Microbiology

Sterilization / Disinfection; Disposal of Biomedical wastes

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Sterilization:

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

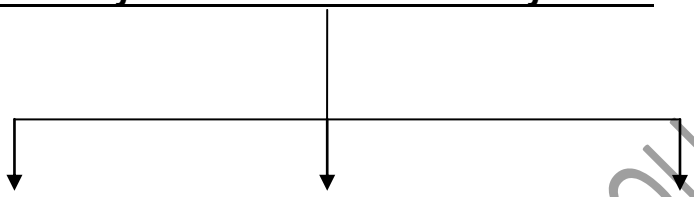
Disinfection:

Disinfection is the killing, inhibition or removal of microorganisms that may cause disease.

Antiseptics:

Antiseptics are chemical agents applied to tissue to prevent infection by killing or inhibiting pathogen growth.

Methods Of Sterilization And Disinfection



Mechanical

i. Scrubbing

ii. Filtration

iii. Sedimentation

Physical

i. Sunlight

ii. Drying

iii. Radiation

iv. Ultrasonic / Sonic vibrations

v. Heat

a) Dry heat

1. Incineration

2. Red heat

Chemical

i. Agents that damage the Cell membranes :

a) Surface Active Disinfectants

b) Alcohol

c) Phenolic compounds

ii. Agents that damage Proteins

a) Acids and Alkalies

b) Alcohols

iii. Agents that modify

3. Hot Air - Sterilizer

4. Microwave Oven

b) Moist Heat

1. Pasteurization

2. Boiling

3. Steam under - Pressure

functional groups of Proteins and Nucleic Acids

a) Heavy Metals

b) Oxidizing Agents

c) Dyes

d) Alkylating Agents

MECHANICAL METHOD:

1. Scrubbing:

It is useful in cleaning of hands, cleaning of skin, scrubbing of hands in operation theathre, cleaning of floor, cleaning of clothed etc.

2. Filtration:

It is used in disinfection of water, disinfection of Culture media (solutions of sugar or antibiotics used for preparation of culture media), disinfection of laboratory liquids (isolation of organisms which are scanty in fluids).

3. Sedimentation:

It is used in purifying water.

PHYSICAL METHODS:

1. Sunlight:

Sunlight has appreciable bactericidal activity. It is a natural method of sterilization in cases of water in tanks, rivers and lakes. It is also used in sterilization of clothes, blankets, mattress etc.

2. Drying:

Drying in air has a deleterious effect on many bacteria.

3. Heat:

It is most reliable and universally applicable method of sterilization. Either dry or moist heat may be applied.

MECHANISM OF ACTION:

I. Dry Heat:

The lethal effect of dry heat or desiccation is usually due to:

- Protein denaturation
- Oxidative damage etc.

II. Moist Heat:

Microorganisms are killed by :

- Coagulation
- Denaturation of their enzymes and structural proteins.

I. Dry Heat Sterilization:

i. Red Heat:

Inoculating wire loops and points of forceps are sterilized by holding them almost vertically on a bunsen flame red heat.

ii. Flaming:

Scapel blades, glass slides, mouth of culture tubes and bottles are exposed to a flame for few seconds.

iii. Incineration:

By this method, infective material is reduced to ashes such as surgical dressings, contaminated materials and other clinical wastes are destroyed by incineration.

iv. Hot Air Oven:

Hot air sterilizer is the most widely used method of sterilization by dry heat. It is used to process materials which can withstand high temperatures for long period of time. Hot air oven is electrically heated. It should be fitted with a fan to provide forced air circulation throughout the oven chamber, a temperature indicator and a timer.

Sterilization Cycle:

The sterilization hold time is set to :

160^{o}C for 2 hours

or

170^{o}C for 1 hour

or

180^{o}C for 30 minutes.

Uses of hot air oven:

It is a method of choice for sterilization of:

- ✓ **Glassware** such as tubes, flasks, glass pipettes, glass petridishes, all - glass syringes etc.
- ✓ **Metal instruments** such as forceps, scissors and scalpels.
- ✓ **Non - Aqueous materials** , powders, oils and greases.

Precautions for handling hot air oven:

- ✓ Hot air oven must be fitted with fan to ensure distribution of hot air.
- ✓ It should not be overloaded.
- ✓ Articles must be completely dry.
- ✓ Oven must be allowed to cool before opening.

II.MOIST HEAT:

Moist heat is divided into 3 forms:

1. At temperature below 100^{0}C. Example: Pasteurization

2. At temperature of 100^0C. Example:
   - i. Boiling
   - ii. Tyndallization

3. At temperature above 100^0C. Example: Autoclave

Pasteurization of Milk:

Disinfection by moist heat at temperature below 100^0C is termed as Pasteurization.

Milk can be Pasteurized in 2 ways:

The Holder Method:

Temperature is employed at 63^0C for 30 minutes.

The Flash Method:

Temperature is employed at 72^0C for 15 to 20 minutes.

Both of these methods are followed by rapid cooling to 13^0C.

Pasteurization is also used for sterilization of Endoscopic Instruments, Vaccine Preparation etc.

Boiling:

Boiling at 100^0C for 10 to 30 minutes kills all vegetative spores and some bacterial spores.

Boiling is used in sterilization of needles, stringer etc; as it destructs pathogenic bacteria, virus and fungi.

Important points which are to be applied during boiling:

- ✓ Articles must be fully immersed in water.
- ✓ Do not add or remove any article during boiling process.
- ✓ Keep the boilers cover with lid.
- ✓ Rubber items should not be boiled for more than 5 minutes.

Tyndallization:

An exposure of steam at 100^0C for 20 minutes on 3 successive days is called ***Tyndallization / Intermitted Sterilization.*** This is Fractional method of sterilization. The instrument commonly used is Koch and Arnold Steamer.

Principle:

Vegetative Cells and some spore cells are killed during Ist heating. The more resistant spore cells germinate and are killed during 2nd or 3rd heating.

Uses:

This method is useful in sterilizing heat sensitive culture media containing such materials as carbohydrates, egg or serum which are damaged by high temperature of autoclave.

Steam Under Pressure (Autoclave):

Steam above 100^0C or Saturated Steam is more efficient sterilizing agent than hot air.

Autoclave is the process of sterilization by saturated steam under high pressure above 100^oC.

Steam sterilization is carried out in a pressure chamber called an autoclave (a device somewhat like a fancy pressure cooker).

PRINCIPLE OF AUTOCLAVE:

The principle of autoclave or steam sterilizer is that H_2O boils when its vapour pressure equals that of surrounding atmosphere.

When pressure inside a closed vessel increases, the temperature at which water boils also increases.

Saturated steam has penetrative power and is a better sterilizing agent than heat.

Steam condenses to water. The H_2O of condensation ensures moist conditions for killing of the exposed micro-organisms.

PROCEDURE:

1. Water: Sufficient H_2O is put in the cylinder (vertical or horizontal cylinder of Gun metal or stainless steel, in supporting sheet iron case.)

2. Lid: The lid is screwed tight with the discharge tap open and the safety valve is adjusted to the required pressure.

3. Air removal: The steam-air mixture is allowed to escape freely till all the air has been displaced. The discharge tap is now closed.

4. Holding period: The steam pressure rises inside and when it reaches the desired set level (15psi), the safety valve opens and the excess steam escapes. From this point, the holding period (15 minutes) is calculated.

6. Autoclave cooling: When the holding period is over, the heater is turned off and the autoclave is allowed to cool.

7. Air entry in the autoclave: The discharge tap is opened slowly and air is allowed to enter the autoclave.

8. Removal of articles: The lid is now opened and the sterilized articles are removed.

USES OF AUTOCLAVE:

i) For sterilization of culture media, aqueous solutions, dressing materials, gowns, gloves etc.

ii) Particularly useful for materials which can't withstand high temperature of hot air oven.

iii) For objects which are wettable.

RADIATONS:

Two types of radiations are used:

a) Non ionizing Radiations:

They include infra- red and UV rays. UV light is lethal and has mutagenic effect. UV radiations can be produced artificially by MECURY VAPOUR LAMPS. They are used to disinfect drinking water and for disinfection of enclosed areas such as operation theatre, hospital wards, entry ways etc.

b) Ionizing Radiations:

They include X- rays, Gamma-rays and Cosmic rays. Ionizing radiations are highly lethal to all cells including bacterial cells. Ionizing radiations damage DNA by various mechanisms. They are used for:

- Sterilization in pharmacy and medicine.
- Packaged disposal articles such as plastic syringes, gloves etc.
- For antibiotics, hormones, sutures, vaccines and to prevent food spoilage.

CHEMICAL METHODS:

S.NO.	NAME	USES
1.	Phenol → 1 : 100	a) Disinfection of faeces, blood, pus - sputum. b) Disinfection of aluminous material.
	Phenol → 1 : 20	a) Carbolization of operation theatre. b) Cleaning of floor. c) Disinfection of contaminated clothes.
2.	Lysol → 1 : 100	a) Disinfection of clothes ; excreta. b) Cleaning of floor, furniture, walls, rubber etc.
	Lysol → Pure Form	a) Disinfection of sharp instruments. b) Disinfection of contaminated objects.

3.	Dettol	For Dressing during Delivery.
4.	Dyes (Gelatin Violet)	For Dressing of infected wounds, boils and burns.
5.	Acridine compounds, Flavin, Proflaxine	a) Dressing of infectious wounds. b) Dressing of Puncture wounds. c) Highly effective against Streptococcus.
6.	Oxidizing Antiseptics/ Hydrogen Peroxide	a) Cleaning of infected wounds and to remove slough. b) used as Mouth Wash [as bleaching agent]
7.	Alcohols, Acids. [Boric Acid]	a) Mouth Wash / Gargles. b)Fermentation to the eyes.
8.	Alkalies [Sodium Bicarbonate, Soaps and Detergents]	a) Sterilization of water. b) Hand Wash. c) Removal of foreign matter from solid surface.

DISPOSAL OF BIOMEDICAL WASTE:

Definition:

According to ***EPA (Environmental Protection Agency)***, the definition of Medical Waste is fairly broad "all waste materials generated at health care facilities, such as hospitals, clinics, physician's offices, dental practices, blood banks and veterinary hospitals/clinics, as well as medical research facilities and laboratories".

According to ***WHO (World Health Organisation)***,"Any waste which is generated during the diagnosis, treatment or immunization of human beings or animal or research activities pertaining thereto or in production or testing of biological or in health camps, including the categories mentioned in schedule I appended to BMW rules 2016".

The WHO classified the Medical waste into eight (8) categories of medical wastes:

1. Infectious Waste:

Lab cultures, Swabs, Equipment and Excreta.

2. Sharps:

Sharp wastes such as Needles, Scalpels, Knives, Blades etc.

3. Pathological:

Human Tissue or Fluids i.e. body parts, blood or other body fluids.

4. Radioactive:

Unused liquid in radiotherapy or lab research, contaminated glassware, etc.

5. Chemical:

Expired Lab Reagents, Film developer, Disinfectant.

6. Pharmaceuticals:

Expired and Contaminated medicines.

7. Pressurized containers:

Gas cylinders and Gas cartridges.

8. General Waste (UMW):

Office paper, wrapper, kitchen waste, general sweeping etc.

Segregation of waste:

Prior to treatment and disposal of waste, it is important to segregate the infectious waste from non - infectious waste and to collect them in appropriate receptacles.

Proper segregation minimize the waste steam needing special treatment.

This practice helps in safe handling and transportation of wastes.

Colour Coding of bags for segregation of biomedical wastes:

COLOUR	TYPE OF CONTAINER	CATEGORY OF WASTE	WASTE TREATMENT

Yellow	non chlorinated plastic bag	1,3,5	Incineration
Red	puncture proof container for sharps	2,4	Autoclaving
Blue	non chlorinated plastic bag/container	5	Chemical - treatment
Black	non chlorinated plastic bag	8	Disposal in muncipal dump sites

SHARPS
Red Sharps Container

- √ Needles
- √ Ampules
- √ Broken Glass
- √ Blades
- √ Razors
- √ Staples
- √ Trocars
- √ Guide Wires
- √ Other Sharps

BIOHAZARD
Red Container or Red Liner in Container

- √ Infectious Waste
- √ Blood Products (albumin.etc)
- √ Contaminated Personal Protective Equipment (PPE)
- √ IV Tubing
- √ Cultures, Stacks

TRACE CHEMO
Yellow Container

- √ Empty vials, ampules
- √ Empty Syringes, Needles
- √ Empty IVs
- √ Gowns
- √ Gloves
- √ Tubing
- √ Aprons
- √ Wipes
- √ Packaging

RCRA HAZARD
Black Container

- √ Hazardous meds (RCRA)
- √ Half/Partial doses (RCRA)
- √ Hazardous bulk meds
- √ P-listed drugs, packaging
- √ Bulk chemo
- √ Pathological Waste (Incineration Only)

PHARMACEUTICAL
Blue Container

- √ Pills
- √ Injectables
- √ Antibiotics

RADIOACTIVE
Shielded Containers with Radioactive Symbol

- √ Fluorine-18 (F-18). 110 minutes half-life.
- √ Technetium-99 (T-99m). 6 hours half-life.
- √ Iodine-131 (I-131). 8 days half-life.
- √ Strontium-89 (Sr-89). 52 days half-life.
- √ Iridium-192 (Ir-192). 74 days half-life.
- √ Cobalt-60 (Co-60). 53 years half-life.

Medical Waste Management