STERILIZATION METHODS

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- Sterilization is the procedure of making objects free from all living organisms. In microbiology, it is employed to remove or destroy all levels of microbes from a particular material such as media and reagents used in laboratory, instruments used in surgical and diagnostic purposes, and also in food and drug manufacturing units to ensure safety from contaminating organisms.

- The commonly used sterilization methods include Heat treatment, Filtration, Radiation, and chemicals.

HEAT TREATMENT: This is the most common and widely used method of sterilization. The temperature and the duration of heating are important factors to achieve complete sterilization. It is done either by dry heat or wet heat.

Dry Heat: A high temperature dry heat can kill the microbes. The instruments which are commonly used by this method of sterilization include:

- Hot air oven
- Microwave
- Radiation
- Flaming
- Incineration/burning

Sunlight also plays an important role in the sterilization of some food items. The raw food materials are dried to such an extent that no microbes can invade there. This is a very old method and still used in villages to preserve the food crops.

Things which are commonly sterilized by dry heating are — Metal instruments, hospital items like syringe, some types of clothings etc., paper-wrapped items, glass wares in laboratory by hot air oven.

Wet Heat: The heat laden with water vapour destroys microorganisms by denaturation of enzymes and structural proteins

This is the most effective method as the pressurized steam can kill the microbes at a lesser time than the dry heat. Autoclaving is one of the effective way which works on time and temperature relationship. The usual method is a time of 30 minutes at a temperature of 121°C (15 pound per square inch pressure can achieve a chamber temperature of at least 121°C).

Autoclaving is used to sterilize items like surgical equipments, laboratory wares, media used to culture microbes, biological and medical wastes.

FILTRATION: Filtration is the only method that uses force to separate the microbes rather than to kill them. The objects are passed through a filter with a porosity sufficient to remove the microorganisms. Pore sizes can be as small as 0.01 μm small enough to stop viruses from passing through, but smaller proteins can still get through. There are even smaller filters called nano-filters, which stop viruses, proteins, and some toxins to pass through the pores.

Filtration is a great option for sterilizing heat-sensitive liquids, which can't be autoclaved or sterilized by other sterilizing methods. These include materials like vaccines, antibiotics, toxins, serum, sugar etc. An ideal filter should follow the following features —

- efficiently remove the particles above the stated size,
- maintaining high flow rate,
- resistance to clogging,
- Steam Sterilizable,
- good flexibility and mechanical strength,
- non-pyrogenic
- biologically inert.

Filters are of two types ie, depth filter and membrane filter.

A depth filter captures contaminants throughout the depth of filter, rather than just on the surface, whereas a membrane filter typically traps contaminants larger than

the pore size on the addressed surface of the membranes. Depth filters are made from sintered glass, compressed fibers or ceramic materials. Membrane filters (also called screen filters) are derived from cast materials, stretched polymers and irradiated plastics.

RADIATION: It is one of the important means of sterilization and has grown tremendously over the last few decades due to its numerous advantages. sterilization by the use of radiation is widely used in medicine and healthcare-related field, for examples during allograft preparation, pharmaceutical packaging and medical device packaging.

The radiation can be achieved by using either electromagnetic radiation or subatomic particles. The electromagnetic radiations are further divided into-

- Ionizing radiation - γ-rays (cobalt-60, Cesium-137)
  X-rays
  They have short-wave length, high energy and high penetrating power.

γ-ray radiation is the most popular form of sterilization. It covers nearly 41% of sterilization market. The rays emitted from radioactive source cobalt-60 is usually used; to a lesser extent cesium 137 is also used.

- Non-Ionizing radiation - Ultraviolet light.
  used to disinfect home, offices, hospitals or any other facility.
  The short-wavelength UV kills or inactivates microorganisms by destroying nucleic acids and disrupting their DNA, leaving them unable to perform vital cellular functions.

- Infrared - Long wavelength, very low levels of radiant energy,
- Microwave - long wavelength, very low levels of radiant energy
  Both the infrared and microwave are not suitable for sterilization because they have very low levels of energy.

The subatomic particles include electrons, neutrons, and protons. Among these, electrons are widely used because when accelerated to very high speed, they have high energy and high penetrating power. The electron beam sterilization has limited application because it has lower penetration ability than gamma rays, therefore used to lower density or smaller products.

Radiation based sterilization has several advantages over traditional heat-based and other means. It can sterilize the product in packaging. Ionizing radiation has great penetration depth and the temperature increase is also the minimum, thus suited for fully sealed and finally packed materials sterilization. Electron beam sterilization is time efficient and can be completed within seconds or minutes

CHEMICAL STERILISATION: It is used for devices that would be sensitive to high heat used in steam sterilization, and for devices that may be damaged by irradiation (rubber, plastic etc become more brittle after irradiation).

Chemicals are often used for those devices which are repeatedly used in laboratories and hospitals. The selection of right sterilizing chemicals help to avoid contamination or even chemical damage to the items that is to be sterilized. The most commonly used chemicals include — ethylene oxide, aldehydes, alcohols, phenols, halogens, peracetic acid, heavy metals, hydrogen peroxide, beta-propiolactone (BPL).

Ethylene oxide is the most commonly used agent. It acts by inactivation of proteins, DNA, and RNA against microorganisms. Its high penetration rate gives it the power to seep through different custom trays. Aldehyde is also a very useful sterilizing agent. A concentration of 4% of formaldehyde (formalin) is used for surface disinfection and fumigation of rooms, chambers, operation theatres, etc. Alcohol gives protection against bacteria and fungi. The 70% aqueous solution is more effective. It dehydrates cells, disrupt membrane and cause coagulation of proteins.

Phenolic compounds such as Cresol (1-5%), lysol (5%), hexachlorophene, chlorhexidine, chloroxlenol (dettol) are commonly used as disinfecting agents. Halogens like chlorine and iodine are the popular microcidals. The iodine is often mixed with alcohol to create tincture of iodine that is used to disinfect skin and small wounds (2% with 70% alcohol). Sodium hypochlorite (1.0%) is used as surface disinfectant in laboratory materials such as seeds and fruit parts. Peracetic acid is sporicidal at low concentration. It has not much harmful health effect. It is mainly used in food industry, where it is applied as cleaner and disinfectant. The bacteriostatic nature of some heavy metals found as mercuric chloride, silver nitrate, copper sulfate, organic mercury salts etc are important in controlling bacterial infection. Copper salts are strong fungicides. Mercuric chloride is highly toxic, therefore precaution is taken in its use. Hydrogen peroxide is used at 6% concentration to decontaminate instruments such as ventilators. At lesser concentration (3%), it is used for skin disinfection. BPL is an alkylating agent and acts through alkylation of carboxyl and hydroxyl groups. It is an effective sporicidal agent, and has broad spectrum activity.

CONCLUSION: The various methods of sterilization are used to provide a microbe free condition. Sterilization is an absolute term meaning complete removal of microorganisms. Depending upon the requirement, different methods are utilized for the purpose of sterilisation. Heat, filtration, radiation and chemical disinfectants are all equally important. Some surface active agents like soaps or detergents are active against vegetative cells of bacteria and enveloped viruses. They act by disrupting membrane resulting in leakage of cell constituents.

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