

# Methods of Sterilization and Disinfection- Part II (PRACTICAL)

## Veterinary Microbiology (Unit-1)

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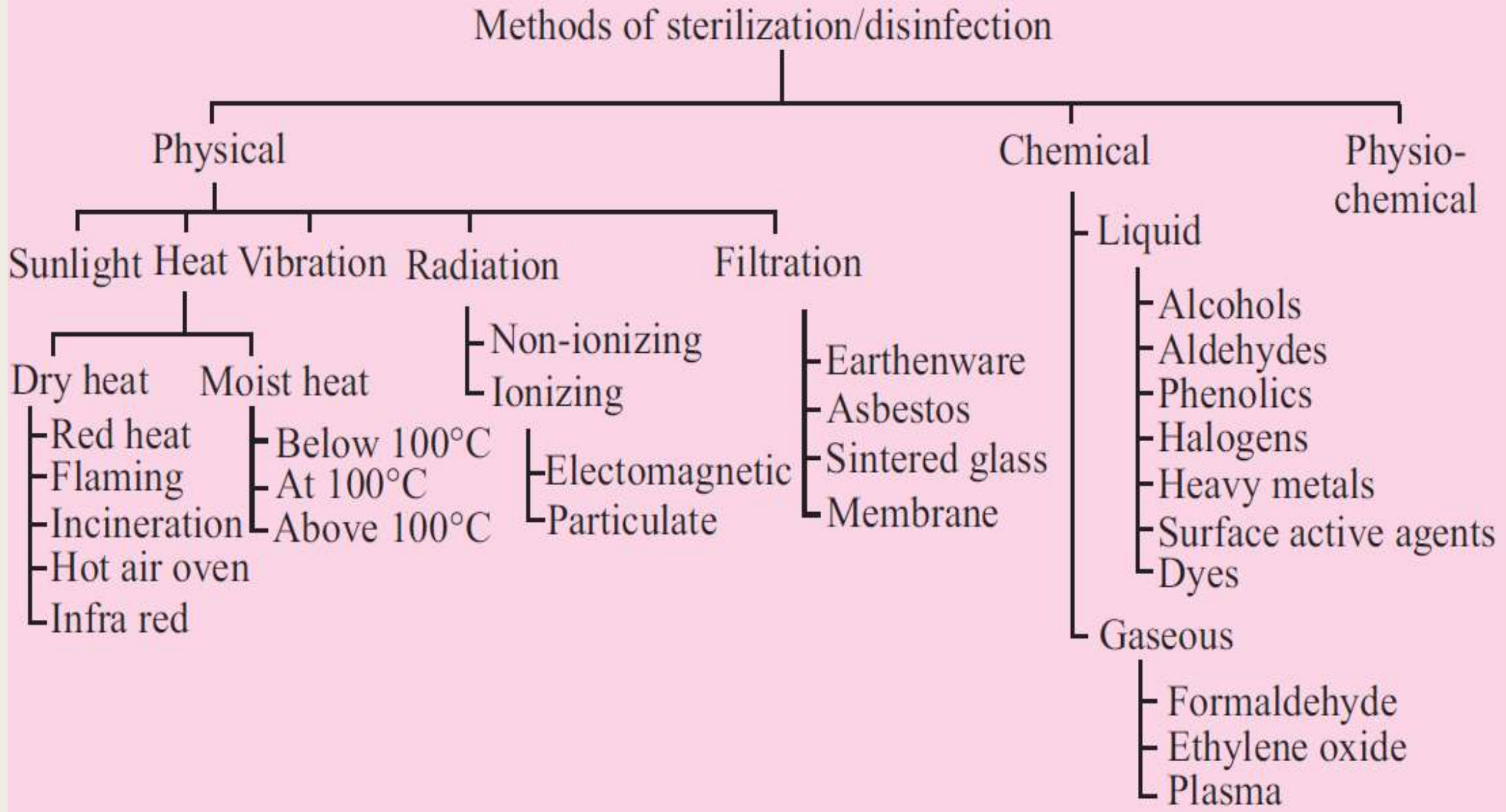
# Sterilization and Disinfection

## ■ Sterilization:

- Process of complete elimination or destruction of all forms of microbial life (i.e., both vegetative and spore forms), which is carried out by various physical and chemical methods
- Article, surface, or medium is freed of all living microorganisms either in the vegetative or in the spore state
- Chemicals used as sterilizing agents are called chemosterilants

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

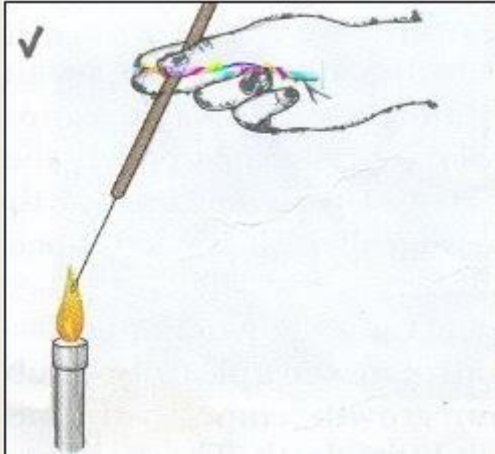
- Reduce the microbial population, not bacterial endospores on inanimate surfaces or in organic materials



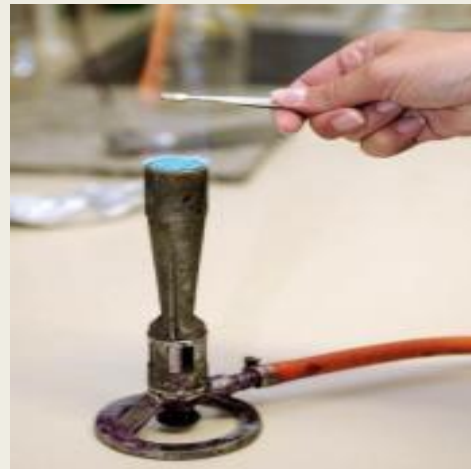
(Source- <https://nios.ac.in>)

## Red Heat

(Image source-Google)



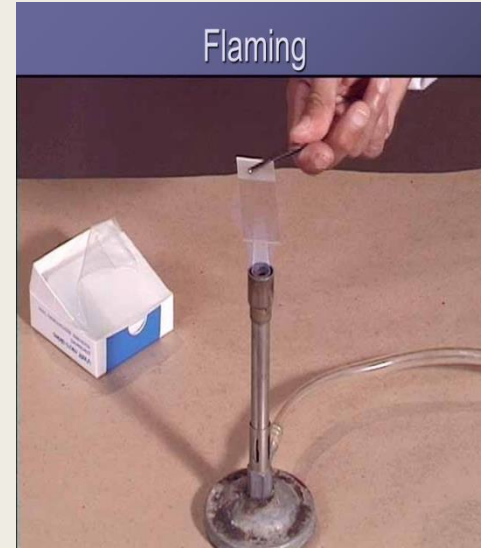
- ❖ Bacteriological loops
- ❖ Straight wires/needles
- ❖ Tips of forceps, spatula



# Flaming:

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- Scalpels
- Mouth of test tube
- Flasks
- Glass slides
- Coverslip



# Hot air oven

- Dry heat sterilization
- Glassware (like petri dishes, flasks, pipettes, and test tubes)
- Powders (like starch, zinc oxide, and sulfadiazine)
- Materials that contain oils
- Metal equipment (like scalpels, scissors, and blades)





# Hot air oven

- Extremely high temperatures for hours to destroy microorganisms and bacterial spores
- Use conduction to sterilize items
- Thermostatically controlled and electrically heated
- The parts of oven:
  - 1. Mechanical part 2. Electric part

■ Temperature (°C)	Minimum sterilization time (min)
160	180
170	60
180	30

# Moist heat sterilization- Autoclave

- Moist heat destroys microorganisms by the irreversible coagulation and denaturation of enzymes and structural proteins
- Operates by using steam under pressure as the sterilizing agent
- High pressures enable steam to reach high temperatures, thus increasing its heat content and killing power, More effective method of sterilization
- At 15 lb per sq. inch pressure, 121 °C temperatures is obtained, kept for 15 minutes for sterilization
- Articles sterilized: Culture media, dressings, certain equipment, linen, rubber (gloves), heat-resistant plastics, liquids etc.



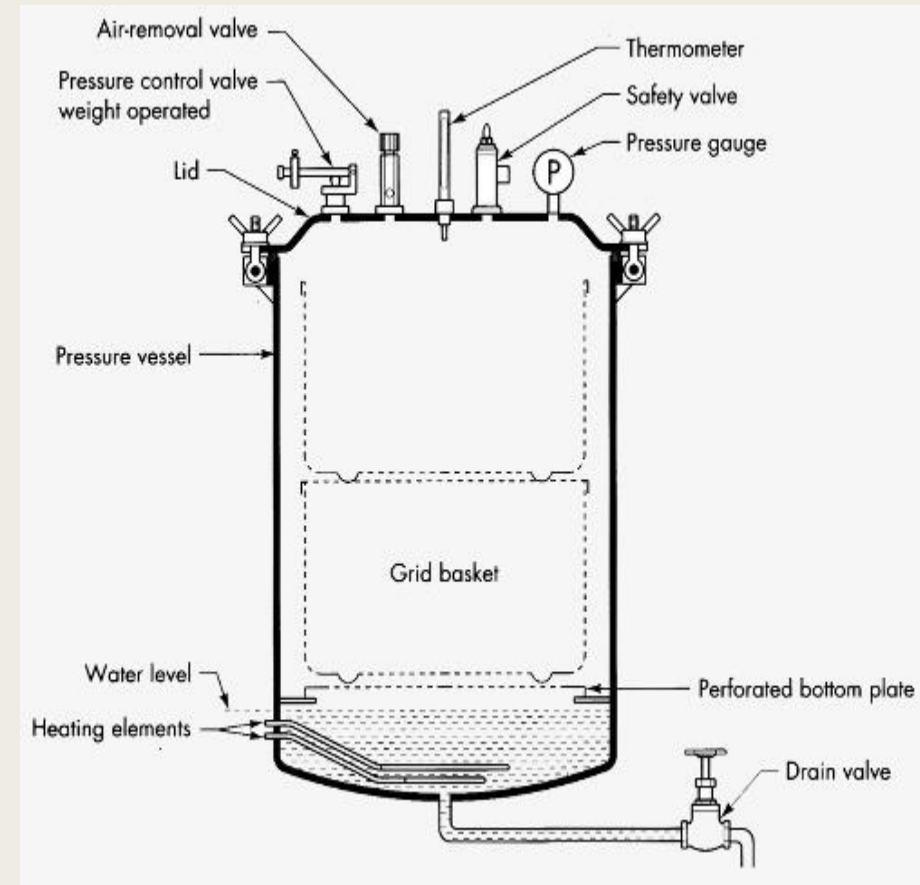
# Autoclave

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# Parts of autoclave

- Vertical or horizontal cylindrical body
- Heating element
- Perforated tray to keep the articles
- Lid that can be fastened by screw clamps
- A pressure gauge
- Safety valve and a discharge tap



# Operation Of Autoclave

- Sufficient amount of water inside chamber
- The articles to be sterilized must not be tightly packed
- The screw caps and cotton plugs must be loosely fitted
- Materials placed inside the chamber, lid closed, and the screws are tightened to ensure an airtight condition
- Electric heater is switched on, safety valves adjusted
- The discharge tap is kept open and the water is heated

## Running an autoclave:

- Water inside the chamber boils, the air-water mixture is allowed to escape
- When all the air displaced and steam start appearing through the discharge tap, the tap is closed
- Steam inside allowed to reach the desired levels (15 lbs in most cases)
- Once pressure reached, the whistle blows to remove excess pressure from the chamber
- Autoclave is run for a holding period, which is 15 minutes in most cases
- Now, the electric heater is switched off, and the autoclave is allowed to cool until the pressure gauge indicates the pressure inside has lowered down to that of the atmospheric pressure
- Lid is opened, and the sterilized materials are taken out of the chamber

# Uses of UV radiation:

- Non-ionizing
- Poor penetrative power
- Inoculation hoods, laminar flow, and operating theaters
- Harmful to skin and eyes



## Filtration:

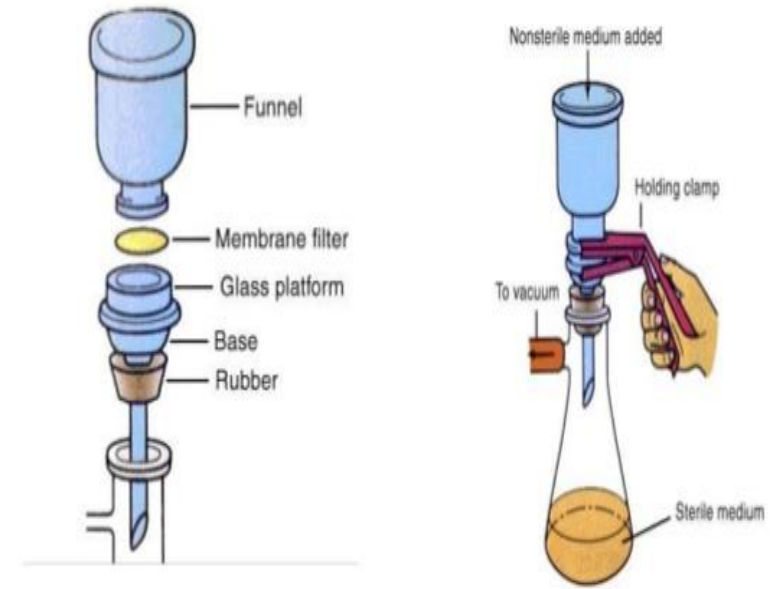
- Preferred method of sterilizing heat sensitive liquid and gases without exposure to denaturing heat
- Choice for sterilizing antibiotic solutions, toxic chemicals, vaccines, serum, sugar solutions, urea solution which are all heat-sensitive
- Preparing suspensions of viruses and phages
- Most commonly used filter composed of nitrocellulose, has a pore size of  $0.22\mu\text{m}$ , retains all bacteria and spores but not all viruses
- Filtration of air is accomplished using high-efficiency particulate air (HEPA) filters designed to remove organisms larger than  $0.3\ \mu\text{m}$  from isolation rooms, operating rooms, and biological safety cabinets
- HEPA filter is a single sheet of borosilicate glass fiber

# Membrane filters

- Most common type of filters used for liquid sterilization in the microbiology laboratory
- Membrane filters are composed of high tensile strength polymers such as cellulose acetate, cellulose nitrate etc.



## Membrane Filtration





- Disinfection - by physical or chemical methods
- Chemicals used in disinfection - **Disinfectants**
- Different disinfectants - different target ranges
- Microorganisms vary in their resistance to destruction by physical or chemical means
- Chemical disinfection does not kill spores, unlike chemical sterilization



# Activity Level

## High Level Disinfection

- High conc. of chemical germicides (ex: conc. sodium hypochlorite, Glutaraldehyde sol.)
- Kills vegetative microorganisms and inactivates viruses
- Does not kill high numbers of bacterial spores
- Used for short periods (10-30 min) for disinfection purposes
- If left for long time periods (6-10 hours) may achieve sterilization
- Not for use on environmental surfaces like floors or lab benches



## Intermediate Level Disinfection

- Kills vegetative microorganisms, including *Mycobacterium tuberculosis*, all fungi, and inactivates most viruses
- May be used for housekeeping and disinfection of laboratory benches
- Ethyl or isopropyl alcohol (70-90%), 10% Chlorine compounds, 0.1-0.2% Iodophor (Intermediate to Low)

## Low Level disinfection

- Kills most vegetative bacteria, some fungi, and inactivates some viruses
- Does not kill *M. tuberculosis*
- Also known as “hospital disinfectants” or “sanitizers”
- Quaternary ammonium compounds, Phenolic compounds

