Sterilisation

It can be defined as the process of removing all forms of all microorganisms including bacteria, spores, fungi and viruses. The term sterilisation can be applied to instruments and not to skin where only antisepsis can be achieved.

Methods of Sterilisation

The term sterilisation can only be applied to instruments, and not to skin, where only antisepsis can be achieved. A general principle is that all items used to penetrate soft tissue or bone, enter into or contact the bloodstream or other normally sterile tissues, should be sterilised and be sterile at the point of use. A sterility requirement for medical products means that the theoretical probability that a living organism will be present on an object after the sterilisation process is equal to or less than one in a million, so-called sterility assurance level (SAL) = 10 degree.

There are several ways of achieving sterilisation. Sterility may be achieved by:

1. Heat
2. Chemical
3. Ionising radiation.

Classification

- Chemical
- e.g: Alcohol, Phenol

- Physical
  - Heat
    - Low Heat.
    - e.g: Water bath, Vaccin bath

    - High Heat
      - Dry Heat.
      - e.g: Red heat, Flaming, Hot air oven, Glass bead sterilization

      - Moist Heat
        - Belos 100'c. e.g:
        - pasturization

        - At 100'c. e.g: Boiling,
        - Tyndalization

        - Above 100'c.
        - Autoclave

  - Radiation.
  - e.g: X-ray, UV-ray

- Filtration

Heat

Autoclave

Front-loading autoclave

A widely used method for heat sterilisation is the autoclave, sometimes called a converter or steam steriliser. Autoclaves use steam heated to 121-134 °C under pressure. To achieve sterility, the article is heated in a chamber by injected steam until the article reaches a time and temperature setpoint. Meantime almost all the air is removed from the chamber, because air is undesired in the moist heat sterilisation process (this is one trait that differs from a typical pressure cooker used for food cooking). The article is then held at that setpoint for a period of time which varies depending on the bioburden present on the article being sterilised and its resistance (D-value) to steam sterilisation. A general cycle would be anywhere between 3 and 15 minutes, (depending on the generated heat) at 121 °C at 15 pps, which is sufficient to provide a sterility assurance level of 10^{-4} for a product with a bioburden of 10^{6} and a D-value of 2.0 minutes. Following sterilisation, liquids in a pressurised autoclave must be cooled slowly to avoid boiling over when the pressure is released. This may be achieved by gradually depressurizing the sterilisation chamber and allowing liquids to evaporate under a negative pressure, while cooling the contents.

Dry heat

Dry heat steriliser

Dry heat was the first method of sterilisation, and is a longer process than moist heat sterilisation. The destruction of microorganisms through the use of dry heat is a gradual phenomenon. With longer exposure to lethal temperatures, the number of killed microorganisms increases. Forced ventilation of hot air can be used to increase the rate at which heat is transferred to an organism and reduce the temperature and amount of time needed to achieve sterility. At higher temperatures, shorter exposure times are required to kill organisms. This can reduce heat-induced damage to food products.

The standard setting for a hot air oven is at least two hours at 160 °C. A rapid method heats air to 190 °C for 6 minutes for unwrapped objects and 12 minutes for wrapped objects.[15][16] Dry heat has the advantage that it can be used on powders and other heat-stable items that are adversely affected by steam (e.g. it does not cause rusting of steel objects).

Flaming

Flaming is done to loops and straight-wires 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 (see Incineration below). 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 less residue than a gas flame.

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 sterilise medical and other biohazardous waste before it is discarded with non-hazardous waste. Bacteria incinerators are mini furnaces used to incinerate and kill off any micro organisms that may be on an inoculating loop or wire.

Tyndallization

Named after John Tyndall, Tyndallization[18] 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. The process involves boiling for a period (typically 20 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 sterilise non-nutritive substrates like water. Tyndallization is also ineffective against prions.

Glass bead sterilisers

Glass bead sterilisers 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 sterilisers were once a common sterilisation method employed in dental offices as well as biologic 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 steriliser since 1997. They are still popular in European as well as Israeli dental practices although there are no current evidence-based guidelines for using this steriliser.

Chemical sterilisation

Chemiclave

Chemicals are also used for sterilisation. Heating provides a reliable way to rid objects of all transmissible agents, but it is not always appropriate if it will damage heat-sensitive materials such as biological materials, fibre optics, electronics, and many plastics. In these situations chemicals, either as gases or in liquid form, can be used as sterilants. While the use of gas and liquid chemical sterilants avoids the problem of heat damage, users must ensure that the article to be sterilised is chemically compatible with the sterilant being used. In addition, the use of chemical sterilants poses new challenges for workplace safety, as the properties that make chemicals effective sterilisers usually make them harmful to humans.

Ethylene oxide

Ethylene oxide (EO, EtO) gas treatment is one of the common methods used to sterilise, pasteurise, or disinfect items because of its wide range of material compatibility. It is also used to process items that are sensitive to processing with other methods, such as radiation (gamma, electron beam, X-ray), heat (moist or dry), or other chemicals. Ethylene oxide treatment is the most common sterilisation method, used for approximately 70% of total sterilisations, and for over 50% of all disposable medical devices.

Nitrogen dioxide

Nitrogen dioxide (NO_2) gas is a rapid and effective sterilant for use against a wide range of microorganisms, including common bacteria, viruses, and spores. The unique physical properties of NO_2 gas allow for sterilant dispersion in an enclosed environment at room temperature and ambient pressure. The mechanism for lethality is the degradation of DNA in the spore core through nitration of the phosphate backbone, which kills the exposed organism as it absorbs NO_2. This degradation occurs at even very low concentrations of the gas. NO_2 has a boiling point of 21 °C at sea level, which results in a relatively high saturated vapour pressure at ambient temperature. Because of this, liquid NO_2 may be used as a convenient

source for the sterilant gas. Liquid NO_2 is often referred to by the name of its dimer, dinitrogen tetroxide (N_2O_4). Additionally, the low levels of concentration required, coupled with the high vapour pressure, assures that no condensation occurs on the devices being sterilised. This means that no aeration of the devices is required immediately following the sterilisation cycle. NO_2 is also less corrosive than other sterilant gases, and is compatible with most medical materials and adhesives.

Ozone

Ozone is used in industrial settings to sterilise water and air, as well as a disinfectant for surfaces. It has the benefit of being able to oxidise most organic matter. On the other hand, it is a toxic and unstable gas that must be produced on-site, so it is not practical to use in many settings.

Glutaraldehyde and formaldehyde

Glutaraldehyde and formaldehyde solutions (also used as fixatives) are accepted liquid sterilising agents, provided that the immersion time is sufficiently long. To kill all spores in a clear liquid can take up to 22 hours with glutaraldehyde and even longer with formaldehyde. The presence of solid particles may lengthen the required period or render the treatment ineffective. Sterilisation of blocks of tissue can take much longer, due to the time required for the fixative to penetrate. Glutaraldehyde and formaldehyde are volatile, and toxic by both skin contact and inhalation. Glutaraldehyde has a short shelf life (<2 weeks), and is expensive. Formaldehyde is less expensive and has a much longer shelf life if some methanol is added to inhibit polymerization to paraformaldehyde, but is much more volatile. Formaldehyde is also used as a gaseous sterilising agent; in this case, it is prepared on-site by depolymerization of solid paraformaldehyde. Many vaccines, such as the original Salk polio vaccine, are sterilised with formaldehyde.

Hydrogen peroxide

Hydrogen peroxide, in both liquid and as vaporised hydrogen peroxide (VHP), is another chemical sterilising agent. Hydrogen peroxide is a strong oxidant, which allows it to destroy a wide range of pathogens. Hydrogen peroxide is used to sterilise heat or temperature sensitive

articles such as rigid endoscopes. In medical sterilisation hydrogen peroxide is used at higher concentrations, ranging from around 35% up to 90%. The biggest advantage of hydrogen peroxide as a sterilant is the short cycle time. Whereas the cycle time for ethylene oxide may be 10 to 15 hours, some modern hydrogen peroxide sterilisers have a cycle time as short as 28 minutes.

Peracetic acid

Peracetic acid (0.2%) is a recognized sterilant by the FDA[39] for use in sterilising medical devices such as endoscopes.

Potential for chemical sterilisation of prions

Prions are highly resistant to chemical sterilisation. Treatment with aldehydes such as formaldehyde have actually been shown to increase prion resistance. Hydrogen peroxide (3%) for one hour was shown to be ineffective, providing less than 3 logs (10^{-3}) reduction in contamination. Iodine, formaldehyde, glutaraldehyde and peracetic acid also fail this test (one hour treatment). Only chlorine, phenolic compounds, guanidinium thiocyanate, and sodium hydroxide (NaOH) reduce prion levels by more than 4 logs; chlorine (too corrosive to use on certain objects) and NaOH are the most consistent. Many studies have shown the effectiveness of sodium hydroxide.

Radiation sterilisation

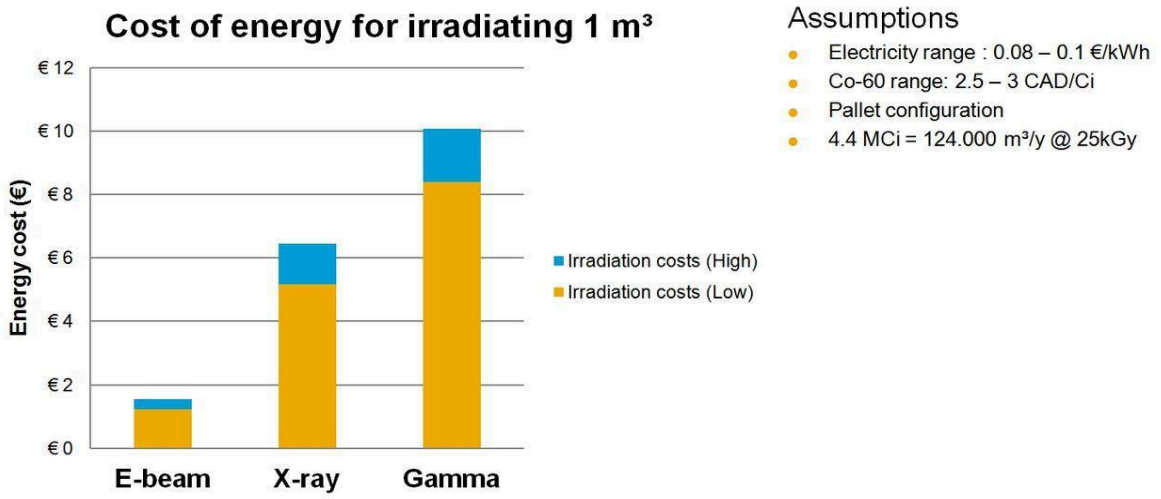
Sterilisation can be achieved using electromagnetic radiation such as electron beams, X-rays, gamma rays, or irradiation by subatomic particles.[41] Electromagnetic or particulate radiation can be energetic enough to ionise atoms or molecules (ionising radiation), or less energetic (non-ionizing radiation).

Non-ionizing radiation sterilisation

Ultraviolet light irradiation (UV, from a germicidal lamp) is useful for sterilisation of surfaces and some transparent objects. Many objects that are transparent to visible light absorb UV. UV irradiation is routinely used to sterilise the interiors of biological safety cabinets between

uses, but is ineffective in shaded areas, including areas under dirt (which may become polymerized after prolonged irradiation, so that it is very difficult to remove). It also damages some plastics, such as polystyrene foam if exposed for prolonged periods of time.

Ionising radiation sterilisation



Efficiency illustration of the different radiation technologies (electron beam, X-ray, gamma rays)

Sterile filtration

Fluids that would be damaged by heat, irradiation or chemical sterilisation, such as drug products, can be sterilised by microfiltration using membrane filters. This method is commonly used for heat labile pharmaceuticals and protein solutions in medicinal drug processing. A microfilter with pore size 0.2 μm will usually effectively remove microorganisms.[45] In the processing of biologics, viruses must be removed or inactivated, requiring the use of nanofilters with a smaller pore size (20 -50 nm) are used. Smaller pore sizes lower the flow rate, so in order to achieve higher total throughput or to avoid premature blockage, pre-filters might be used to protect small pore membrane filters.

Membrane filters used in production processes are commonly made from materials such as mixed cellulose ester or polyethersulfone (PES). The filtration equipment and the filters themselves may be purchased as pre-sterilized disposable units in sealed packaging, or must be sterilised by the user, generally by autoclaving at a temperature that does not damage the fragile filter membranes. To ensure proper functioning of the filter, the membrane filters are integrity tested post-use and sometimes before use. The non-destructive integrity test assures the filter is undamaged, and is a regulatory requirement.[46] Typically, terminal pharmaceutical sterile filtration is performed inside of a cleanroom to prevent contamination.

Preservation of sterility

A curette in sterile packaging.

Instruments that have undergone sterilisation can be maintained in such condition by containment in sealed packaging until use.

Aseptic technique is the act of maintaining sterility during procedures.