# Microbiology Laboratory

## Lab 4: sterilization

### What is Sterilization?

**Sterilization** is the removal of all forms of microorganisms from the surface of an object. It includes both spore and vegetative forms. Here, let's glance at the definition and classification of sterilization notes. Sterilization is a process mainly used to kill all forms of microorganisms and its spores. This process is carried out to maintain a sterile environment. It is usually done through combinations of heat, irradiation, filtration, high pressure etc.Sterilization is carried out by various physical and chemical methods such that it eliminates around $10^6$ log colony-forming units.

Sterilization is done to avoid the growth of microorganisms which may grow on the surface of an object if left without killing the germs. It is, however, different from disinfection or sanitisation where only reduction of the microorganisms takes place, rather than total elimination. After sterilization, an object becomes sterile or aseptic.

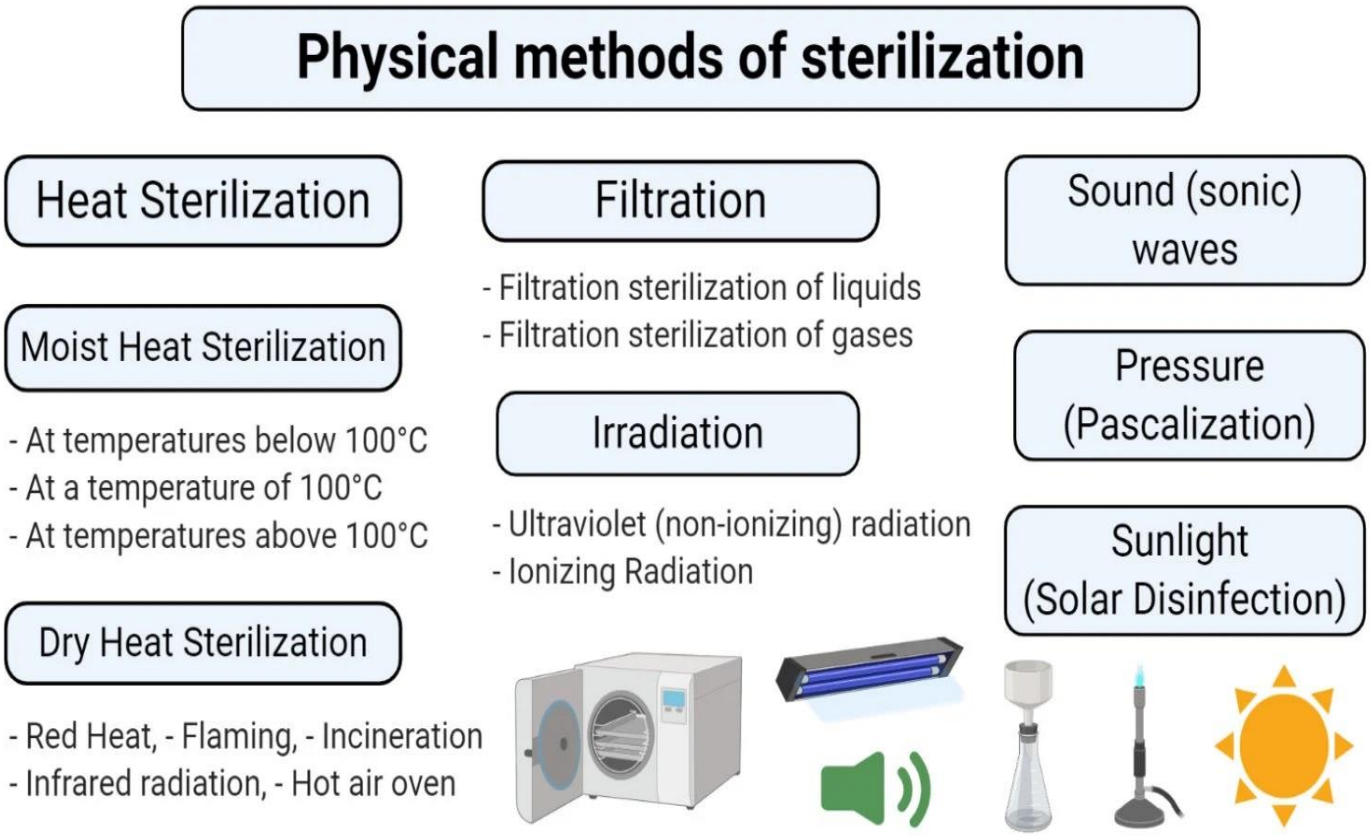
### Classification of Sterilization:

Sterilization is achieved by different **physical** and **chemical** methods in microbiology. Sterilization is classified into 2 types – **physical sterilization and chemical sterilization**.

### Physical Methods of Sterilization

Physical sterilization includes the following methods:

- Heat Sterilization. 1. Moist Heat Sterilization. 2. Dry Heat Sterilization.
- Filtration
- Irradiation. 1. Non-ionising Radiation. 2. Ionising Radiation.
- Sound Waves Vibration
- Fractional Sterilization




- **Heat Sterilization**

Heat sterilization is the most effective method of sterilization, where the elimination of microbes is achieved by the destruction of cell constituents and enzymes. It is done by two methods:

1. **Moist Heat Sterilization:** It is one of the best methods of sterilization. Moist heat sterilization is done with the help of an instrument called an autoclave. An autoclave works on the principle of producing steam under pressure. Thus, moist heat sterilization is also known as steam sterilization. The most commonly used standard temperature-time cycles for clinical porous specimens (e.g. surgical dressings) and bottled fluids are 134°C for 3 minutes and 121°C for 15 minutes, respectively. An autoclave is a device that works on the principle of moist heat sterilization through the generation of steam under pressure. In this method, the microorganisms are killed by coagulating their proteins, and this method is much more effective than dry heat sterilization where microbes are killed through oxidation. Moist heat can be used in sterilization at different temperatures:

❖ **At temperatures below 100°C** like **pasteurization**, in this process the temperature of 63°C for 30 minutes (the holder method) or 73°C for 20 seconds (the flash method. n pasteurization not

all the pathogenic organisms are killed. The principle of pasteurization is the logarithmic reduction in the number of viable microbes so that they can no longer cause diseases. The milk is not heated above its boiling point as the milk might curdle, and its nutritional value might be destroyed. Besides milk, other fluids and equipment like vaccines of non-sporing bacteria are also pasteurized at 60°C for 1 hour in special water baths. Similarly, serum and body fluids with congealable proteins are also sterilized at 56°C for 1 hour in water baths.

- **At a temperature of 100°C** like **tyndallization** is a method that is used for sterilization of media with sugar and gelatin at 100°C for 30 minutes on three successive days so as to preserve sugar which might be decomposed at a higher temperature. Moist heat at 100°C is applicable for contaminated dishes, beddings, pipettes, and other instruments that are not soiled or contaminated as well as for objects that are temperature sensitive. Also, **Distilled water** is preferred because hard water might result in the formation of a film of calcium salts on the instruments.
- **At temperatures above 100°C**: Moist heat sterilization above 100°C involves sterilization by steam under pressure. As a result, the steam under pressure has a higher penetrating power. When this steam comes in contact on the surface, it kills the microbes by giving off latent heat. The condensed liquid ensures the moist killing of the microbes. Autoclaves are used for the sterilization of contaminated instruments along with different culture media as it ensures complete sterility.

2. **Dry Heat Sterilization:** This method is used on objects that are sensitive to moisture. Dry moisture-less heat destroys microorganisms by causing denaturation of proteins and also lyses the proteins in many organisms, causes oxidative free radical damage, causes drying of cells, and can even burn them to ashes, as in incineration. Dry heat sterilization is used for the sterilization of materials which are difficult to sterilize by moist heat sterilization for several reasons. Substances like oil, powder, and related products cannot be sterilized by moist heat because moisture cannot penetrate into deeper parts of oily materials, and powders are destroyed by moisture. There are different types of dry heat sterilization which are explained like: Red Heat, Flaming, Incineration, Infrared radiation, Hot air oven

- **Filtration**

This is a mechanical method of sterilization in microbiology. This method uses membranous filters with small pores to filter out the liquid so that all the bigger particles and microbes cannot pass through. The three steps of filtration are sieving, adsorption and trapping.

- **Irradiation**

  Irradiation is the process of exposing surfaces or objects to different kinds of radiation for sterilization. It is of two types:

  A. Non-ionising Radiation like Ultraviolet radiation
  B. Ionising Radiation like gamma rays and X-rays

- **Sound Waves Vibration**

  Sonix sound waves ranging from 20-40 kHz in frequency are applied across the fluid to be sterilized. The antibacterial activity of airborne sound waves is directly related to the sound intensity, the period of irradiation, and the distance of the sample from the sound source. This method is routinely used by healthcare facilities to clean surgical and dental instruments before the terminal sterilization.

- **Pressure (Pascalization)**

  Pascalization or High-Pressure Processing (HPP) is a method employed for preservation and sterilization of food, in which products are processed under very high pressure (hundreds of megapascal At 400-600 MPa), leading to the death of specific microorganisms and inactivation of enzymes in the food & proteins are easily denatured, and cell morphology is altered, and ribosomes are destroyed. Pascalization is not particularly effective against spores, but combined treatment with heat is found to be effective in the inactivation of spores. Pascalization is especially useful on acidic foods, such as yogurts and fruits, because spores which are pressure-tolerant don't have the ability to live in environments with low pH.

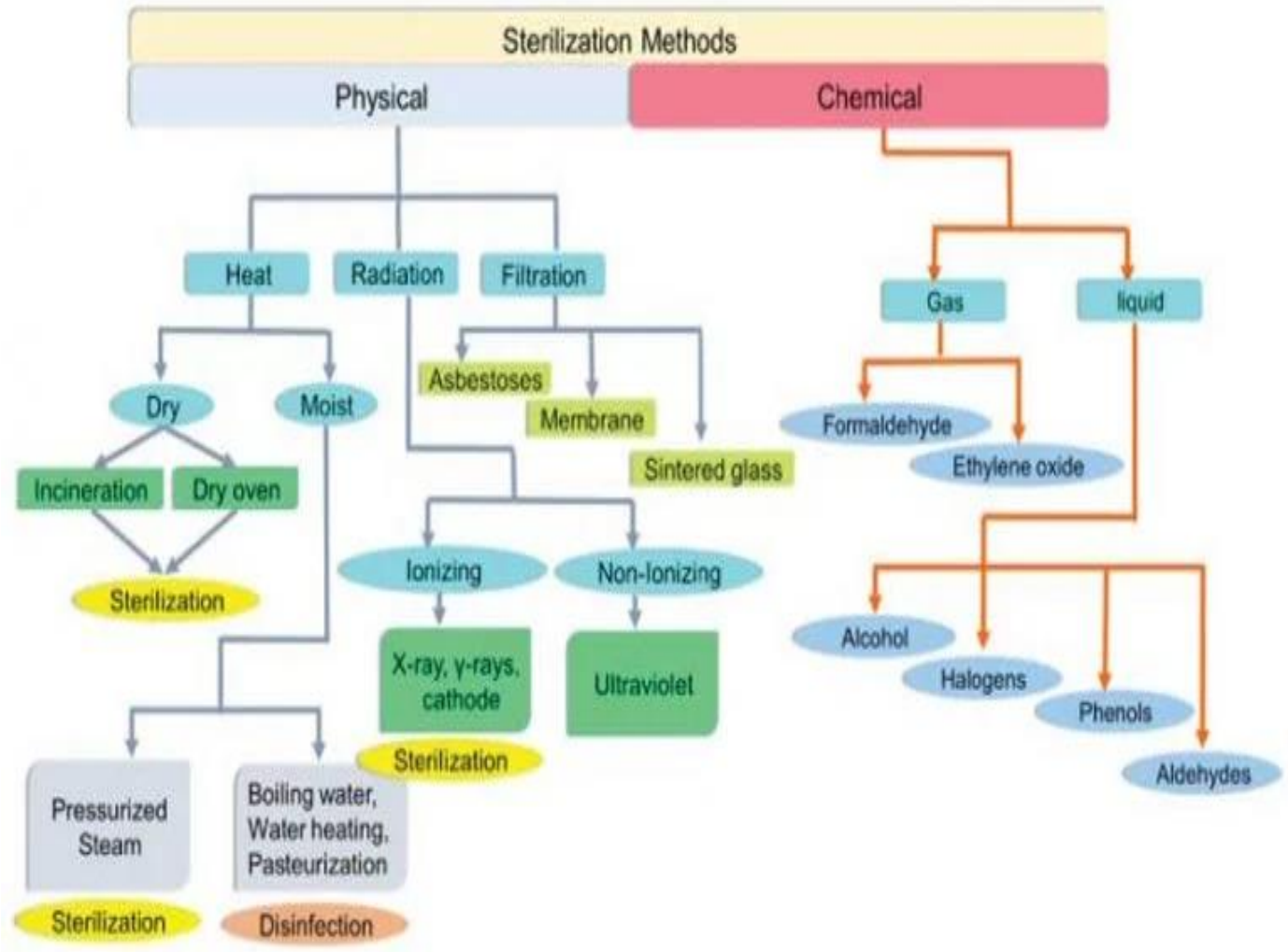
- **Sunlight (Solar disinfection)**

  Solar disinfection is a process used for the removal of microorganisms with the help of sunlight. The principle of solar disinfection is similar to that of radiation sterilization; however, the efficacy of solar disinfection is significantly low as it requires a long period of exposure. Solar disinfection is based on the inactivation of pathogenic organisms as a result of the UV-A (wavelength 320–400 nm) part of the sunlight, which reacts with oxygen dissolved in the water and releases highly reactive forms of oxygen (oxygen free radicals and hydrogen peroxides). These metabolites damage pathogens, while it also interferes with metabolism and destroys bacterial cell structures, and simultaneously the full band of solar energy (from infrared to UV) heats the surface.

**Chemical Methods of Sterilization**

Chemical methods of sterilization are used in microbiology for biological specimens and plastic equipment. In this method, several chemicals work as bactericidal agents. They can be of two types: gaseous or liquid.

- **Gaseous Sterilization**: The gaseous chemical agents used for sterilization include ethylene oxide, formaldehyde, nitrogen dioxide and ozone.
- **Liquid Sterilization**: This method is less effective than gaseous sterilization and is used to remove low levels of contamination. Common liquid chemical agents that are used for sterilization include hydrogen peroxide, glutaraldehyde and hypochlorite solution.



## Exam question:

Q1\ **What do you mean by sterilization?**

Sterilization is a process mainly used to kill all forms of microorganisms and their spores. It is carried out to maintain a sterile environment. It is usually done through combinations of filtration, heat, irradiation, high pressure etc.

Q2\ **Explain sterilization principles.**

Sterilization is a process which uses physical or chemical agents by which an article, object or medium is freed of microbes.

Q3\ **What is the principle of moist heat sterilization?**

The process of moist heat sterilization is based on the principle that high temperature coagulates the proteins of the microorganisms such that it effectively dies.

Q4\ **What is the classification of sterilization?**

There are two types of sterilization methods: physical method and chemical method.

Q5\ **Define cold sterilization.**

In microbiology, cold sterilization is defined as a process in which sterilization is carried out at low temperatures with the help of chemicals, filters, radiation and all other means excluding high temperature.

Q6\ **What is the principle of dry heat sterilization?**

Dry heat sterilization works on the principle of conduction. Here, the heat is absorbed by an object's outer surface and then transferred inside to the next layer. Eventually, the entire object reaches the required temperature for sterilization.

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