

Sterilization

Sterilization is the process of destroying or physically removing all forms of microbial life including vegetative cells, spores and viruses from a surface, a medium or an article. The principal reasons for controlling microorganisms are:

1. To prevent transmission of disease and infection
2. To prevent contamination by undesirable microorganisms
3. To prevent deterioration and spoilage of materials by microorganisms

The methods of sterilization employed depend on the purpose for which sterilization is carried out, the material which has to be sterilized and the nature of the microorganisms that are to be removed or destroyed. The various agents used in sterilization can be grouped into physical and chemical agents

Physical Agents (Physical Methods)

- *Sunlight*

Direct sunlight has an active germicidal effect due to the combined effect of the ultraviolet radiation and heat. This is a natural method of sterilization.

- *Drying*

Moisture is essential for the growth of bacteria. Drying in air has therefore a deleterious effect on many bacteria. But spores are unaffected by drying. Hence this is very unreliable method.

- *Heat*

Heat has a killing effect on microorganisms and is one of the most popular reliable methods to destroy.

Microorganisms has a minimum, optimum and maximum growth temperatures. Temperature below the minimum usually produces static (inhibition of metabolism).

Temperature above the maximum, generally kill microorganisms. This is because biochemical changes in the cells organic molecules result in its death. These changes arise from alterations in enzyme molecules or chemical break down of structural molecules especially in the cell membranes. Heat also drives off water and this loss of water may be lethal to the organisms.

The killing rate of heat may be expressed as a function of time and temperature. Each microbial species has a Thermal Death Time (TDT). It's the minimum time required to kill a population of microorganism in a microbial suspension at a given temperature and under defined condition. Each species also has a Thermal Death Point (TDP), the temperature at which it dies in a given time.

In determining the time and temperature for microbial destruction with heat, the following factors are considered.

1. Type of organism to be killed
2. Type of material to the treated
3. Presence of organic matter
4. Acidic or basic nature of the material

Nature of heat:

a) Dry heat

Many objects are best sterilized in the absence of water by dry heat sterilization; killing by dry heat is due to protein denaturation, oxidative damage and toxic effect of elevated levels of electrolytes.

Methods of Sterilization by Dry Heat

1. Flaming

Inoculating loops and points of forceps may be heated in the Bunsen flame, till they are red-hot. Articles such as mouth of the culture tubes, cotton wool plugs, glass slides etc. are passed over the flame without allowing it to become red hot.

2. Incineration

This is an excellent method for rapidly destroying, animal carcasses, pathological material and disposables.

3. Hot Air Oven

This is the most widely adopted method of sterilization by dry heat. The hot air oven utilizes radiating dry heat for sterilization. This type of energy does not penetrate materials easily and thus, long periods of exposure to high temperature are necessary. For example, at a temperature of 160°C, a period of two hours is required for the destruction of bacterial spores. Hot air oven is used to sterilize glassware, forceps, scissors, scalpels, glass syringes, liquid paraffin, dusting powder etc. A holding period of 160°C for an hour is used. The oven is usually heated by electricity, with heating elements in the wall of the chamber and it must be filled with a fan to ensure even distribution of hot air and elimination of air pockets. The materials should be arranged in a manner which allows free circulation of hot air in between the objects. It should not



Fig. 3. Hot Air Oven

be over-loaded. Glass wares should be perfectly dry before being placed in the oven. Test tubes, flasks etc. should be wrapped in craft paper. Oven must be allowed to cool slowly for about 2 hours before the door is opened, since the glasswares may get cracked by sudden or uneven cooling.

Sterilization control: The spores of a non – toxigenic strain of *Clostridium tetani* are used as a microbiological test of dry heat efficiency. Paper stripes impregnated with 10^6 spores are placed in envelop and inserted into suitable packs. After sterilization is over, the strips are removed and inoculated into thioglycollate or cooked meat media and incubated for sterility test under strict anaerobic conditions for five days at 37°C.

b) Moist heat

Moist heat kills microorganisms by coagulating their proteins and is much more rapid and effective than dry heat because water molecules conduct heat better than air. Lower temperature and less time of exposure are therefore required than for dry heat. Moist heat readily kills viruses, bacteria, fungi etc.

a) Temperature below 100°C

i. Pasteurization of milk

For pasteurization of milk, there are two methods

- *Holding Method or Low Temperature Holding Method (LTH)*

In this method, the milk is exposed to a temperature of 63°C (145°F) for 30 minutes in an appropriately designed equipment. This is followed by sudden cooling to 13°C or below.

- *Flash Process or High Temperature Short Time (HTST)*

In this method, the milk is exposed to a temperature of 72°C for 15 seconds in the equipment. This is followed by sudden cooling to 13°C or below. The finished product should be stored at a low temperature to retard growth of microorganisms and pasteurization removes the pathogenic bacteria in milk. By these

processes all non-sporing pathogens such as mycobacteria, salmonellae and brucella are destroyed 'Coxiella burnetii' is relatively heat resistant and may survive the holder method.

ii. *Vaccine bath*

It's used for killing non-sporing bacteria which may be present in vaccine. In vaccine bath, the vaccine is treated with moist heat for one hour at 60°C.

iii. Serum containing coagulable proteins can be sterilized by heating for one hour at 56°C in a water bath for several successive days.

b) Temperature at 100°C

i. *Boiling*

Most of the vegetative forms of bacteria, fungi etc. are killed almost immediately at 90-100°C, but sporing bacteria required considerable periods of boiling. Boiling water is not considered as a sterilizing agent because destruction of bacterial spores and inactivation of viruses cannot always be assured. Under ordinary circumstances, most species of microbes can be killed within 10 minutes. However, spores of bacteria and fungi, protozoa cysts and large concentrations of Hepatitis A viruses requires up to 30 minutes exposure or more because inadequate information exists on the heat tolerance of many microorganisms, boiling water is not reliable for sterilization purpose especially the sterilization of instruments and for surgical procedures.

In cases where boiling is considered adequate, the material should be immersed in water and boiled for a period of 10-30 minutes. The lid of the sterilizer should not be opened during that period. Addition of little acid, alkali or washing soda will increase the efficiency of the process.

ii. *Steam under atmospheric pressure (100°C)*

Steam under atmospheric pressure is used to sterilize culture media which may decompose if subjected to higher temperature. A Koch or Arnold sterilizer is an instrument that generates free floating steam.

The container and the medium are simultaneously sterilized and evaporation from the medium is prevented one exposure of 90 minutes usually ensures complete sterilization of the medium. This is an inexpensive method.

iii. *Sterilization above 100°C (steam under pressure)*

Heat in the form of saturated steam under pressure is the most practical and dependable agent for

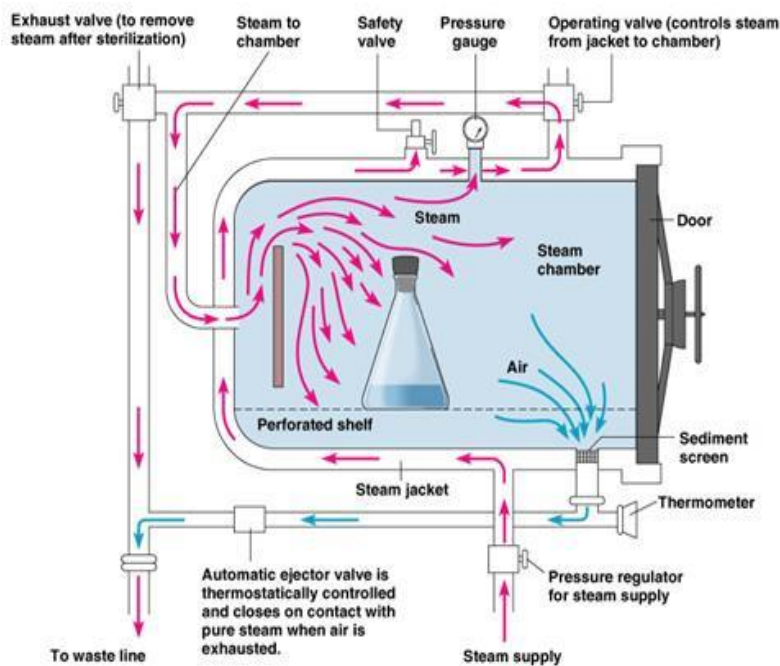


Fig. 4. Autoclave

sterilization. Steam under pressure provides temperature above those obtainable by boiling. Moreover, it has advantages of rapid heating, penetration and moisture in abundance, which facilitates the coagulation of the protein of microorganisms, resulting in complete destruction of all forms of microbial life, including bacterial endospores. It is important to note that the sterilizing agent is the moist heat not the pressure. The laboratory apparatus designed to use steam under regulated pressure is called an autoclave. It is essentially a double jacketed steam chamber equipped with devices which permit the chamber to be filled with saturated steam and maintained at a designed temperature and pressure for any period of time. The articles to be sterilized are placed in the sterilizing chamber and steam is maintained in the steam jacket into the sterilizing chamber, cool air is forced out and a special valve increases the pressure to 15 pounds/square inch above normal atmospheric pressure. The temperature rises to 121.5°C and the superheated water molecules rapidly conduct heat into microorganisms and will be killed. The time for destruction of the most resistant bacterial spore is reduced to 15 minutes. For denser objects, up to 30 minutes of exposure may be required.

Autoclave is an essential equipment in every microbiology laboratory. It's used to sterilize many media, solutions, discarded cultures, glass wares, metal wares etc.

- **Filtration**

Filtration is the process of removal of microorganisms from liquid or gases using a mechanical device known as filter. This is an excellent way to reduce the microbial population in solution of thermo labile materials such as sera, antibiotic solutions, intravenous solutions, carbohydrates solutions used in the preparation of culture media etc. As fluid passes through the filter, microorganisms are trapped in the pores of the filtering material. The solution that drips through the filter is collected in a previously sterilized container. Porosity, electric charges of the filter, electric charge carried by the organisms, nature of the fluid being filtered etc. can influence efficiency of filtration.

Types of filters: Seltz Filter, Berkefeld Filter, Membrane Filter, High Efficiency Particulate Air (HEPA) filter

- **Irradiation**

The process of exposing organisms to any one of the radiation such as UV-rays, X-rays, gamma rays etc. is known as irradiation. Irradiation is an effective method of sterilization. Two types of radiations are used for sterilization.

- a) Non ionizing radiation

- UV radiation
- Infrared radiation

- b) Ionizing radiation

- X rays
- Gamma rays

Chemical Methods

The physical agents for controlling microorganisms are generally intended to achieve sterilization. Instead, they are expected only to destroy the pathogenic organisms on or in or removal of pathogenic microorganisms is called disinfection. An agent, usually a chemical that kills the pathogenic microorganisms on/in animate objects is known as a disinfectant. A disinfectant does not necessarily sterilize an object because a few microorganisms and viable spores may remain. The chemical agents that are applied to the body to prevent infection or species are called antiseptic. The antiseptic prevents the growth or action of microorganisms either by destroying them or by inhibiting their growth and metabolism.

An antimicrobial agent is generally called as a germicide. A disinfectant or antiseptic can be particularly effective against a specific group called as bactericides, fungicides or algacides. Some chemicals do not kill microorganisms, but they temporarily prevent from multiplication. Many different chemicals are available for use as disinfectants and each has its own advantages and disadvantages.

Characteristics of a desirable disinfectant

The disinfectant must be effective against a wide variety of infections/agents, at high dilutions and in the presence of organic matter. The chemical must be toxic for infection agents but it should not be toxic to people or corrosive for common upon storage, colourless with a pleasant odors, soluble in water and lipids for penetration into microbes and have a low surface tension so that it can cracks in surface. If possible, the disinfectant should be relatively inexpensive.

The factor influencing the effectiveness of chemical disinfectants:

1. Size of the microbial population
2. Nature of microbes present
3. Concentration and nature of the disinfectant
4. Duration of exposure
5. Temperature
6. Local environment

The main modes of action of disinfectant:

1. Protein coagulation
2. Disruption of cell membrane, thus resulting in exposure of the contents of the cell to the adverse environment and loss of the constituents of the cell and changes in the composition of the contained cytoplasm. These cause death of cell.
3. Removal of free sulfhydryl groups which are essential for the functioning of the enzymes and thus the life of the cells.
4. Inhibition of respiration of catabolic/anabolic reactions.
5. Loss of membrane integrity resulting in leakage of essential constituents such as potassium cations, inorganic phosphate, pentose, nucleotides and nucleosides and proteins.

Major Group of Chemical Agents: Phenol and Phenol compounds, Alcohols, Heavy metals and their compounds, Dyes, Soaps and Detergents, Aldehydes, Gaseous agents