Sterilization and Disinfection

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Introduction

Sterilization

- Process by which an article, surface or medium is freed of all living microorganisms either in vegetative form or spore state including prions without affecting its quality and quantity
- It results in reduction of at least 10^6 log colony-forming units of microorganisms and their spores
- It can be achieved by a physical agent or a chemical agent

contd

- **Disinfection**

- Process that eliminates most or all disease causing microorganisms ,viruses and bacteria but not usually bacterial spores
- It results in reduction of at least 10^3 log colony-forming units of microorganism, but not spores
- Disinfection can be achieved by a physical agent or a chemical agent (called disinfectant)
- Disinfectants are normally used only on inanimate objects, not on body surfaces

contd

Asepsis

It is a process where the chemical agents (called antiseptics) are applied on to the body surfaces (skin), which kill or inhibit the microorganisms present on skin.

- They prevent the entry of pathogens into sterile tissues and thus prevents infection or sepsis.

- However, they are generally not as toxic as disinfectants as they must not destroy too much of host tissue.

Methods of sterilization/disinfection

Physical methods	
Drying	
Heat	
Dry heat: - Flaming - Incineration - Hot air oven	Moist heat: - Temperature below 100°C, e.g. pasteurization, water bath and inspissation - Temperature at 100°C, e.g. boiling, steaming and tyndallization - Temperature above 100°C, e.g. autoclave
Filtration: Depth filters and membrane filters	
Radiation - Ionizing radiation: γ rays, X-rays and cosmic rays - Nonionizing radiation: Ultraviolet (UV) and infrared rays	
Ultrasonic vibration	
Chemical methods	
Alcohols: Ethyl alcohol, isopropyl alcohol	
Aldehydes: Formaldehyde, glutaraldehyde, Ortho-phthaladehyde	
Phenolic compounds: Cresol, lysol, chlorhexidine, chloroxylenol, hexachlorophene	
Halogens: Chlorine, iodine, iodophors	
Oxidizing agents: Hydrogen peroxide, peracetic acid	
Salts: Mercuric chloride, copper salts	
Surface active agents: Quaternary ammonium compounds and soaps	
Dyes: Aniline dyes and acridine dyes	
Gas sterilization: - Low temperature steam formaldehyde - Ethylene oxide (ETO) - Betapropiolactone (BPL) - Plasma sterilization	

Factors affecting efficacy of sterilizing methods/agents

1. **Organism load:** Larger microbial population requires a longer time to die than a smaller one.
2. **Nature of organisms:** It greatly influences the efficacy of the disinfectants or sterilizing agents.

The decreasing order of resistance of microorganisms to disinfectant or sterilizing agents is as follows—

Prions (highest resistance) > *Cryptosporidium* oocysts > Bacterial spores > Mycobacteria > Other parasite cysts (e.g. *Giardia*) > Small non-enveloped viruses > Trophozoites > Gram-negative bacteria > Fungi > Large non-enveloped viruses > Gram-positive bacteria > Enveloped viruses.

3. **Concentration** of the chemical agent or the temperature of heat sterilization.
4. **Nature of the sterilant/disinfectant such as its:**
   - Microbicidal ability
   - Rapidity of action
   - Ability to act in presence of organic matter
   - Residual activity

contd

5. **Duration of exposure:** More is the exposure time to sterilant/disinfectant, better is the efficacy.
6. **Temperature:** An increase in the temperature at which a chemical acts often enhances its activity. A lower concentration of disinfectant or sterilizing agent can be used at a higher temperature.
7. **Local environment:** The microorganisms to be controlled are not isolated but surrounded by environmental factors that may either offer protection or aid in its destruction, e.g.
   - **pH:** Heat kills more readily at an acidic pH.
   - **Organic matter:** Presence of organic matter, such as pus, blood, and stool can protect the organisms against heating and chemical agents.
     - It may be necessary to mechanically clean an object (e.g. syringes and medical equipments) before it is disinfected or sterilized.
     - Materials containing organic substances require more time or volume or concentration of chemical agent for sterilization/disinfection. More chlorine must be added to disinfect drinking water if a city's water supply has a high content of organic material.
   - **Biofilm:** Formation of biofilm is another mechanism which prevents the entry of disinfectants to act on the microorganisms which are embedded inside the biofilm.

Ideal antiseptic or disinfectant

- Wide spectrum of activity
- Able to destroy microbes within practical period of time
- Active in the presence of organic matter
- Make effective contact Active in any pH
- Stable and have long shelf life
- High penetrating power
- Non-toxic, non-allergenic, non-irritative or non-corrosive
- No bad odour
- Should not leave non-volatile residue or stain
- Efficacy should not be lost on reasonable dilution
- Should not be expensive and must be available easily

Dry Heat

Principle

- Kills the microorganisms by:

➢ **Denaturation of proteins**

➢ **Promoting oxidative destruction of essential cell constituents**

➢ **Toxic effects of elevated levels of electrolytes.**

contd

1.Red Heat:

- Inoculating wires loops and points of forceps are sterilized by holding them almost vertically in a Bunsen burner until red hot along their whole length

2. Flaming

- Scalpels, mouth of test tubes, culture bottles are sterilized by passing through Bunsen flame without allowing them to become red hot
- Method is limited to those articles that can be exposed to flame

contd

3.Incineration

- high-temperature dry oxidation process
- Waste converted into ashes, flue gas and heat
- Two heating chambers: one set at 1100°C and other at 800°C
- Pathological waste, surgical dressings, contaminated material, animal carcasses and other clinical waste are safely destroyed

- Incineration should not be done for:

- Reactive chemical waste
- Halogenated plastics
- Radiographic wastes

4. Hot air oven

- Comprises of :

✓Heating chamber: electrically heated

✓Thermostat: maintains the chamber air at a chosen temperature

✓Timer

✓Temperature indicator

✓Fan: ensures even distribution of hot air

Different temperature-time relations for holding time

1) 60 minutes at 160°C
2) 40 minutes at 170°C
3) 30 minutes at 180°C

Contd Materials to be sterilized

- Glassware like glass syringes, petri dishes, flasks,
- Surgical instruments like scalpel, forceps
- Chemicals such as liquid paraffin, glycerol

- Control of hot air oven:
- Chemical- Brownes tape
- Biological control:

✓spores of non toxigenic strain of *Clostridium tetani*

Moist heat

1. **Moist heat at a temperature below 100°C**

a. Pasteurization

b. Water bath

c. Inspissation (fractional sterilization)

2**. Moist heat at 100°C**

a. Boiling

b. Steaming

c. Tyndallization or intermittent sterilization

contd

3. **Moist heat at temperature above 100°C**

a. Autoclave

Mechanism of inactivation by moist heat

- Damaging the outer membrane
- RNA breakdown
- Protein coagulation
- Damage to bacterial chromosome

Pasteurization

- **Pasteurization:** It is method used for control of microorganisms from beverages like fruit and vegetable juices, beer, and dairy products, such as milk.
  - Two methods are available–Holder method (63°C for 30 minutes) and Flash method (72°C for 20 seconds followed by rapid cooling to 13° or lower).
  - All nonsporing pathogens, including mycobacteria, brucellae and salmonellae are killed except *Coxiella burnetii* which being relatively heat resistant may survive in holder method.

Water bath

- ➢Method of sterilization by moist heat below 100°C
- ➢Kills microorganisms by denaturation and coagulation of proteins
- ➢used for disinfection of serum, body fluids and vaccines
- ❖Bacterial vaccines: 60°C for 1 hour
- ❖Serum or heat labile body fluids: 56°C for one hour.

Inspissation

Inspissation (fractional sterilization): It is a process of heating an article on 3 successive days at **80–85°C for 30 minutes** by a special instrument called inspissator

- Working principle: In inspissator, the first exposure kills all the vegetative forms, and in the intervals between the heatings the remaining spores germinate into vegetative forms which are then killed on subsequent heating.
- Uses: Inspissation is useful for sterilization of egg and serum based media which generally get destroyed at higher temperature.
  - Egg based media—e.g. Lowenstein-Jensen medium and Dorset's egg medium.
  - Serum based media—e.g. Loeffler's serum slope.

Boiling

- **Boiling:** Boiling of the items in water for 15 minutes may kill most of the vegetative forms but not the spores, hence not suitable for sterilziation of surgical instruments. Though boiling is a simple, easily available option to most people; however, boiling can be hazardous and not effective; hence should not be used if better methods are feasible.

Steaming

Steaming: Koch's or Arnold's steam sterilizer are useful for those media which are decomposed at high temperature of autoclave.

- The articles are kept on a perforated tray through which steam can pass.
- They are exposed to steam (100°C) at atmospheric pressure for 90 minute.
- Most of the vegetative forms are killed by this method except thermophiles and spores.

Tyndallization

Tyndallization or intermittent sterilization (named after John Tyndall) involves steaming at 100°C for 20 minutes for 3 consecutive days.

- The principle is similar to that of inspissation, except that here, the temperature provided is 100°C, instead of 80°C.
- It is used for sterilization of gelatin and egg, serum or sugar containing media, which are damaged at higher temperature of autoclave.

Autoclave

❖Sterilization by saturated steam under high pressure(boiling point of water increases when pressure increases)

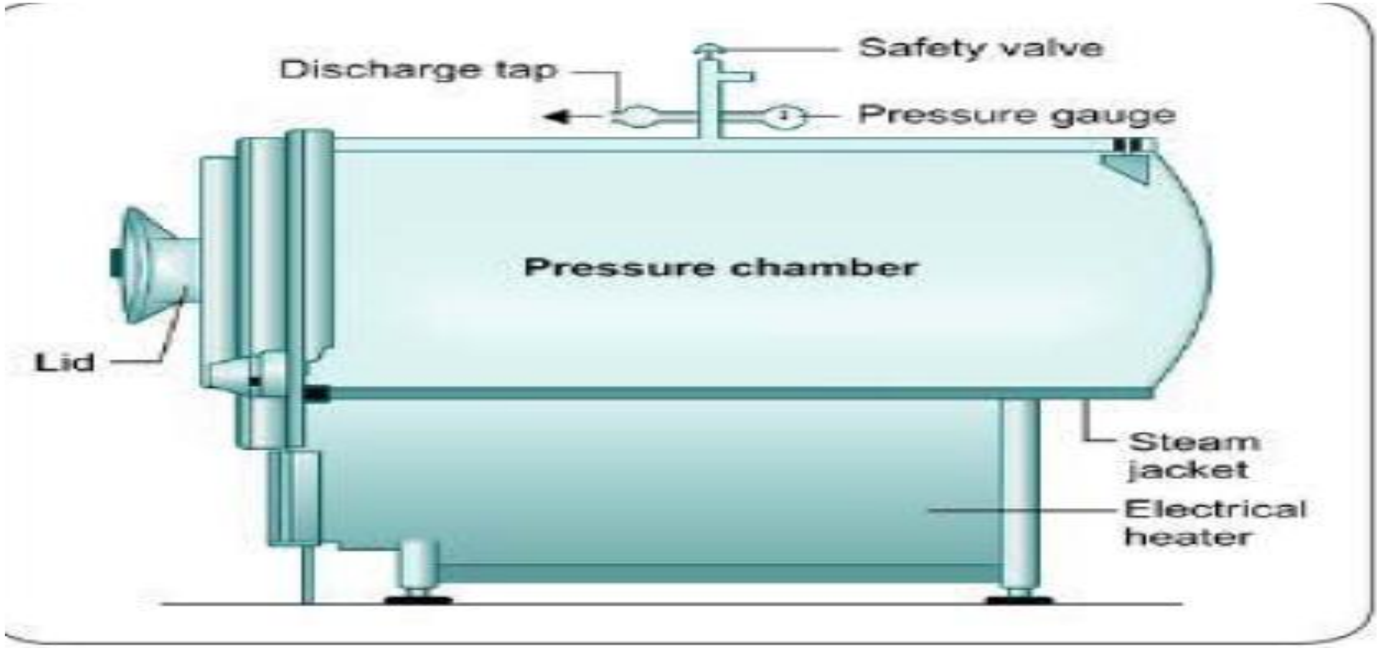

Fig. 3.4: Schematic diagram of horizontal autoclave

Procedure

- The cylinder is filled with sufficient water
- Material to be sterilized is placed inside the pressure chamber
- Lid is closed
- Electrical heater is put on
- Safety valve is adjusted to required pressure

❖Sterilize at 121°C in 15minutes. Pressure is 15lbs

❖Materials that can be autoclaved :Surgical instruments, Culture media, Autoclavable plastic containers, Biohazardous waste

Control for autoclave

- Browne's tube
- Spores of Geo*bacillus stearothermophilus*
- Autoclave tapes
- Thermocouple

Filtration

- Does not kill microbes, it separates them out.
- Membrane filters with pore sizes 0.2-0.45 μm are commonly used .

❖ Types of filter:

1. **Earthenware filters**
2. **Asbestos filter**
3. **Sintered glass filter**
4. **Membrane filter**

contd

Applications

- Removing bacteria from ingredients of culture media.
- Preparing suspensions of viruses and phages free of bacteria.
- Used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions, urea solution
- Coliform count for water quality testing

Filtration of air

Air filters are used to deliver bacteria-free air. Air can be filtered by various methods:

- Surgical masks (that allow air in but keep microorganisms out) are the simplest examples.
- There are two important air filters that are used in biological safety cabinets and laminar airflow systems):
    - **HEPA filters** (High-efficiency particulate air filters): HEPA filter removes 99.97% of particles that have a size of 0.3 μm or more.
    - **ULPA filters** (Ultra-low particulate/penetration air): An ULPA filter can remove from the air at least 99.999% of dust, pollen, mold, bacteria and any airborne particles with a size of 0.12 μm or larger.

Radiation

- **Radiation does not generate heat, so termed "cold sterilization"**
- Two types of radiations are available

1)Ionizing: UV rays and infrared rays

- UV rays induce formation of thymine-thymine dimers (DNA), which ultimately inhibits DNA replication
- Rays are employed to disinfect hospital wards, operation theatres, virus laboratories, corridors, etc.

2)Nonionizing: Gamma rays

Non-ionizing radiation

- High-energy radiations damage the nucleic acid of the microorganism.
- Used commercially to sterilize disposable petridishes, plastic syringes, antibiotics, vitamins, hormones, glasswares and fabrics
- Irradiation of food (permitted in some countries)
- *Bacillus pumilus* is used to evaluate sterilization process.

Classification of disinfectants

1) High-level disinfectants are chemical sterilants that may be used for a shorter exposure period than would be required for sterilization to kill all microorganisms with the exception of high numbers of bacterial spores.

- Used for items involved with invasive procedures that cannot withstand sterilization procedures (e.g., certain types of endoscopes and surgical instruments with plastic
- Example: glutaraldehyde, hydrogen peroxide, peracetic acid, and chlorine compounds

contd

2) Intermediate-level disinfectants will kill mycobacteria (causes of tuberculosis and leprosy), vegetative bacteria, most viruses (such as poliovirus), and most fungi but do not kill all bacterial spores.

- Used to clean surfaces or instruments where contamination with bacterial spores and other highly resilient organisms is unlikely
- Example: alcohols, iodophor compounds, phenolic compounds

contd

3) Low-level disinfectants kill most vegetative bacteria, some fungi, and some viruses

- Quaternary ammonium compounds are used to treat noncritical instruments and devices

4)Chemical sterilants are those that kills all forms of life. Eg: formaldehyde, glutaraldehyde

Alcohols

Mode of action:

- dehydrate cells
- disrupt membranes
- coagulation of protein.

Examples: Ethyl alcohol, isopropyl alcohol and methyl alcohol

Application:

- Isopropyl alcohol: clinical thermometers
- 70% ethyl alcohol(spirit) is used as antiseptic on skin.

Aldehydes

Mode of action:

- Acts through alkylation of amino-, carboxyl- or hydroxyl group
- Kills all microorganisms, including spores
- Examples: Formaldehyde, Glutaraldehyde

Formaldehyde

- 40% Formaldehyde (formalin) is used for surface disinfection and fumigation of rooms, chambers, operation theatres,wards etc.
- Preservation of anatomical specimen
- Preparation of toxoid from toxin

❖Glutaraldehyde:

➢used to sterilize instruments like endoscope, cystoscope

➢Used at 2% concentration(called cidex)

➢Kills in 20 minutes but may take as long as 12hrs to kill spores

Phenol and phenolic compounds

Mode of action: Act by disruption of membranes, precipitation of proteins and inactivation of enzymes

- Are bactericidal, fungicidal, mycobactericidal but are inactive against spores and most viruses.
- Antiseptics: chlorhexidine, chloroxylenol
- Disinfectants: Lysol, cresol(Used for disinfection of ward floors, in discarding jars in laboratories and disinfection of bedpans)

Halogens

Mode of action: cause damage by oxidation of essential sulfydryl groups of enzymes

1) Chlorine compounds(sodium hypochlorite, calcium hypochlorite): used in municipal water supplies and swimming pool, laboratory disinfectant
2) Iodine compounds(tincture of iodine and povidone iodine): used as antiseptics

Heavy metals

Mode of action:

 Act by precipitation of proteins and oxidation of sulfydryl groups.

- Copper sulphate is an effective fungicide (algicide) in lakes and swimming pools
- Thiomersal (merthiolate) is used as preservative in vaccines, sera and other immunoglobulin preparations.

Surfactants

- Quaternary ammonium compounds are the most popular cationic surfactant
- Disrupt microbial membranes and may also denature proteins
- Disinfectants for food utensils and small instruments and as skin

antiseptics.

- Examples of quaternary ammonium are cetrimide, benzalkonium cloride

Hydrogen peroxide

- **Mode of action:**
- Hydrogen peroxide produces hydroxyl-free radical that damages proteins and DNA.
- **Application:** is used at 6% concentration to decontaminate the instruments, equipments such as ventilators

Ethylene oxide

- MOA- alkylation of protein, DNA, and RNA
- Used in combination with carbon dioxide
- Rapidly penetrates packing materials, even plastic wraps
- Ethylene oxide sterilizer required
- Used to sterilize heat labile articles such as bedding, textiles, rubber, plastics, syringes, disposable petri dishes, respiratory and dental equipments.
- *B.globigi* is the recommended biological indicator

Beta propiolactone

- **Mode of action:** An alkylating agent and acts through alkylation of carboxyl- and hydroxyl- groups.
- **Used** to sterilize vaccines, tissue grafts, surgical instruments and enzymes

Plasma Sterilization

Plasma sterilization is a recently introduced sterilization method; increasingly used now a days.

- **Principle:** Plasma refers to a gaseous state consisting of ions, photons and free electrons and neutral uncharged particles (such as O and OH). These active agents present in plasma such as photons of ultraviolet rays and radicals (e.g. O and OH) are capable of killing microorganisms and spores efficiently.
- **Plasma sterilizers:** It is a special device use to create the plasma state (commercial brands, such as Sterrad and Plazlyte). They maintain a uniform vacuum inside the chamber.
- Chemical sterilants, such as H_2O_2 alone or a mixture of H_2O_2 and peracetic acid are used which provide O and OH.
- Low temperature is maintained (<50°C) throughout the process which preserves the integrity of heat labile items.
- It is used for sterilization of surgical instruments.
- **Sterilization control:** Efficacy of plasma sterilization is tested by using *Bacillus stearothermophilus, Bacillus subtilis* subsp. *niger*.