
5. pH of media: At neutral pH, organisms are resistant to heat and so the media should be acidic or basic.

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FILTRATION

It is a method for removing microbes from fluid or air by using mechanical porous devices, known as filter papers. The fluids are passed through two types of filter paper. Most common is nitrocellulose filter, having pore with size of 0.22µm.

USES:

- For sterilization of IV solutions, pharmaceuticals and baverages.
- For sterilization of sera (plural of serum), anti-sera, anti-toxins etc
- For sterilization of air and water

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RADIATION

There are two types of radiations:

1. **Non-ionizing radiations**: In this type we have uv and infrared rays. They cause damage to the formation of thymine dimer of DNA of animals. Thymine dimer leads to inhibition of replication. They do not affect spores of bacteria.
2. **Ionizing Radiations**: They are more powerful and form hydroxyl free radicals due to hydrolysis of water, which damages cells. They have more penetrating power e.g beta rays, X-rays and gamma rays.

CHEMICAL METHODS:

Different types of chemicals have property of rapid onset of action. There are many chemicals used for this purpose. They should have following properties:

- They should be non-toxic and non-irritant.
- They should have a wide range of safety and storing activity against gram positive and gram negative bacteria.
- They should not get inactivated by organic matter.

They are divided into three main groups:

1. Chemicals causing damage to cell membrane
2. Chemicals causing modification of protein
3. Chemicals causing modification of nucleic acids

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CHEMICALS CAUSING DAMAGE TO CELL MEMBRANE

ALCOHOL

It is used as ethanol, 70% solution of isopropyl alcohol is also used but it is toxic. It is used as skin antiseptic.

DETERGENTS

They act as surface active agents e.g. benzalkonium chloride (skin antiseptic). They are not effective against gram negative bacteria.

PHENOL AND DERIVATIVES

They include:

- Cresol which include Lysol, sudol, steriol, cleurosal
- Chloroxylenol (Dettol)
- Chlorohexidine (habitane)
- Hexachlorophene

CHEMICALS CAUSING MODIFICATION OF PROTEIN

These proteins may be structural or enzymatic proteins which are affected by these chemicals.

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HALOGENS

Iodine (oxidant), povidine iodine, tincture iodine (2% iodine + K iodine in ethanol). They are used as skin antiseptic

Chlorides: chlorine (powerful oxidant) used in water supply and swimming pools. They are also used as bleaching powder as hypochlorate (bbleach, Clorox)

HEAVY METALS

Silver: silver sulphadizaine is used in burn wounds and silver nitrate drops are used to prevent gonococcal ophthalmia neonatorum.

Mercury: it is used as skin antiseptic in the form of thiomerosal (merthiolate) and merbromin (mercurochrome).

OXIDIZING AGENTS

They include:

- Hydrogen peroxide, which is also sporicidal
- Potassium permanganate
- Aldehydes which may be formalin or glutaraldehyde. Glutaraldehyde is sporicidal.

GASEOUS DISINFECTANTS

- Formaldehyde but it is irritant
- Ethylene oxide which is highly inflammable. It is mainly used for sterilization of plastic material.

CHEMICALS CAUSING MODIFICATION OF NUCLEIC ACIDS

They mainly contain dyes including acridine dyes and aniline dyes

USES OF STERILIZATION

- Culture media, suspending fluids, reagents for use in microbiology.
- Medical and surgical instruments.
- Decontamination of objects before disposal or re-use.
- Pre-operative disinfection of skin
- Fumigation of a room, airplanes, ships, operating theatre etc.

STERILIZATION METHODS FOR DIFFERENT OBJECTS

1. Dressing, gloves, surgical instruments – Autoclave
2. Rubber and plastic catheters – Boiling
3. Plastic syringe – ionizing radiation
4. Thermometer – iodine or phenolic compounds
5. Hospital rooms/ OT – hot formalin vapors
6. Skin – tincture iodine
7. Culture media – autoclave or tyndalization
8. Cannulas – ionizing radiation
9. Milk – pasteurization

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10. Water supply – filtration and treated with hypochloride

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