Methods of Sterilization and Disinfection

Veterinary Microbiology (Unit-1)

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Terms:

- Sterilization: Article, surface, or medium is freed of all living microorganisms either in the vegetative or in the spore state

- Used only in absolute sense

- Disinfection: Destruction of microorganisms, especially potential pathogens, on the surfaces of inanimate objects or in the environment

- Reduce the microbial population, not bacterial endospores on inanimate surfaces or in organic materials

- Antisepsis: Destruction or inhibition of microorganisms on living tissues by chemicals (non-toxic and non-irritating)

- Chemical agents- Antiseptics

- **Germicide/microbicide:** chemical agent that kills pathogenic microorganisms
- **Sepsis:** growth of microorganisms in the body or the presence of microbial toxins in blood and other tissues
- **Asepsis:** Practice to prevent entry of infectious agents into sterile tissues and thus prevents infection
- **Sanitization*:*** Cleansing technique that mechanically removes microorganisms to reduce the level of contaminants
- Sanitizer- compound (e.g., soap or detergent)

Uses of Sterilization

- 1. Sterilization for Surgical Procedures and medicines: Gloves, aprons, surgical instruments, syringes, drugs and other supplies etc.

  2. Sterilization in Microbiological works: Preparation of culture media, reagents and equipments

METHODS

Sterilization and disinfection are done by :

(A). Physical methods

(B). Chemical methods

(a) Heat:

(i) Dry heat

(ii) Moist heat

(b) Radiation:

(i) Non-ionising radiation (Ultraviolet radiation)

(ii) Ionising radiation (X-ray, gamma ray)

(c) Filtration

(Berkfeld, Chamber land, Seitz, sintered glass, cellulose membrane filters etc)

Heat

- Mostly used method
- Highly effective and most reliable process
- Two major methods:
- Dry heat and moist heat
- Dry heat induces:
- Denaturation of protein, oxidative damage and toxic effect due to the high level of electrolytes
- Also damage the DNA of the microorganism
- As a result, the microorganism got killed

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- ## Moist Heat:

kills the microorganisms by denaturation and coagulation of proteins

- Temperature required to kill microbe by dry heat more than moist heat

- Thermal death time-

Minimum time required to kill a suspension of organisms at a predetermined temperature in a specified environment

Factors affecting sterilization by heat

- Nature of heat: Moist heat is more effective
- Temperature and time: inversely proportional
- Number of microorganisms: More number- higher temperature or longer duration
- Nature of microorganism: Species and strain, Spores highly resistant
- Type of material: heavily contaminated, higher temperature/prolonged exposure
- Certain heat sensitive articles sterilized at lower temperature
- Presence of organic material: Organic materials (protein, sugars, oils and fats) increase the time required

DRY HEAT:

- Red heat:
- sterilized by holding them in Bunsen flame till they become red hot
- bacteriological loops, straight wires, tips of forceps and searing spatulas
- limited to those articles that can be heated to redness in flame
- Flaming:
- method of passing the article over a Bunsen flame, but not heating it to redness
- e.g. scalpels, mouth of test tubes, flasks, glass slides and cover

(Image source-Google)

Incineration:

- Method of destroying contaminated material by burning them in incinerator
- safely destroying infective materials by burning them to ashes
- e.g. soiled dressings; animal carcasses, pathological material, bedding
- Suitable only for those articles to be disposed

(Image source-Google)

Hot air oven:

- Introduced by Louis Pasteur
- Metallic instruments (like forceps, scalpels, scissors)
- Glasswares (such as petri-dishes, pipettes, flasks, all-glass syringes)
- Swabs, oils, grease, petroleum jelly and some pharmaceutical products
- Unsuitable for rubber and plastics

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- Exposed to high temperature in an electrically heated oven
- Air poor conductor of heat, even distribution of heat throughout the chamber by a fan
- fitted with a thermostat control, temperature indicator, meshed shelves or trays
- Oven not overloaded
- Materials perfectly dry and arranged to allows free circulation of air inside the chamber
- Mouths of flasks, test tubes and both ends of pipettes must be plugged with cotton
- Petri dishes and pipettes wrapped in a paper

- 160^0C for two hours, 170^0C for 1 hour and 180^0C for 30 minutes

- Increasing temperature by 10 degrees shortens the sterilizing time by 50 percent

- The hot air oven must not be opened until the temperature inside has fallen below 60^o C to prevent breakage of glassware

- To determine the efficacy of sterilization

\- Thermocouples, chemical indicators, and bacteriological spores of *Bacillus subtilis* as sterilization controls

Infra red rays:

- Sterilization by generation of heat
- Articles placed in a moving conveyer belt and passed through a tunnel that is heated by infrared radiators to a temperature of 180^{o} C
- The articles are exposed to that temperature for a period of 7.5 minutes
- Articles sterilized: metallic instruments and glassware
- Requires special equipment
- Efficiency can be checked using Browne’s tube No.4 (blue spot)

Sterilization by moist heat

- In the form of
- hot water
- boiling water
- steam (vaporized water)

- In practice, the temperature of moist heat usually ranges from 60 to 135°C

- Adjustment of pressure in a closed container- regulate the temperature of steam

- kills microorganisms by denaturation and coagulation of proteins

- Sterilization by moist heat:
- temperature <100°C
- temperature of 100°C
- temperature >100°C

At temperature below 100° C

- **Pasteurization:**
- originally employed by Louis Pasteur
- employed in food and dairy industry
- Two methods of pasteurization
- holder method (heated at 63° C for 30 minutes)
- flash method (heated at 72° C for 15 seconds) followed by quickly cooling to 13° C
- Suitable to destroy most milk borne pathogens (Mycobacteria, Streptococci, Staphylococci, Brucella etc.)
- Inactivates most viruses and destroys the vegetative stages of 97–99% of bacteria, fungi, does not kill endospores or thermoduric species
- Efficacy tested by phosphatase test and methylene blue test
- Newer techniques: Ultra-High Temperature (UHT), 134°C for 1–2 second

Vaccine bath:

- The contaminating bacteria in a vaccine preparation can be inactivated by heating in a water bath at 60^{o} C for one hr
- Only vegetative bacteria are killed and spores survive

Serum bath:

- The contaminating bacteria in a serum preparation can be inactivated by heating in a water bath at 56^{o} C for one hour on several successive days
- Proteins in the serum will coagulate at higher temperature
- Only vegetative bacteria are killed and spores survive

Inspissation:

- Egg and serum containing media(Lowenstein- Jensen's; Loeffler's serum)
- Inspissation means stiffening of protein without coagulation
- Placed in an inspissator and heated at 80-85^{o} C for 30 minutes on three successive days
- On the first day, the vegetative bacteria would die and those spores that germinate by next day are then killed the following day
- The process depends on germination of spores in between inspissation

At temperature 100^o C

Boiling:

- Boiling water (100^o C) for 10–30 minutes kills most vegetative bacteria and viruses
- Certain bacterial toxins such as Staphylococcal enterotoxin heat resistant
- Some bacterial spores are resistant to boiling and survive
- Not a substitute for sterilization
- The killing activity can be enhanced by addition of 2% sodium bicarbonate
- Certain metal articles and glasswares disinfected by placing them in boiling water for 10-20 minutes
- The lid of the boiler must not be opened during the period
- Syringes, forceps, scissors etc.

Steam at 100^o C

- Subjected to free steam at 100^o C
- Traditionally Arnold's and Koch's steamers were used
- A steamer is a metal cabinet with perforated trays to hold the articles and a conical lid
- The bottom of steamer is filled with water and heated
- The steam generated sterilizes the articles when exposed for a period of 90 minutes
- Media such as TCBS, DCA and Selenite broth are sterilized by steaming

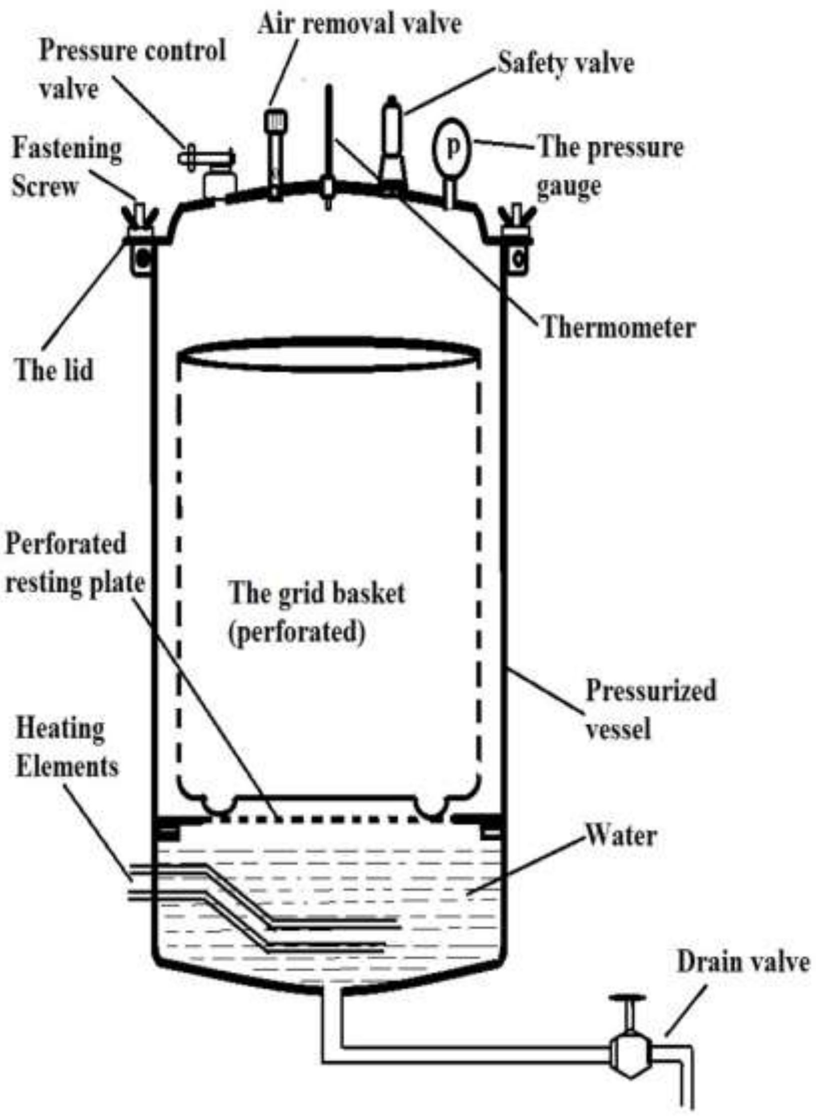
Tyndallisation:

- Heat labile media like those containing sugar, milk, gelatin
- Tyndallisation (after John Tyndall)/ fractional sterilization/ intermittent sterilization
- Steaming at 100°C is done in steam sterilizer for 20 minutes followed by incubation at 37°C overnight
- Repeated for another 2 successive days
- The vegetative bacteria are killed in the first exposure and the spores that germinate by next day are killed in subsequent days
- The success of process depends on the germination of spores

At temperature above 100° C:

- Sterilization by steam under pressure
- When a gas is compressed, its temperature rises in direct relation to the amount of pressure
- 5 psi above normal atmospheric pressure- 109°C
- 10 psi above normal- 115°C
- 15 psi - 121°C
- Pressure–temperature combinations achieved with special device “Autoclave”
- At 15 lb per sq. inch pressure, 121°C temperatures is obtained, kept for 15 minutes for sterilization

Autoclave



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Autoclave

- Vertical or horizontal cylindrical body

- Articles sterilized: Culture media, dressings, certain equipment, linen, rubber (gloves), heat-resistant plastics, liquids etc.

- Ineffective for sterilizing substances that repel moisture (oils, waxes, or powders)

- Kills all the vegetative as well as spore forms of bacteria

- Sterilization controls:

(*a) Thermocouples*

(b) Chemical indicators- Brown's tube No.1 (black spot)

(*c) Bacteriological spores- Bacillus stearothermophilus*

RADIATION:

- Ionizing and non-ionizing

Non-ionizing rays

- low energy rays, poor penetrative power
- Rays of wavelength longer than visible light
- Microbicidal
- wavelength of UV rays: 200-280 nm, 260 nm most effective
- UV rays induce formation of thymine-thymine dimers, ultimately inhibits DNA replication
- UV readily induces mutations in cells
- Don’t kill spores

Uses of UV radiation:

- Disinfection of closed areas in microbiology laboratory, inoculation hoods, laminar flow, and operating theaters
- Harmful to skin and eyes
- Doesn't penetrate glass, paper or plastic
- Of use in surface disinfection
- Generated using a high-pressure mercury vapor lamp

Ionizing rays

- High-energy rays, good penetrative power
- Radiation does not generate heat- "cold sterilization“
- e.g. (*a) X-rays,* (*b) gamma rays, and (c) cosmic rays*
- *Gamma radiation from* cobalt-60 source-

  sterilization of antibiotics, hormones, vitamins, sutures, catheters, animal feeds, metal foils, and plastic disposables, such as syringes, petri dishes
- A dosage of 2.5 megarads kills all bacteria, fungi, viruses and spores
- Also used for meat and other food items
- Damage the nucleic acid of the microorganism

FILTRATION:

- Does not kill microbes, it separates them out
- Membrane filters with pore sizes between 0.2-0.45 µm
- used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions, urea solution
- For removing bacteria from ingredients of culture media, preparing suspensions of viruses and phages
- Aided by using either positive or negative pressure using vacuum pumps
- Older filters made of earthenware or asbestos- depth filters

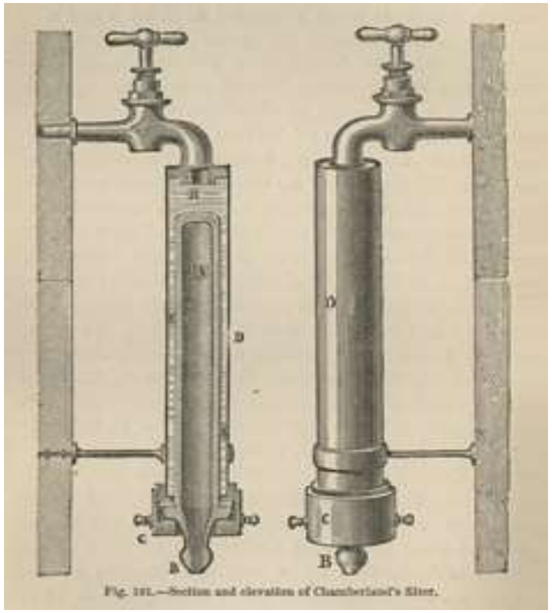
Types of filters

1. **Earthenware filters:**

- Made up of diatomaceous earth or porcelain
- usually baked into the shape of candle

a. Pasteur-Chamberland filter:

- Candle filters from France, of porcelain
- Various porosities, graded as L1, L1a, L2, L3, L5, L7, L9 and L11
- Similar filter from Britain is Doulton; P2, P5 and P11



(Image source: https://www.nlm.nih.gov)

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b. Berkefeld filter:

- made of Kieselguhr, a fossilized diatomaceous earth found in Germany

- Three grades depending on their porosity (pore size)
- V (veil), N (normal) and W (wenig)

c. Mandler filter:

- from America
- made of kieselguhr, asbestos and plaster of Paris

Asbestos filters:

- Made up of asbestos such as magnesium silicate
- Examples- Seitz and Sterimat filters
- Disposable, single-use discs in different grades
- Tend to alkalinize the filtered fluid
- Use limited, carcinogenic potential of asbestos

Sintered glass filters:

- Made up of finely powdered glass particles, which are fused together
- Available in different pore sizes

Membrane filters:

- made up of
- (a) Cellulose acetate
- (b) Cellulose nitrate,
- (c) Polycarbonate
- (d)Polyvinylidene fluoride,
- (e) Other synthetic materials

- Widely used, circular porous membranes, usually 0.1 mm thick

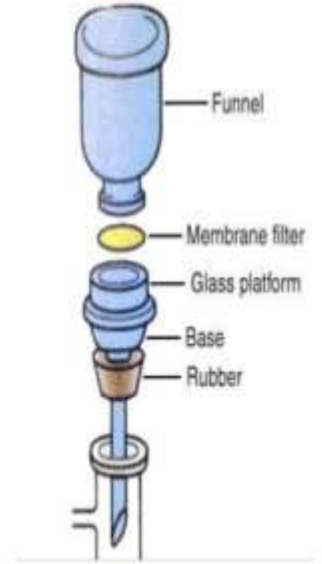
- Variety of pore sizes (0.015–12 µm)

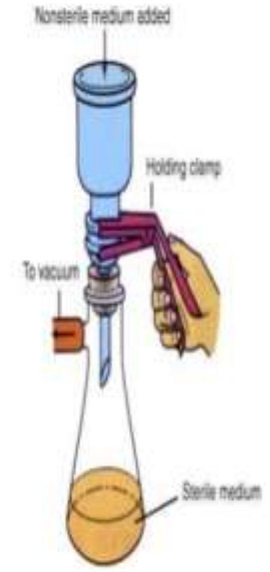
- Membranes with pores about 0.2 µm are used, smaller than the size of bacteria

Uses of membrane filters

- Sterilize pharmaceutical substances, ophthalmic solutions, liquid culture media, oils, antibiotics, and other heat-sensitive solutions
- To obtain bacterial free filtrates of clinical specimens for virus isolation
- To separate toxins and bacteriophages from bacteria

Membrane Filtration





Chemical Methods of Sterilization

- Several chemical agents- antiseptics/ disinfectants

Properties of Ideal Disinfectant

- Wide spectrum of antimicrobial activity
- Should act in the presence of organic matter
- Non-toxic, non-corrosive
- Stable upon storage, no chemical change
- Odorless or with a pleasant odor
- Soluble in water, lipids for penetration into microorganisms
- Effective in acidic as well as in alkaline media
- Speedy action, relatively inexpensive

Action of Disinfectants

- Damage to the cell wall and alter permeability of the cell membrane, resulting in exposure, damage, or loss of the cellular contents
- Alter proteins and form protein salts or cause coagulation of proteins
- Inhibit enzyme action, nucleic acid synthesis or alter nucleic acid molecules
- Cause oxidation or hydrolysis

Factors Influencing Activity of Disinfectants

- Temperature: Increase in temp. increases the efficiency of disinfectants
- Type of microorganism: Vegetative cells more susceptible than spores. Spores may be resistant
- Physiological state of cell: Young and metabolically active cells more sensitive
- Environment: Physical or chemical properties of medium or substance e.g., pH of the medium and presence of extraneous materials

Types of Disinfectants

- (a) phenolic compounds
- (b) halogens
- (c) alcohols
- (d) aldehydes
- (e) gases
- (f) surface active agents
- (g) oxidizing agents
- (h) dyes
- (i) heavy metals
- (j) acids and alkalis

ALCOHOLS:

Mode of action:

- Alcohols dehydrate cells, disrupt membranes and cause coagulation of protein

Examples:

Ethyl alcohol, isopropyl alcohol and methyl alcohol

Application:

- 70% ethyl alcohol (spirit) is used as antiseptic on skin
- Isopropyl alcohol is preferred to ethanol
- Also used to disinfect surfaces
- Used to disinfect clinical thermometers
- Methyl alcohol kills fungal spores, hence is useful in disinfecting inoculation hoods

Disadvantages:

- Skin irritant, volatile (evaporates rapidly), inflammable

ALDEHYDES:

Mode of action:

Acts through alkylation of amino-, carboxyl- or hydroxyl group, damages nucleic acids. It kills all microorganisms, including spores

Examples:

Formaldehyde, Glutaraldehyde

Application:

- Bactericidal, sporicidal, and also effective against viruses
- Can also be used as chemical sterilants

40% Formaldehyde (formalin): for surface disinfection and fumigation

- 10% formalin with 0.5% tetraborate sterilizes clean metal instruments

2% glutaraldehyde

- To disinfect hospital and laboratory equipments
- An exposure of at least 3 hours at alkaline pH is required for action by glutaraldehyde
- Especially effective against tubercle bacilli, fungi, and viruses

PHENOL:

- Mode of action:
- Act by disruption of membranes, precipitation of proteins and inactivation of enzymes
- Examples:
- 5% phenol, 1-5% Cresol, 5% Lysol, hexachlorophene, chlorhexidine, chloroxylenol
- Applications:
- As disinfectants at high concentration and as antiseptics at low concentrations
- Bactericidal, fungicidal, but are inactive against spores and most viruses
- Effective in the presence of organic material and remain active on surfaces long after application

HALOGENS:

- Mode of action:
- Oxidizing agents and cause damage by oxidation of essential sulfydryl groups of enzymes
- Chlorine reacts with water to form hypochlorous acid, microbicidal
- Examples:
- Chlorine compounds (chlorine, bleach, hypochlorite) and iodine compounds (tincture iodine, iodophores)
- Application:
- Effective disinfectants and antiseptics
- Microbicidal
- Also sporicidal with longer exposure

HEAVY METALS:

- Mode of action:
- Act by precipitation of proteins and oxidation of sulfydryl groups
- They are bacteriostatic

- Examples:
- Mercuric chloride, silver nitrate, copper sulfate, organic mercury salts (e.g., mercurochrome, merthiolate)

- Applications:
- Silver compounds as antiseptics
- Silver sulfadiazine for burns
- Silver nitrate in eye infection
- Merthiolate in 1:10000 -preservation of serum
- Copper salts as a fungicide

SURFACE ACTIVE AGENTS:

- Mode of actions:
- Disrupt membrane resulting in leakage of cell constituents

- Examples:
- Soaps or detergents, anionic or cationic
- Anionic detergents- soaps and bile salts

- Cationic detergents are known as quaternary ammonium compounds (or quat)
- Cetrimide and benzalkonium chloride act as cationic detergents

- Application:
- active against vegetative cells, Mycobacteria and enveloped viruses
- As disinfectants at dilution of 1-2% for domestic use and in hospitals

DYES:

- Acridine dyes, Aniline dyes
- Act by interfering with the synthesis of nucleic acids and proteins in bacterial cells
- Acridine dyes such as acriflavin and aminacrine
- More effective against gram positive bacteria than gram negative bacteria and are more bacteriostatic in action
- Aniline dyes (such as gentian violent, crystal violet, and malachite green)
- Also more active against Gram-positive bacteria than against Gram-negative organisms
- Applications:
- Topically as antiseptics, on skin treat bacterial skin infections
- The dyes are used as selective agents in certain selective media

HYDROGEN PEROXIDE:

- Mode of action:
- Release of nascent oxygen, that damages proteins and DNA of microorganisms

- Application:
- 6% concentration to decontaminate the instruments, equipments such as ventilators

- 3% for skin disinfection and deodorising wounds and ulcers

- Strong solutions are sporicidal

BETA-PROPIOLACTONE (BPL):

Mode of action:

- Acts through alkylation of carboxyl- and hydroxyl- groups

Application:

- Effective sporicidal agent, broad-spectrum activity, 0.2% to sterilize biological products

- Carcinogen

ETHYLENE OXIDE (EO):

- Mode of action:
- by alkylating sulfydryl-, amino-, carboxyl- and hydroxyl- groups

- Application:
- Effective chemisterilant, capable of killing spores rapidly

- Highly flammable, used as 10% CO_2+ 90% EO) or dichlorodifluoromethane

- Used to sterilize heat labile articles such as bedding, textiles, rubber, plastics, syringes, disposable petri dishes, respiratory and dental equipments

- Highly toxic, irritating to eyes, skin, highly flammable, mutagenic and carcinogenic