Sterilization and Disinfection

Sterilization is a process in which all microorganisms (vegetative and spore forming) are destroyed or killing or removal . Sterilization is an absolute term, i.e. the article must be sterile meaning the absence of all microorganisms.

Disinfection is a process in which vegetative and non sporing microorganisms are destroyed , but not all microorganisms . It is a process of reduction of number of contaminating organisms to a level that cannot cause infection.

Disinfectants are chemicals agents that are used for disinfection. Disinfectants should be used only on inanimate objects.

Antiseptics are mild forms of disinfectants that are used externally on living tissues to kill microorganisms, e.g. on the surface of skin and mucous membranes.

Uses of Sterilization.

1- Sterilized of Surgical Procedures : Gloves, aprons, surgical instruments, syringes *etc*. are to be sterilized.

2- Sterilization in Microbiological works like preparation of culture media, reagents and equipments where a sterile condition is to be maintained.

Factors affecting of Disinfection and Sterilization :-

* Resistance of microorganism
* Concentration and potency of Disinfectants
* Physical and chemical factors
* Organic and inorganic matter structure
* Duration of exposure
* Number and Location of microorganism .

Types of sterilization .

Two main types of sterilization are used :-

A- Physical Methods

- Heat
- Filtration
- Radiation

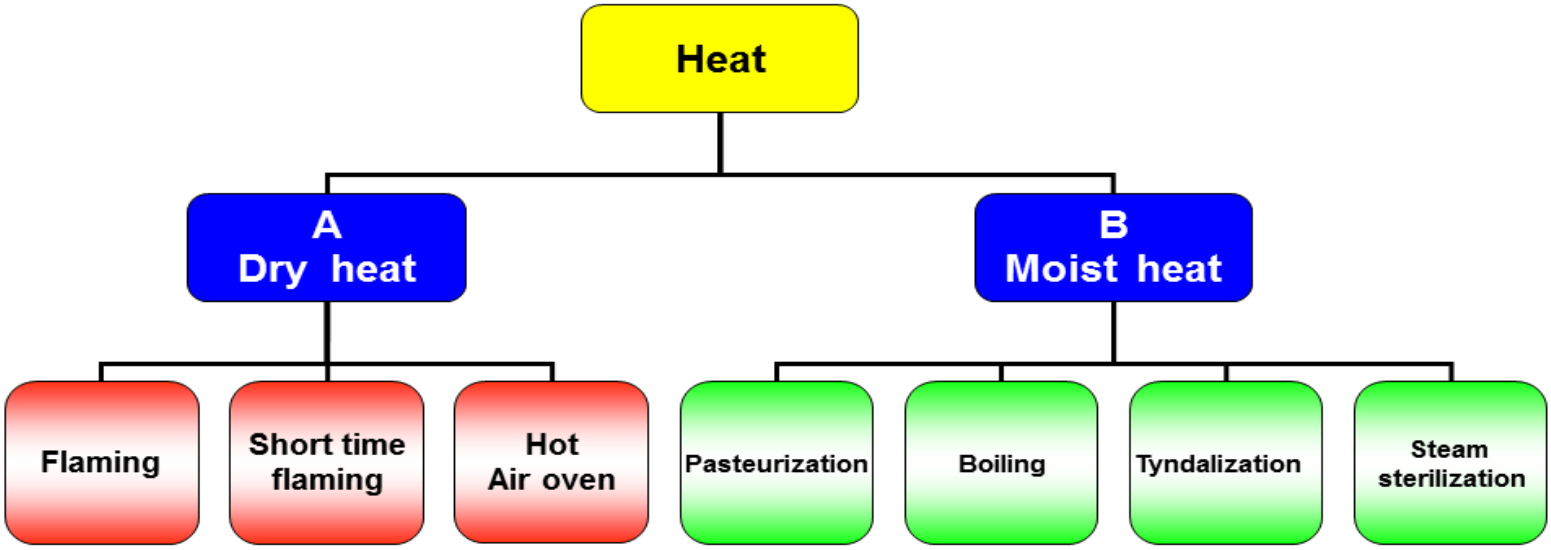




B- Chemical agents (Chemical Methods) .

- Phenol and Phenolic
- Alcohols
- Halogens
- Heavy metals
- Gaseous agents
- Soap and detergents





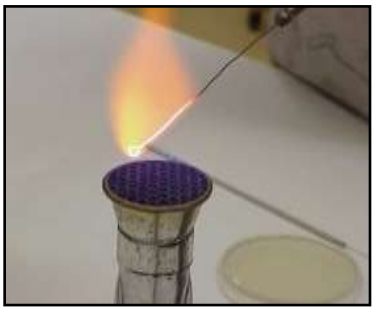
1- Heat .

The most effective method for destroying microorganisms, it's of two types, dry and moist heat .

A- Dry heat

1- Flaming .

- This method is commonly used in microbiology labs.
- Used for small metal or glass objects Inoculating loops, needles, forceps and scissors, but not for large objects.
- Inoculating loops and needles should be heated until they are red-hot. Before they are introduced into cultures, they must be allowed to cool down sufficiently to prevent killing organisms that are to be transferred.



2- Short time flaming .

- Used for flaming test tube openings, flasks and pipettes in order to prevent contamination .

3- Hot air oven .

- This method used for sterilizing dry glass ware, forceps, scalps and scissors.
- Not suitable for heat sensitive materials like many plastic and rubber items
- The standard setting for a hot air oven is at least 2 hours at 160 °C .

B- Moist heat .

Classified according to the temperature of water to :-

1- Pasteurization .

- The process was named after its creator, French chemist and microbiologist Louis Pasteur.
- It is a process which slows microbial growth in food.
- Pasteurization usually destroy all the non-spore former pathogens.

There are two mainly methods for pasteurization :-

- Holding method (batch pasteurization): Involves heating large batches of milk to a lower temperature, typically (63 - 66 ºC for 30 minutes) followed by quick cooling to about 4 C^0 .
- Flash method: Involves heating to 72 ºC for 20 seconds.

2- Boiling .

- Boiling is a simple process, It is an option available to most people, requiring only water, enough heat, and a container.
- Boiling in water at 100 ºC for 15 minutes will kill most vegetative bacteria and inactivate viruses, but boiling is ineffective against many bacterial and fungal spores.
- It is useful for reducing viable levels if no better method is available.

3- Tyndallization .

- Named after John Tyndall, it is a lengthy process designed to reduce the level of activity of sporulating bacteria that are left by a simple boiling water method.
- The process involves boiling for a period (typically 20 minutes) at atmospheric pressure, cooling, incubating for a day This process repeated two times. (totally 3 times), finally boiling again.
- The three incubation periods are to allow heat-resistant spores surviving the previous boiling period to germinate to form the heat-sensitive vegetative (growing) stage, which can be killed by the next boiling step.
- This is effective because many spores are stimulated to grow by the heat shock.
- The procedure only works for media that can support bacterial growth.

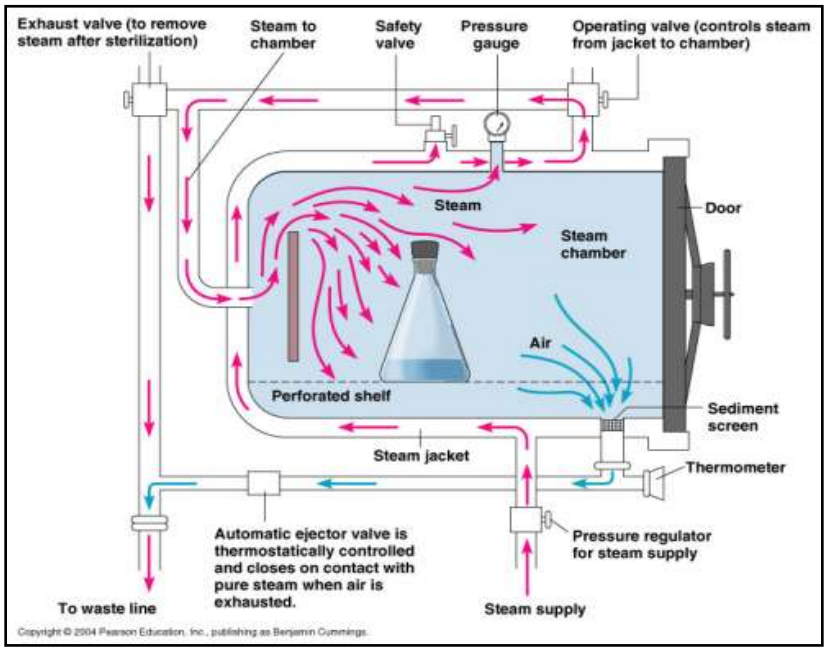
4- Steam sterilization .

A widely-used method for heat sterilization. It is carried out with an **Autoclave**, a device , somewhat like a pressure cooker.

The principle .

- Steam under pressure.
- Pressure, 15 lbs/in2 (1 atmosphere).
- Temperature, 121ºC.
- Time,15-20 min.

This method used for sterilization of culture media, clothes and surgical materials.

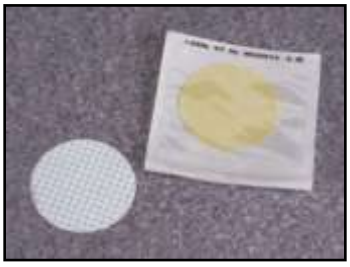


Factors affect sterilization by autoclave

- Temperature: Considered as the main factor that affects the process of autoclaving.
- Steam: Used for coagulation of bacterial protoplasm (proteins and enzymes) this could be occurs at (121^0 C) .
- Pressure: It has no effect on autoclaving, but it is necessary for rising temperature over 100 ºC.
- Time: Mainly 15 -20 minutes, additional sterilizing time is usually required for liquids and instruments packed in layers.
- Trapped air: Must be exhausted to the outside as it affects the sterilization quality.
- Material nature: Packed materials need more time, make sure to leave adequate distances between Loaded materials, don't secure the bottles, leave them loose to prevent explosion .

2- Filtration .

- Clear liquids that would be damaged by heat, irradiation or chemical sterilization can be sterilized by mechanical filtration.
- It depends on separation by special filters, A filter with pore size 0.2 µm will effectively remove bacteria. If viruses must also be removed, a much smaller pore size around 20 nm is needed.
- This method is commonly used for sensitive pharmaceuticals and protein solutions that can be affected by heat like toxins, serum, sugar solutions and antibiotics .



Types of Filter used :-

1. **Membrane filters .**

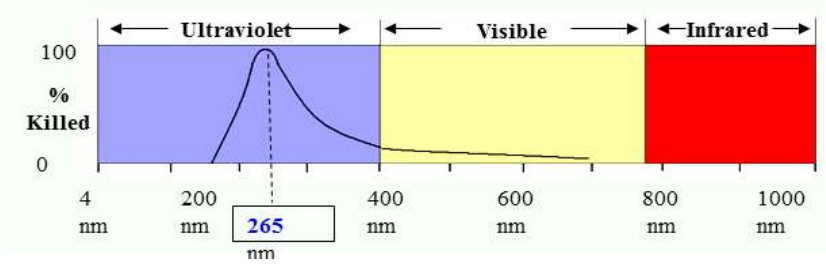
- It is most common used filters in microbiology lab.
- Membrane filters remove microorganisms by screening them out.
- It is made of cellulose acetate, cellulose nitrate, chemically inert and autoclavable.
- Wide vary of pore sizes are available, the most used one is 0.22 µm with a special holder.

2. **Seitz filters :-** Made of asbestos pad layers.
3. **Sintered glass filters : -** Made of glass like plates with pores that materials can pass through.
4. **Chamber land filters :-** Made of kaolin.

3- Radiation .

1- Ultraviolet light or U.V. light (240 – 280 nm)

- Ultraviolet light irradiation is useful only for sterilization of surfaces(benches, biological safety cabinets BSC).
- It doesn't penetrate glass, dirt films, water and other substances effectively (ineffective in shaded areas, including areas under dirt).
- It burns the skin and damages eyes.
- Make sure the UV lamps are off when the areas are in use .



2- Ionizing radiation .

- It has a very short wavelength like X rays and Gamma rays.
- They induce damage in DNA by various mechanisms including the production of free radicals (H2O2, HO2 and HO) .
- It is an excellent sterilizing agent and penetrates deep in to objects .
- Gamma radiation is used in the cold sterilization of antibiotics, hormones, plastic disposable supplies such as syringes that could be damaged by heat .

B - Chemical Agents .

- Many different chemicals are available for use as disinfectants or antiseptics, and each has its own advantages and disadvantages.
- Often called “cold sterilization".
- Ideally the disinfectant must be effective against a wide variety of infectious agents at high dilutions and in the presence of organic matter.
- The effect of chemical agents is either bactericidal that can kill all microorganisms or bacteriostatic that just inhibit the growth of bacteria.

1- Phenol and Phenolics .

- The pure phenol are not used now due to its irritation effect and bad odor.
- Phenol derivatives like cresols, Lysol and hexachlorophene are most popular antiseptics.
- Phenolics act by denaturating proteins and disturbing cell membranes .
- Effective in the presence of organic material.
- Remain active on surfaces long after application.

2- Alcohols .

- Alcohols are among the most widely used disinfectant and antiseptics.
- They are bactericidal and fungicidal but not sporicidal ; some lipid-containing viruses are also destroyed.
- Ethanol and isopropanol alcohol are used in about 70 -80% concentration.
- They act by denaturating proteins and dissolving membrane lipids.

3- Halogens

- They are important antimicrobial agents like chlorine and iodine.
- Act by oxidation of cellular materials.
- Chlorine (sodium hypochlorite) is the usual disinfectant for diary and food industries, municipal water supplies and swimming pool.
- Iodine can be used as a dye in concentration of 2%.
- More recently iodine has been complexed with an organic carrier to form iodophor which is water soluble, non staining and used in hospitals and laboratories for disinfecting .

4- Heavy metals

- Most of heavy mineral disinfectants contains mercury and silver, including organic and inorganic compounds.
- Commercial compound (mercurochrome) used as antisepsis.

5- Gaseous agents

- The formaldehyde and ethylene oxide are considered the most common gases used in sterilization.
- The formaldehyde (formalin solutions 40%) is used for disinfection of halls, heat sensitive materials such as plastic tools. It is also used in vaccine industries.
- Ethylene oxide penetrates well, moving through paper, cloth, and some plastic films and is highly effective.

6- Soap and detergents

- It reduces the surface tension and characterized by moisture with highly water solubility .