T-33

STERILIZATION

Sterilization(or **sterilisation**)refers to any process by which all living cells, spores, and acellular entities (e.g., viruses, viroids, and prions) are either destroy edor removed from an object or habitat. A sterile object is totally free of viable microorganisms, spores, and other infectious agents.

Sterilization can be achieved through various means, including heat, chemicals, irradiation, and filtration, requires the use of aseptic techniques which ensure that sterilized products or materials are not reconta⇔minated by microorganisms. Sterilization is distinct from disinfection, sanitization, and pasteurization in that sterilization kills or eliminates all forms of life and other biological agents which are present.

METHODS EMPLOYED IN STERILIZATION

The physical methods used in sterilization include th following:

A. Heat:

I. Moist heat

II. Dry heat

B. Radiations

I. Ultraviolet radiations

II. Ionizing radiations

C. Filtration through a bacteria- proof filters

A. Sterilization by Heat

Heat sterilization involves the use of moist heat and dry heat.

I. Moist Heat sterilization

Moist heat destroys viruses, bacteria, and fungi moist heat kills by degrading nucleic acids and denaturing enzymes and other essential proteins. It also disrupts cell membranes. Some techniques that involve the use of moist heat include:

1 Steam under pressure

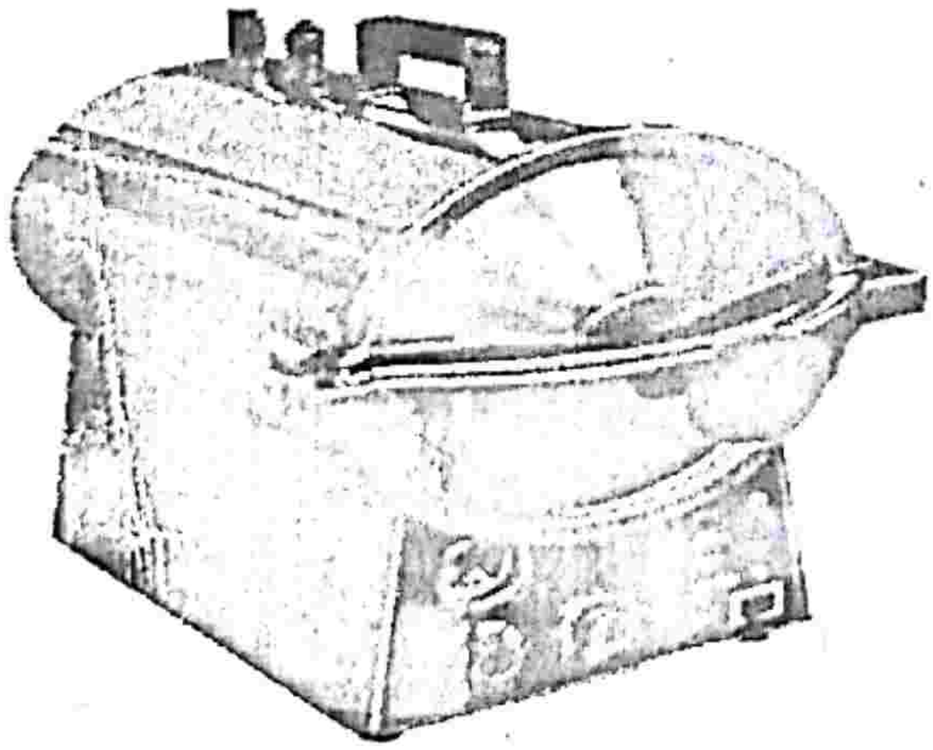
Exposure to boiling (100°C) for 10 - 30 minutes is sufficient to destroy vegetative cells and eukaryotic spores. Unfortunately, the temperature is not sufficient to destroy bacterial spores, which may survive hours of boiling. To destroy bacterial spores, moist heat sterilization must be carried out at temperatures above 100°C, and this requires the use of saturated **steam under pressure**. Steam sterilization is carried out with an **autoclave.**

The most widely used method for heat sterilization is the autoclave. In autoclaving, water is boiled to produce steam, which is released into the autoclave's chamber. The air initially present in the chamber is forced out until the chamber is filled with saturated steam and the outlets are closed. Hot, saturated steam continues to enter until the chamber reaches the desired temperature and pressure, usually 121°C and 15 pounds of pressure. At this temperature, saturated steam destroys all vegetative cells and spores in a small volume of liquid within 10 to 15 minutes.

Autoclaving must be carried out properly or the processed materials will not be sterile. If all air has not been flushed out of the chamber, it will not reach 121°C, even though it may reach a pressure of 15 pounds. The chamber should not be packed too tightly because the steam needs to circulate freely and contact

everything in the autoclave. Bacterial spores will be killed only if they are kept at 121°C for 10 to 15 minutes. When a large volume of liquid must be sterilized, an extended sterilization time is needed because it takes longer for the center of the liquid to reach 121°C; 5 liters of liquid may require about 70 minutes.

Biological indicators can also be used to independently confirm autoclave performance. Simple bioindicator devices are commercially available based on microbial spores. Most contain spores of the heat resistant microbe *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*), which is extremely resistant to steam sterilization. Biological indicators may take the form of glass vials of spores and liquid media, or as spores on strips of paper inside glassine envelopes. Sometimes either special indicator tape or paper that changes color upon sufficient heating is autoclaved with a load of material. These approaches are convenient and save time but are not as reliable as the killing of bacterial spores. These indicators are placed in locations where it is difficult for steam to reach to ascertain steam penetration.



An automated Autoclave

2. Tyndallization

Named after John Tyndall, Tyndallization is an obsolete and lengthy process designed to reduce the level of activity of sporulating bacteria that are left by a simple boiling water method. It involves the use moist heat. The process involves boiling for a period (typically 30-60 minutes) at atmospheric pressure, cooling, incubating for a day, then repeating the process a total of three to four times. The 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, and will not sterilize non-nutritive substrates like water. Tyndallization is also ineffective against prions.

3. Heating with a bactericide

This method is described in British Pharmacopoeia (1973). It is proposed for products that are not stable at the temperatures reached in sterilizing with an autoclave but may withstand heating at 98-100°C for 30 minutes.

It is based on the principle that bactericides are most active at elevated temperatures. The inclusion of appropriate concentration of suitable bactericides followed by heating at 98-100°C for 30 minutes would destroy the cells and spores of contaminating bacteria. The only officially permitted bactericides used for this purpose are chlorocresol at 0.2 % and phenylmercuric nitrate or phenylmercuric acetate at 0.002 %.

This method is relatively simple and is used for sterilizing injections in their final containers i.e. in sealed ampoules and vials. The products are immersed in water in a covered water bath and heated up at the specific conditions. The apparatus is inexpensive, but its application is highly limited. Not many medicaments are stable at the operational temperature of 98-100°C. The technique is not suitable for oily products or suspensions, which need to be sterilized at temperatures higher than 100°C. It should not be used for intravenous injections of volume more than 15 ml as the required concentration of the bactericide will add to the toxic amounts in the large volume injections. The bactericide may cause irritation, inflammation or damage to the tissues or organs involved in these modes of administration. The bactericide must be compatible with the medicaments and other adjuvant present in the product and, therefore, should be included after a thorough consideration of the possibilities of incompatibilities.

II. Dry heat sterilization

Many objects are best sterilized in the absence of water by dry heat sterilization. Microbial death results from the oxidation of cell constituents and denaturation of proteins. Dry heat is less effective than moist heat. For instance, the spores of *Clostridium botulinum,* the cause of botulism, are killed in 5 minutes at 121°C by moist heat but only after 2 hours at 160°C with dry heat. The temperature of sterilization and time of exposure depend on the nature of the products being sterilized and its size and packing in the oven.

APPLICATIONS OF DRY HEAT

1. Dry heat is applied in the sterilization of glasswares required for aseptic and bacteriological techniques such as flasks, beakers, tubes, containers (e.g ampoules, pipettes, petri dishes and all glass syringes).
2. Equipments for aseptic processing including mortars, pestles, evaporating dishes (porcelain articles) and metals (stainless steel dishes, scissors, scalpels, ointment tubes) can be sterilized by heat.
3. It is employed in the sterilization of powders, vehicles for oily injections (fixed oil), ingredients of ointment bases (liquid, soft and hard paraffin, wool fat, wool alcohol and beeswax and medical lubricant (glycerol).

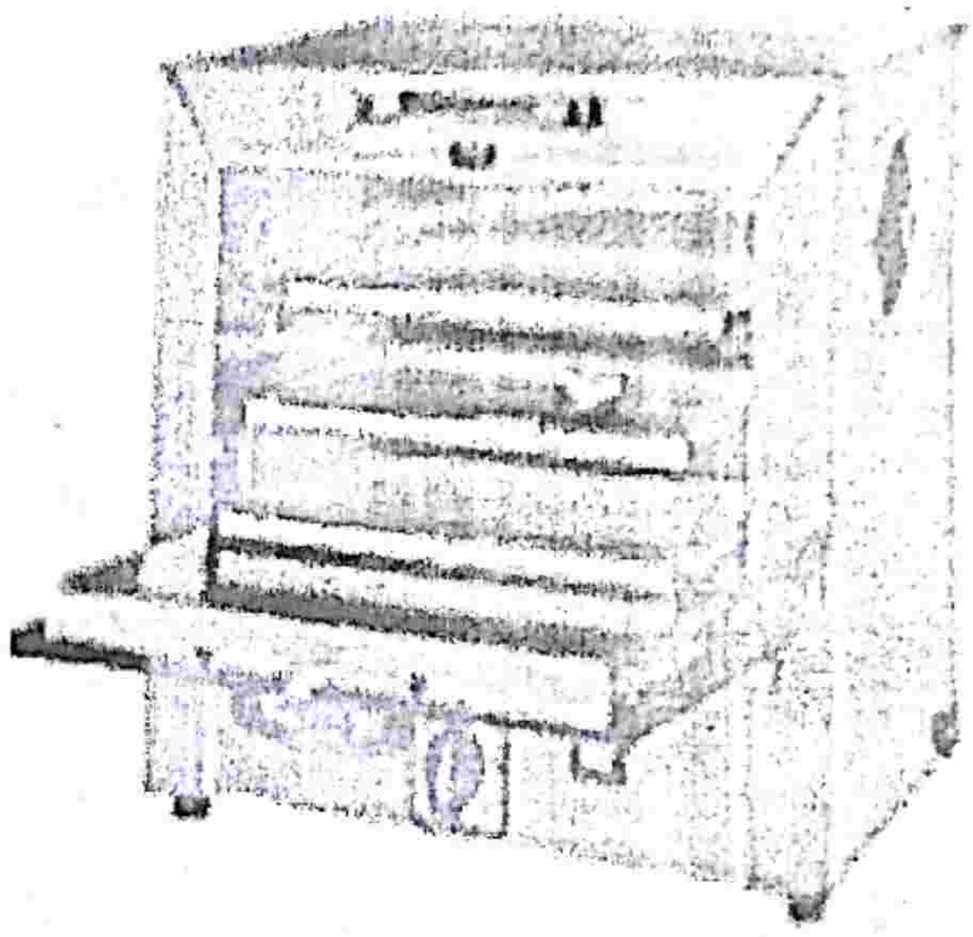
Some techniques that involve the use of dry heat includes hot- air oven, flaming, incineration and the use of glass bead sterilizers.

1. Hot Air Oven

In a hot- air oven, a high temperature of 140-170°C is required and a time of exposure of 2-4 hours. With longer exposure to lethal temperatures, the number of killed microorganisms increases. At higher temperatures, shorter exposure times are required to kill organisms. In hot air oven, the loads are placed in such a way as to permit the circulation of hot air. As the temperature rises from room temperature, the ventilating slide is opened to allow for the escape of residual moisture. Above 110°C, the vent is closed and the temperature rose to the desired sterilization level. Due to slow heat transfer, the exposure time need to be kept at not less than 2 hours.

There are basically two types of hot air oven: **the natural convention oven and the forced convention oven.**

In natural **convention oven,** air circulation within the oven chamber occur by the rising of heated air and the descent of cool air while in **the forced convention type,** circulation of heated air within the oven chamber and around the load occur by means of a fan.



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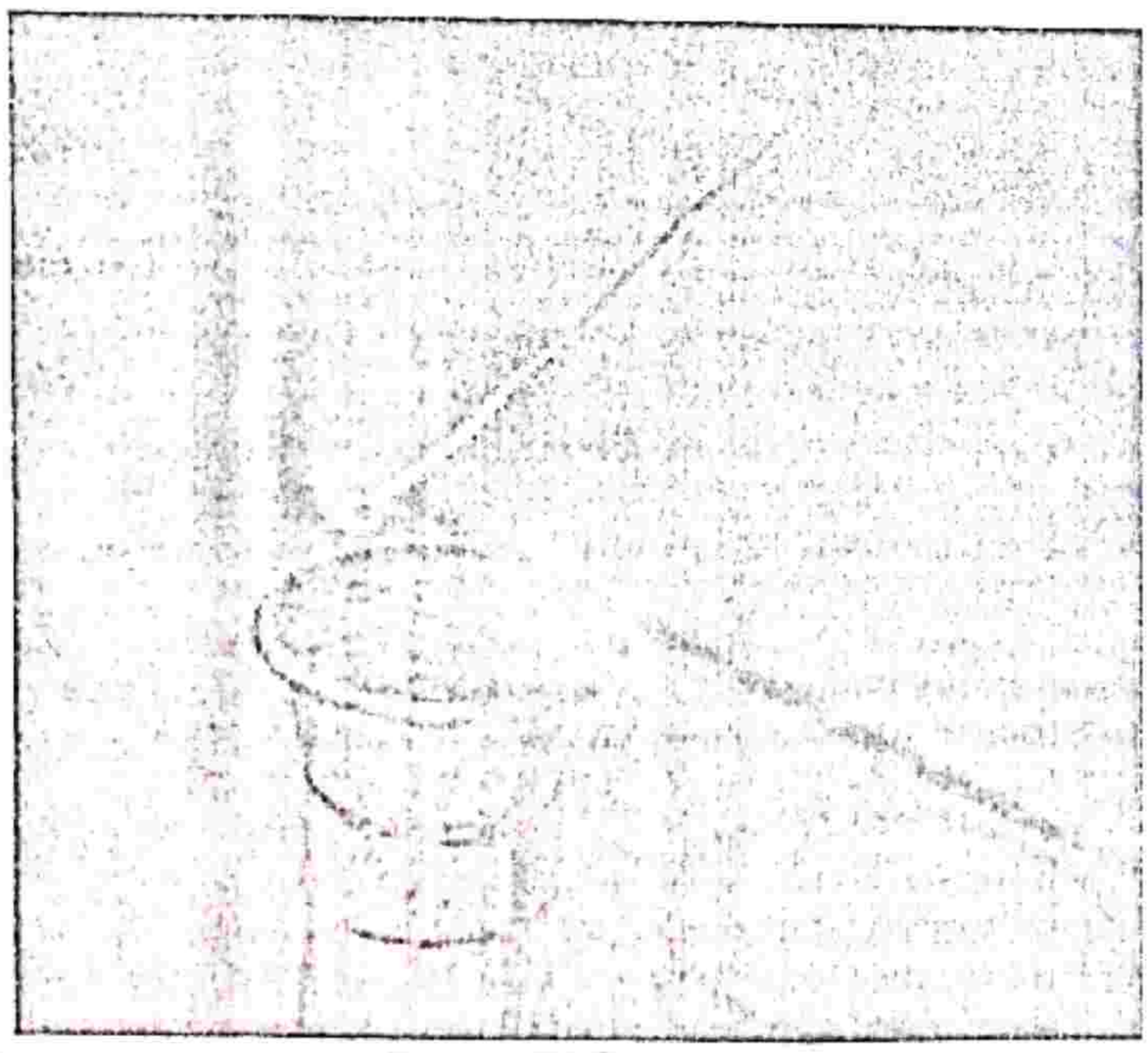
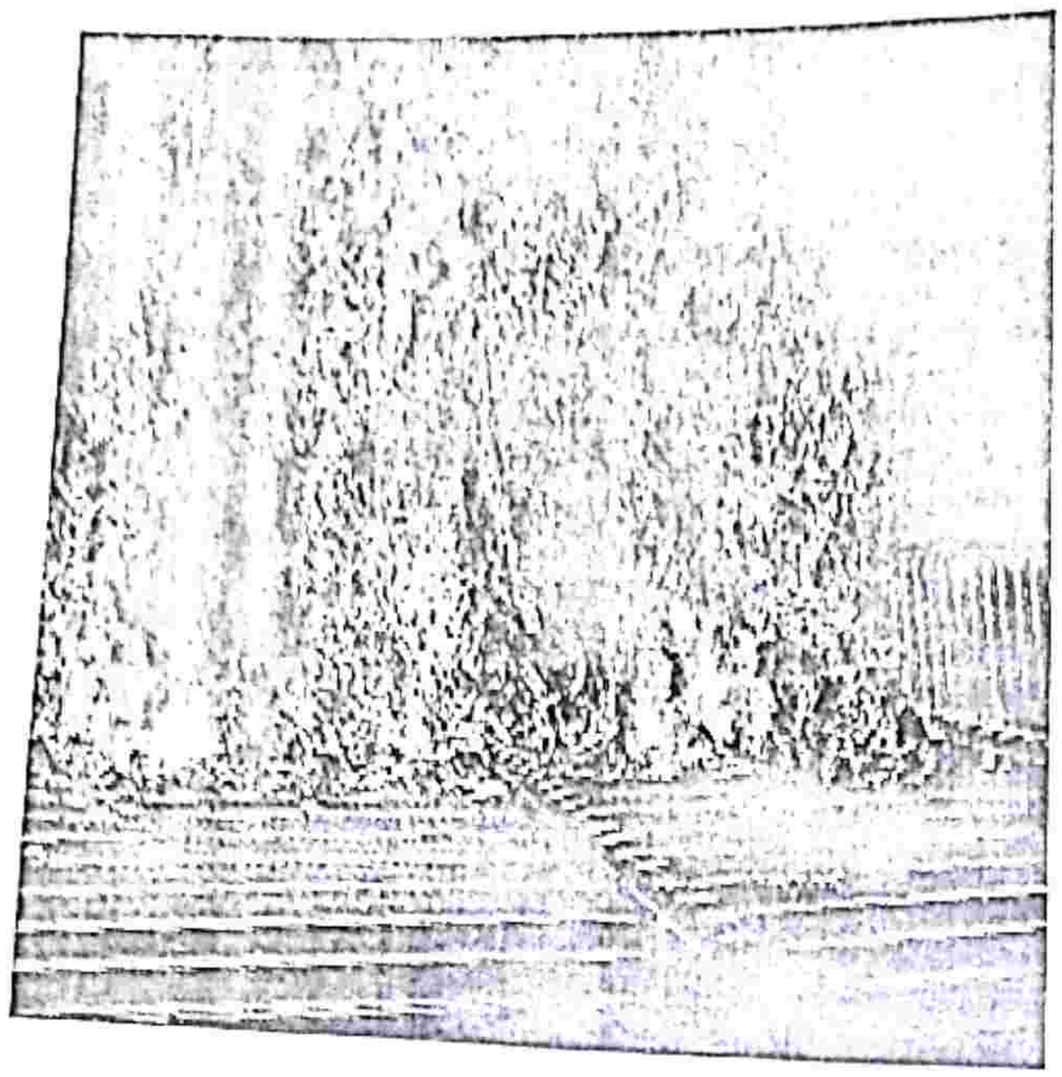
A. Flaming

Flaming is done to wire loops and straight-wires used in microbiology labs. Leaving the loop in the flame of a Bunsen burner or alcohol lamp until it glows red ensures that any infectious agent is inactivated. This is commonly used for small metal or glass objects, but not for large objects. However, during the initial heating infectious material may be sprayed from the wire surface before it is killed, contaminating nearby surfaces and objects. Therefore, special heaters have been developed that surround the inoculating loop with a heated cage, ensuring that such sprayed material does not further contaminate the area. Another problem is that gas flames may leave carbon or other residues on the object if the object is not heated enough. A variation on flaming is to dip the object in 70% or higher ethanol, then briefly touch the object to a Bunsen burner flame. The ethanol will ignite and burn off rapidly, leaving fewer residues than a gas flame.



B. Incineration

Incineration is a waste treatment process that involves the combustion of organic substances contained in waste materials. This method also burns any organism to ash. It is used to sterilize medical and other biohazardous waste before it is discarded with non-hazardous waste. An incinerator removes all infectious components and does not have long term environmental impact such as landfills. In – situ disposal can be done in an incinerator without having to worry about transportation over long distances. However, Incineration facilities are expensive to build, operate and maintain. They also require skilled staff to run and maintain them.Smoke and ash emitted by the chimneys of incinerators include acid gases, nitrogen oxide, heavy metals, particulates, and dioxin, which is a carcinogen.

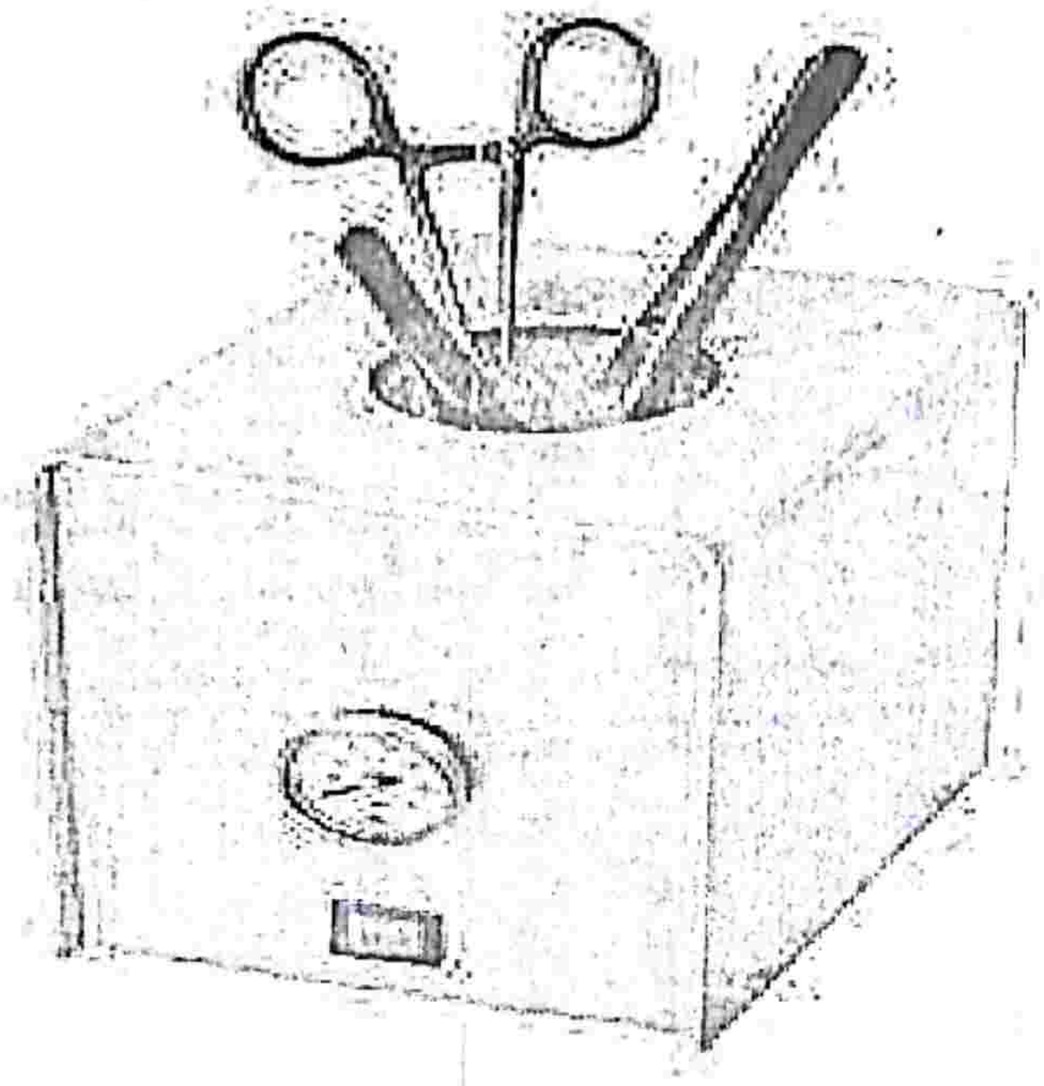


INTERIOR PART

C.Glass bead sterilizers

Glass bead sterilizers work by heating glass beads to 250 °C. Instruments are then quickly doused in these glass beads, which heat the object while physically scraping contaminants off their surface. Glass bead sterilizers were once a common sterilization method employed in dental offices as well as biological laboratories, but are not approved by the U.S. Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) to be used as a sterilizers since.

They are still popular in European as well as Israeli dental practices although there are no current evidence-based guidelines for using this sterilizer.



GLASS BEAD STERILIZATION

Advantages of Dry-heat sterilization

1. It can be used for substances that could be harmed by moisture e.g oily materials and powders.
2. It provides sufficient time for penetration of materials and therefore, suitable for assembled equipment such as glass syringes.

3. It is less damaging to glass and metal equipment than Moist heat. Repeated exposure of glass to moisture at high temperature clouding and alkali extraction.

Disadvantages of Dry-heat sterilization

1. The high temperature, long exposure and very long heating up times are not suitable for most medicaments, rubbers and plastics which are too thermo labile for sterilization by this process.
2. It is not suitable for surgical dressing.

B. Radiation

Sterilization can be achieved using electromagnetic radiation such as electron beams, X-rays, gamma rays, or irradiation by subatomic particles. The varying effects of radiation on cells depend on its wavelength, intensity and duration of exposure. There are two types of radiation which causes bactericidal effects on microbes: ionizing radiation and non-ionizing radiation.

Non-ionizing radiation sterilization

The most widely used form of non-ionising radiation is ultraviolet (UV) light. Wavelengths around 260nm are used because these are absorbed by the purine and pyrimidine components of nucleic acids, as well as certain aromatic amino acids in proteins. The absorbed energy causes a rupture of the chemical bonds, so that normal cellular function is impaired. UV light causes the formation of *thymine dimers* where adjacent thymine nucleotides on the same strand are linked together, inhibiting DNA replication. Viruses are much more susceptible. UV lamps are

commonly found in food preparation areas, operating theatres and specialist areas such as tissue culture facilities, where it is important to prevent contamination. Because they are also harmful to humans (particularly the skin and eyes), UV lamps can only be operated in such areas when people are not present. UV radiation has very poor penetrating powers; a thin layer of glass, paper or fabric is able to impede the passage of the rays. The chief application is therefore in the sterilisation of work surfaces and the surrounding air, although it is increasingly finding an application in the treatment of water supplies.

Ionizing radiation sterilization

Ionizing radiations are excellent sterilizing agents and penetrate deep into objects and can destroy bacterial spores and vegetative cells both prokaryotic and eukaryotic; however, they are not effective against viruses. Ionising radiations have a shorter wavelength (size<1 nm) and much higher energy, giving them greater penetration powers. Examples include electron beams, X-rays, gamma rays. The effect of ionising radiations is due to the production of highly reactive free radicals, which disrupt the structure of macromolecules such as DNA and proteins. Surgical supplies such as syringes, catheters and rubber gloves are commonly sterilised employing gamma (γ) rays from the isotope cobalt 60 (60Co). Gamma radiation has been approved for use in over 40 countries for the preservation of food, which it does not only by killing pathogens and spoilage organisms but also by inhibiting processes that lead to sprouting and ripening. Gamma radiation is used in situations where heat sterilisation would be inappropriate, because of undesirable effects on the texture, taste or appearance of the product. This mainly relates to fresh produce such as meat, poultry, fruit and vegetables. Irradiation is

not suitable for some foodstuffs, such as those with a high fat content, where unpleasant tastes and odours result. Ionising radiations have the great advantage over other methods of sterilisation that they can penetrate packaging.

C. Sterile filtration through a bacteria- proof filter

Many liquids such as solutions of antibiotics or certain components of culture media become chemically altered at high temperatures, so the use of any of the heat regimes described above is not appropriate. Rather than killing the microorganisms, an alternative approach is simply to isolate them. This can be done for liquids and gases by passing them through filters of an appropriate pore size. Filters used to be made from materials such as asbestos and sintered glass, but have been largely replaced by membrane filters, commonly made of nitrocellulose or polycarbonate. These can be purchased ready-sterilized and the liquid passed through by means of pressure or suction. Supplies of air or other gases can also be filter-sterilized in this way. A pore size of 0.22μm is commonly used; this will remove bacteria and yeasts; however, mycoplasma and viruses are able to pass through pores of this size. With a pore size 10 times smaller than this, only the smallest of viruses can pass through, so it is important that an appropriate pore size is chosen for any given task. A drawback with all filters, but especially those of a small pore size, is that they can become clogged easily. Filters in general are relatively expensive, and are not the preferred choice if alternative methods are avaiiable.

Air also can be sterilized by filtration. Two common examples are **N-95 disposable masks** used in hospitals and labs, and **cotton plugs** on culture vessels that let air in but keep microorganisms out. N-95 masks exclude 95% of particles

that are larger than 0.3 μm. Other important examples are **laminar flow biological safety cabinets ,** which employ **high-efficiency particulate air (HEPA) filters** (a type of depth filter) to remove 99.97% of particles 0.3 μm or larger. Laminar flow biological safety cabinets or hoods force air through HEPA filters, and then project a vertical curtain of sterile air across the cabinet opening. This protects a worker from microorganisms being handled within the cabinet and prevents contamination of the room. A person uses these cabinets when working with dangerous agents such as *M. tuberculosis* and tumor viruses. They are also employed in research labs and industries, such as the pharmaceutical industry, when a sterile working environment needed

Tests for sterility

Test for sterility seeks to determine whether the sterilized product is totally free of viable organisms. The practical parameter for judging the result of sterilization process is the level of **sterility assurance,** which is the probability that there is no contaminant in the product after the sterilization process. These tests are integral part of the preparation of sterile products such as parenteral products and water for injections.

Since the entire product batch cannot be used for the test, it is important to carry out the test on samples of the product selected randomly from the batch. The acceptability of conclusion made on the sterility status of the batch depends, to a large extent, on the number of items sampled.

However, below is the summary of BP 2001 specifications for the sterility test of parenteral products.

1. Size of samples for the tests.

The number of items to be used for the test depends on the batch sizes for various categories of sterile product. For parenterals, not less than 4 or 10 % is tested for batches having up to 100 items, at least 10 items for batches having between 101-500 items and at most 20 for batches having more than 500 items are recommended. For quantity required from each sample container, the BP states that if liquid and the volume is equal to 1 or 2 ml, the entire content should be used-half for aerobic bacteria and fungi and the remaining half for anaerobic bacteria.

2. Media

Three classes of media are essential for sterility tests- media for growing fungi, those for aerobic bacteria and media for anaerobic bacteria. These media must be adequately enriched to support the growth of even damaged cells.

Fungi- malt extract medium, sabouraud dextrose medium and soya bean medium.

Aerobic bacteria- peptone broth, protein digest medium, etc.

Anaerobic bacteria- cooked meat medium, thioglycollate medium, liver broth, etc.

The media for anaerobic bacteria must be heated at 100 °C for at least 10 minutes to expel dissolved oxygen, and then cooled to about 30°C before inoculating with the test sample.

3. Temperature of incubation

The inoculated media should be incubated at temperatures between 30- 35°C for 14 days for bacteria and at 20-25 °C for 14 days for fungi.

4. The p^H

No specific p^H is recommended for the incubation of heat- treated cells. However, p^H conditions between 6.0 and 8.0 should be used.

5. Presence of growth inhibitors (bactericides or preservatives)

If microbial growth inhibitors are present in the product being tested, it must be inactivated using specific quenching agents.

In carrying out sterility test, strict aseptic techniques are required in order to eliminate any chance of contamination from outside sources. For instance, special screens sterilized with ethylene oxide, uv radiation or sprayed with disinfectant can be used. Presently, laminar flow chamber is used. The required volume of the sample is transferred into the fungal, aerobic and anaerobic media using a sterile pipette. If the product to be tested is a solid, a solution or suspension of the solid is prepared by adding suitable sterile diluents such as water to the container. A volume equivalent to the weight specified for the test is used.

Control test should be carried out at the same time as the sample tests. The BP specifies the use of typed strains of *Staphylococcus aureus* or *Bacillus subtilis* for aerobic tests, *Clostridium sporogenes* for anaerobic tests and *Candida albicans* for fungi. Then, both the test and control are incubated as specified above for 14 days. The product is said to have passed the sterility test if growth of microorganism is not detected at the end of the test. If growth occurs, the sterility test is repeated using fresh samples obtained from the same number of containers and from the same batch. There is need for a second repeat test if there is no growth. The batch failed the test if growth of the same organism occurs in more than one test. If in two or more tests different organisms are detected, contamination may have occurred during the test. In any case, all the positive control cultures must show growth for the test to be valid.