STERLIZATION AND DISINFECTION

Definition:

Sterilization means freeing of an article from all of its burden of microorganisms. It is an absolute term meaning if an article is sterilized not a single living microorganism should be present in it. Otherwise, it cannot be called sterilized.

Disinfection, on the other hand, means freeing of an article from all or a part of its burden of microorganisms. It is a relative terms, means two articles may be compared in terms of their relative level or degree of disinfection. Although by definition it is not necessary, sometimes disinfection processes may lead to complete removal or destruction of all living microorganisms in an article. In that case, it may be regarded as equivalent to sterilization.

Methods of sterilization:

The methods of sterilization can broadly be categorized as follows:

1. Sterilization by heat
2. Sterilization by filtration
3. Sterilization by irradiation
4. Sterilization by chemical disinfection

Sterilization by heat:

Sterilization by heat is one of the most commonly used methods in the microbiology laboratory. It is influenced by a number of intrinsic and extrinsic factors, such as:

(i) **Time and temperature relationship**: Time required for effective sterilization of an article is inversely proportional to the temperature to which it is heated. For speedy sterilization, the article has to be exposed to a very high temperature and on the other hand, low temperature sterilization will need a longer exposure. Therefore, the time of exposure shall be variable according to the temperature at which sterilization is done. However, while determining the temperature of exposure one has to take care of the heat tolerance of the material intended to sterilize. Usually, under dry heat the most effective time - temperature relationship is 160°C for 1 hour or 180°C for 30 minutes, and under moist heat the corresponding values are 121°C for 15 minutes or 110°C for 30-45 minutes.

(ii) Microbial load: The efficacy of a sterilization method employing heat directly depends on the initial microbial load of the article being sterilized. More the load higher is the requirement of exposure temperature or longer time of exposure. If the total microbial load of the article is apparently very high, it is better to reduce it by first employing methods like cleaning and washing with detergents before exposing the article to heat for sterilization.

(iii) Species, strain and spore forming ability of the microorganisms: The efficacy of heat sterilization is directly influenced by the type of microorganisms present in the article, i.e. species, strain and spore forming ability of the microorganisms, as there is usually a great variation among the microorganisms in respect of their heat tolerance.

Most of the vegetative bacteria (i.e. growing bacteria without spores) are killed under moist heat conditions when exposed to about 60-70°C for 15-30 minutes. Among the pathogens, one of the

most heat sensitive bacteria may be *Treponema pallidum*, which is killed at 60°C for 15 minutes. However, heat-resistant bacteria like *Bacillus stearothermophilus* may be resistant to around 80° for 15 minutes and can be killed only by boiling. Similarly among the pathogens, the most heat-resistant bacteria may be *Mycobacterium tuberculosis* and *Brucella abortus* that may be killed only at about 65°C when exposed for 30 minutes.

Bacterial spores are highly heat-resistant and require a higher temperature to destroy them. While spores of *Bacillus anthracis* may be destroyed by boiling at 100°C for 10 minutes, the most heat-resistant ones like *Clostridium botulinum* spores may be destroyed only when exposed to a very high temperature, 121°C for 15 minutes or 110°C for 30 minutes.

As dry heat less efficient that moist heat a means of sterilization, killing of bacteria and their spores may be done effectively only when exposed to a very high temperature, usually 160°C for 1 hour or 180°C for 30 minutes.

(iv) **Type and nature of material in which sterilization is done**: Presence of some substances like acids, bases, alcohols, aldehydes and detergents etc. enhances the efficacy of sterilization by heat and on the other hand, presence of organic compounds like carbohydrates, proteins and fats retards the process the sterilization. Hence, the efficacy of sterilization by heat also depends on the type and nature of material being sterilized.

There are two types of methods for sterilization by heat: methods employing dry heat (hot air) and methods employing moist heat (hot steam).

Sterilization by dry heat: There are basically four different methods of sterilization employing dry heat. They are:

(1) Application of red heat: Inoculation loops, points of scissors, forceps, scalpels etc. may be heated by exposing directly to the flame until red hot. The advantage of the method is in its promptness; however, the method is limited to highly heat-resistant metallic items only.

(2) Flaming: Surface of scalpels, scissors, searing spatula etc. may be instantly sterilized by holding directly over the flame for a few seconds moving gently to & fro so that the surface is sufficiently heated to kill all microorganisms present.
(3) Infra red radiation: Table tops and working surfaces may be sterilized by exposing to direct rays from an infra red lamp. Here the heating potential of infra red not the direct effect of the radiation is used as the sterilizing agent.

(4) Hot air oven: It is an electrically-operated thermostatically controlled equipment used commonly in the microbiology laboratory to routinely sterilize glassware, metallic items, pestle & mortar, mineral oils, sand for grinding tissue samples etc. It employs hot dry air as the sterilizing agent and highly effective when used at a temperature of 160°C for period of 1 hour. Care should be taken to make the glassware dry before putting them in the inner chamber of the hot air oven, as water droplets remaining on the surface of glassware may result in appearance of cracks in them. The glassware should also be plugged (in case of open mouthed vessels) and wrapped properly with paper before putting in the oven.

Mechanism of action of dry heat: Dry heat kills microorganisms by dehydration and destructive oxidation.

Hot air oven

Sterilization by moist heat:

Methods of sterilization by moist heat can broadly be discussed under three categories:

(i) Methods employing a temperature < 100°C
(ii) Methods employing a temperature of 100°C
(iii) Methods employing a temperature > 100°C

Methods employing a temperature < 100°C

(a) Pasteurization: This method was originally developed by Louis Pasteur as a means of sterilization of wine. However, today pasteurization is commonly used as the method of choice for sterilization of milk and dairy products. There are two methods of pasteurization of milk.
Holder or Batch method: In this method, milk is held at a temperature of 63-66°C for a period of 30 minutes. As a quantity of milk has to be held for a period and can be held in batches, so it is called holder or batch method.
High temperature short time (HTST) or Flash method: In this method, milk is sterilized by exposing to 72.4°C for 20 seconds. As sterilization is achieved within a few seconds or at a flash, it is called flash method.

Although traditionally milk has been sterilized in the household method by boiling in an open vessel, it usually results in deterioration of quality of milk due to denaturation of water soluble vitamins (Vitamin B complex and Vitamin C) and milk protein. Boiled milk also develops a burn flavor which is not relished by many people and therefore palatability is reduced. Pasteurization employs a time-temperature relationship that ensures killing of the most heat-resistant microorganisms (like *Mycobacterium tuberculosis* and *Brucella abortus*) that are commonly transmitted through milk and at the same time, allow maintenance of nutritional quality of milk and milk products.

(b) Hospital linens and clothes used by the patients are usually sterilized routinely by immersing them in hot water at about 70°C for 30-45 minutes in presence of a detergent. Detergents enhance the process of microbial killing and all common microbial contaminants can be easily eliminated by this process without destroying the fabrics.

Methods employing a temperature of 100°C: Methods of sterilization employing moist heat at a temperature of 100°C may broadly be categorized into two:

(a) Boiling at 100°C and (b) Steaming at 100°C

Boiling at 100°C: Metallic or glass instruments and appliances commonly used in a dispensary or hospital like scalpels, scissors, forceps, catheters, all-glass syringes, etc. are usually sterilized in the instrument-

sterilizer by boiling at 100°C for at least 10 minutes. Care should be taken so as to avoid the warm-up time in the period of exposure.

Steaming at 100°C: Hot steam is a very efficient sterilizing agent because of its high penetration power and can be employed in two different ways:

(i) Continuous steaming: Some laboratory media like Robertson's Cooked Meat Broth (RCMB) that cannot be sterilized by autoclaving owing to sensitivity of some of the ingredients to the high temperature of autoclave (121°C) can be sterilized by continuous exposure of the media to hot steam for a period of 90 minutes.

(ii) Intermittent steaming: Laboratory media can be sterilized by an alternative method in which media are exposed to hot steam for a period of 30-45 minutes daily for 3 consecutive days. This method is commonly called tyndallization in recognition to the scientist, John Tyndall who discovered it. On the first exposure, all vegetative organisms are killed. If spores are present, they germinate in the intervening period as the flask containing the media is kept at room temperature. These newly grown vegetative organisms are killed during the second exposure. Even if some spores remain after the second exposure to steam, their killing is ensured by the third exposure.

Methods employing a temperature > 100°C: Under normal atmospheric pressure, the maximum temperature of water is 100°C (boiling temperature) that remains constant even on further heating. However, according to Charles' Law, keeping the volume constant the temperature of a gaseous substance is directly proportional to pressure exerted on it. Water vapour or steam behaves like a gas and this property of steam is utilized to enhance the temperature beyond 100°C in a method called autoclaving. An autoclave is an electrically-operated equipment with a provision of adjusting the inner pressure with the help of a pressure gauze. The mechanism of the equipment is almost same as that of a household pressure cooker. Release of hot steam generated by heating water inside the autoclave is regulated through a valve and thereby inside pressure may be regulated with the help of the pressure gauze fitted on the lid. A pressure equivalent to 15 lb can give rise to a temperature of 121°C (similarly 10 lb is equivalent to 110°C) at which the materials intended to be sterilized are kept for 15-20 minutes. Laboratory media and reagents, drapers and aprons, metallic appliances with sharp edges, surgical instruments, rubber & plastic items, etc. are commonly sterilized by autoclaving. However, some laboratory media which contain heat-sensitive ingredients like sugar, antibiotics, blood, serum or urea cannot be sterilized by this method.

Mechanism of action of moist heat: Moist heat kills microorganisms by coagulation of proteins and enzymes. It is more efficient than dry heat due to the high penetration power of hot water or steam.

Autoclave

Sterlization by filtration:

Filtration is a process of sterilizing liquids by eliminating or removing the microorganisms present without necessarily killing them. There are four different types of microbial filters:

(i) Earthenware candles: Filter candles made of earth like Berkefield filter, Chamberlain filter etc. are commonly used in the household as drinking water filters. The pore diameter of the earthenware candles usually vary from 0.75 to 1 μm and hence can trap most of the bacteria and large viruses from water.

(ii) Asbestos filters: Filters made of asbestos pads are very efficient to remove all bacteria and viruses as the pore diameter is sufficiently less as to trap smaller microorganisms. Asbestos filter pads are used in Seitz filter, which is commonly used in microbiology laboratory to sterilize heat-sensitive liquids like solutions and media containing sugar, blood, serum, antibiotic, urea etc. Seitz filter is also commonly used to sterilize growth and maintenance media used for cell culture.

(iii) Cellulose membrane filters: Highly efficient microbial filters made of cellulose membrane are commercially available, which are now commonly used in the laboratory to sterilization of media and heat-sensitive liquids by filtration. They are available in various sizes and with variable pore diameters ranging from 0.01 to 0.1 μm.

(iv) Sintered glass filters: This type of filters is made of sintered glass or glass powder. Although efficient, not commonly available.

In addition to these four types of microbial filters, there is a type of air filter ***H***igh ***E***fficiency ***P***articulate ***A***ir (HEPA) filter, which is commonly used in the laminar flow which facilitates laboratory works conducted in an aseptic germ-free condition.

Laminar flow

Sterilization by irradiation: In addition to the visible spectrum of sunlight (VIBGYOR), it also contains other rays with lesser wavelength, which have great penetration power and microbicidal effect. Shorter the wavelength more is the microbicidal effect of these radiations. The range of microbicidal radiations can be classified into two categories:

(a) Non-ionizing radiation and
(b) Ionizing radiation

Non-ionizing radiation: Non-ionizing radiations are so called because they do not possess sufficient energy so as to ionize atoms on hitting. However, they do have high penetration power and microbicidal property. Example: ultra-violet ray.

Ultra-violet (UV) radiation is frequently used as a sterilizing agent particularly for sterilization of surfaces and for decontamination of a closed laboratory room or cubical. UV radiation to be effective as a

sterilization agent should have a wavelength of 280 nm or less. However, the wavelength of UV rays in sunlight varied between 10 to 400 nm. The lowest wavelength of UV radiation that reaches earth surface is usually above 280 nm and therefore sunlight has little microbicidal property. Living creatures on earth are protected from the deleterious effect of UV radiation with shorter wavelength by a number of factors including the ozone layer, cloud and smoke. Ultraviolet radiation with wavelength shorter than 2650 AU can be generated artificially with the help of Mercury vapour lamp and can be utilized for sterilization of surfaces and laboratories as mentioned above.

Ionizing radiation: Radiations of shorter wavelength such as gamma ray, cobalt ray etc. possess energy content > 100 eV and therefore can ionize atoms on hitting them. So they are called ionizing radiations. They have a very high microbicidal property and can be used effectively as sterilizing agents. However, owing to the carcinogenic and other deleterious effect on health on direct exposure to these radiations and also due to the high cost of installation of such facilities, they are not commonly used for day-to-day laboratory purposes. Although x-ray has high penetration power and is microbicidal, it is not commonly used as a sterilization agent owing to the difficulty in pointing or converging specifically at certain points. Ionizing radiation like gamma ray, cobalt ray, etc. are now-a-days commonly used by large manufacturing industries for instantaneous sterilization of prepackaged disposable plastic items in large-scale.

Mechanism of action: A common mode of microbicidal action of radiation is related to their capacity to destroy nucleic acids by inducing formation of thymine dimmers. UV radiation damages DNA by knocking electrons around and disrupting the chemical bonds that hold the DNA molecule's atoms together. Bacteria can repair DNA damage, but when the damage is massive, as is the case after a high dose of UV radiation, the cell dies before the damage can be repaired. A short exposure to UV light is not enough to kill a large population of bacteria. A moderate dose will kill many, cause permanent DNA mutations in some and leave others unharmed.

Sterilization by chemical disinfection:

Disinfection

Decontamination of laboratory benches, furniture, equipment and other materials requires the use of chemical disinfectants. Their activity is related to the following factors:

- concentration
- pH
- contact time
- humidity
- temperature
- presence of organic matter

Microorganisms show variable degree of resistance to chemical disinfectants and no single disinfectant is effective in all situationsThe following points should be considered when selecting a disinfectant:

- type of microorganisms, numbers and presence of spores
- physical situation (surface type, suspension, etc.)
- contact available between disinfectant and microorganisms
- possible interaction between disinfectant and materials
- contact time allowable
- concentration

Activity of Different Types of Disinfectants						
Toxicity Against	**Disinfectant**					
	Phenolic Compounds	**Hypochlorites**	**Alcohols**	**Formal-dehyde**	**Glutaral-dehyde**	**Iodophors**
Fungi	good	slight	nil	good	good	good
Bacteria (gram +/-)	good	good	good	good	good	good
Mycobacteria	fair	fair	good	good	good	good
Spores	nil	fair	nil	good (<40ºC)	good (<20ºC)	slight
Lipid Viruses	slight	slight	slight	slight	slight	slight
Non-Lipid Viruses	variable	slight	variable	slight	slight	slight
Skin	slight	slight	nil	slight	slight	slight
Eyes	slight	slight	slight	slight	slight	slight
Lungs	nil	slight	nil	slight	slight	nil

Common Disinfectants

Chlorine Compounds: Generally used in the form of sodium hypochlorite.

- Effective against a wide variety of microorganisms (vegetative bacteria and viruses). Preferred disinfectant for HIV and hepatitis viruses.
- Use at 0.1% as a general disinfectant.
- Less suitable in the presence of organic matter (such as blood). Concentration must be increased to retain action (0.5%).
- Effective between a pH range of 6-8.
- Strength decreases on standing (make fresh solutions daily).
- High concentrations corrode metal surfaces, and bleach and damage fabrics.

Alcohols: Ethanol (80% v/v ethyl alcohol) or 2-propanol (60-70% v/v iso-propyl alcohol) solutions are used to disinfect skin and decontaminate clean surfaces.

- Effective against fungi, vegetative bacteria, Mycobacterium species and some lipid-containing viruses.
- Not effective against spores.
- Most effective at 70% in water.
- May swell rubber or harden plastics.
- Do not use near flames due to flammability.

Iodine: Iodine vapour is highly toxic and is absorbed through the skin. Iodine is used in aqueous or alcoholic solution.

- Rapidly effective against most microorganisms.
- Usually diluted to 1% w/v free iodine, optimum pH neutral to acid.
- Not suitable in the presence of organic matter.
- Stains skin and may cause irritation.
- Dilute in alcohol for washing hands, or use as a sporicide.
- Prepare dilutions daily.
- Most commonly used for skin disinfection and decontaminating clean surfaces.
- Decomposes when heated above 40ºC.
- Do not use on aluminium or copper.

Formaldehyde: Precautions are required when handling formaldehyde. Formalin is 37% w/v formaldehyde gas in water.

- Active against most microorganisms.
- 13% v/v formalin is a good decontaminant (but has an irritating odour).
- 8% v/v formalin in 80% v/v alcohol is effective against vegetative bacteria, spores and viruses.
- Does not corrode stainless steel.
- Use to disinfect equipment such as centrifuges or biosafety cabinets.
- Formaldehyde can be used to disinfect rooms, cubicles and safety cabinets:
- Generated by heating paraformaldehyde suspended in silicone oil to 160ºC.
- A quantity of 5 grams formaldehyde should be used per cubic metre of space to be decontaminated.
- Requires relative humidity between 70% and 90% (humidity can be raised by evaporating water into the room).
- Formaldehyde can react with free chlorine to produce toxic gas. Remove hypochlorite solutions and hydrochloric acid from spaces to be decontaminated.
- Effect of formaldehyde can be neutralized with ammonia following decontamination.

Glutaraldehyde: Glutaraldehyde is known to cause dermatitis and asthma. Glutaraldehyde is commercially available as 2% w/v aqueous solution which must be made alkaline to "activate" (e.g. by addition of 0.3% sodium bicarbonate).

- Active against vegetative bacteria, spores, fungi and many viruses.
- Less irritating than formaldehyde, but may cause dermatitis. One should wear protective gloves when handling materials which have been immersed in glutaraldehyde.
- It should be discarded if turbid.

Chorohexidine: Chlorhexidine as chlorhexidine gluconate is dissolved in 70% alcohol.

- Use as antiseptic. Apply alcoholic chlorhexidine to the skin in the event of accidental contamination.
- Effective against Gram-positive organisms and HIV.
- Not recommended as a general disinfectant.
- Not active against sporulating bacteria or non-lipid-containing viruses.
- Active in ph range 5.5 - 8.0.
- Incompatible with soap and anionic detergents.

Hydrogen Peroxide: A concentration of 3% w/v generally used for disinfection.

- Active against a range of microorganisms.
- Fungi, spores and enteric viruses require higher concentration.
- No toxic end-products of decomposition.
- Do not use on aluminium, copper, zinc or brass.

Phenolics: Synthetic phenolics (clear soluble fluids) can be used as general disinfectants in the laboratory.

- Active against bacteria and lipid-containing viruses.
- Not active against spores and non-lipid-containing viruses.
- Active in presence of organic matter.
- Use for disinfecting floors, walls, benches and other furniture.

Quaternary Ammonium Compounds: Quaternary ammonium compounds are positively charged (cationic) surface-active disinfectants.

- Effective against Gram-positive bacteria and lipid-containing viruses.
- Not recommended as general disinfectants (they have a narrow antibacterial spectrum).
- Inactivated by proteins, soap and anionic detergents.