**Pharmaceutical Science and Technologies**

**Course number: 614**

**Assigned Topic: Sterilization.**

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Introduction to Sterilization:

Sterilization is defined as the process in which all microorganisms and bacterial spores are killed after germination. Sterilization can be achieved by chemical, physical and physio chemical mean.

Difference between disinfection and sterilization is Disinfection can kill pathogenic microorganisms but it can’t kill bacterial spores. While Sterilization method is used to kill such pathogenic microorganisms as well as bacterial spores. We can assume that sterilization is an absolute condition. The major difference between them is disinfection have different target range.

Some terminologies we used related to Sterilization:

* **Chemisterilants:**

These are chemicals used as sterilizing agent.

* **Disinfectants:**

These are chemical used in disinfection.

* **Decontamination:**

It is the process of removal of contaminating pathogenic microorganisms from the articles by a process of sterilization or disinfection.

* **Sanitization:**

It is the process of chemical or mechanical cleansing, applicable in public health systems.

* **Asepsis:**

It is the employment of techniques to achieve microbe-free environment.

* **Antisepsis:**

It is the use of chemicals to make skin or mucus membranes devoid of pathogenic microorganisms.

* **Bacteriostasis:**

It is a condition where the multiplication of the bacteria is inhibited without killing them.

* **Bactericidal:**

It is that chemical that can kill or inactivate bacteria. Such chemicals may be called variously depending on the spectrum of activity, such as bactericidal, virucidal, fungicidal, microbicidal, sporicidal, tuberculocidal or germicidal.

* **Antibiotics:**

These are substances produced by one microbe that inhibits or kills another microbe. Often the term is used more generally to include synthetic and semi-synthetic antimicrobial agents.

Some methods of disinfection such as filtration do not kill bacteria, they separate them out.

Types of Sterilization:

There are three types of sterilization.

1. Physical
2. Chemical
3. Physio-Chemical
4. Physical method of Sterilization:

This method is further divided into following sub classes.

1. **Sunlight:**

Sunlight is main source of sterilization due to the presence of ultra violet rays in it. It is responsible for spontaneous sterilization in natural conditions. In tropical countries, the sunlight is more effective in killing germs due to combination of ultraviolet rays and heat. By killing bacteria suspended in water, sunlight provides natural method of disinfection of water bodies such as tanks and lakes. But sunlight can’t kill spores.

1. **Heat:**

Heat acts by oxidative effects as well as denaturation and coagulation of proteins. Those articles that cannot withstand high temperatures can still be sterilized at lower temperature by prolonging the duration of exposure. That’s beneficial because articles which are heat sensitive can be exposed for short time period.

This class is further divided into two types.

1. **Moist heat:**

Moist heat acts by coagulation and denaturation of proteins.

This class is further divided into sub classes on the basis of temperature ranges.

1. **Temperature below 100°C:**

* **Pasteurization:**

There are two methods of pasteurization, the holder method (heated at 63°C for 30 minutes) and flash method (heated at 72°C for 15 seconds) followed by quickly cooling to 13°C.

This method is suitable to destroy most milk borne pathogens like Salmonella, Mycobacteria,Streptococci,Staphylococci and Brucella, however Coxiella may survive pasteurization. Efficacy is tested by phosphatase test and methylene blue test.

* **Vaccine bath:**

The contaminating bacteria in a vaccine preparation can be inactivated by heating in a water bath at 60°C for one hour. Only vegetative bacteria are killed and spores survive.

* **Serum bath:**

The contaminating bacteria in a serum preparation can be inactivated by heating in a water bath at 56°C for one hour on several successive days. Proteins in the serum will coagulate at higher temperature. Only vegetative bacteria are killed and spores survive.

* **Inspissation:**

This is a technique to solidify as well as disinfect egg and serum containing media. The medium containing serum or egg are placed in the slopes of an inspissator and heated at 80-85°C for 30 minutes on three successive days. On the first day, the vegetative bacteria would die and those spores that germinate by next day are then killed the following day. The process depends on germination of spores in between inspissation. If the spores fail to germinate then this technique cannot be considered sterilization.

1. **Temperature at 100°C:**

* **Boiling:**

Boiling water (100°C) kills most vegetative bacteria and viruses immediately. Certain bacterial toxins such as Staphylococcal enterotoxin are also heat resistant. Some bacterial spores are resistant to boiling and survive; hence this is not a substitute for sterilization. The killing activity can be enhanced by addition of 2% sodium bicarbonate. When absolute sterility is not required, certain metal articles and glassware can be disinfected by placing them in boiling water for 10-20 minutes. The lid of the boiler must not be opened during the period.

* **Steam at 100°C:**

Instead of keeping the articles in boiling water, they are subjected to free steam at 100°C. An autoclave can also serve the same purpose. A steamer is a metal cabinet with perforated trays to hold the articles and a conical lid. The bottom of steamer is filled with water and heated. The steam that is generated sterilizes the articles when exposed for a period of 90 minutes. Media such as TCBS, DCA and selenite broth are sterilized by steaming. Sugar and gelatin in medium may get decomposed on autoclaving, hence they are exposed to free steaming for 20 minutes for three successive days. This process is known as tyndallisation or fractional sterilization or intermittent sterilization. The vegetative bacteria are killed in the first exposure and the spores that germinate by next day are killed in subsequent days. The success of process depends on the germination of spores.

1. **Temperature above 100°C:**

* **Autoclave:**

Sterilization can be effectively achieved at a temperature above 100°C using an autoclave. Water boils at 100°C at atmospheric pressure, but if pressure is raised, the temperature at which the water boils also increases. In an autoclave the water is boiled in a closed chamber. As the pressure rises, the boiling point of water also raises. At a pressure of 15 lbs. inside the autoclave, the temperature is said to be 121°C. Exposure of articles to this temperature for 15 minutes sterilizes them. To destroy the infective agents associated with spongiform encephalopathies (prions), higher temperatures or longer times are used; 135°C or 121°C for at least one hour are recommended.

Advantages of steam:

It has more penetrative power than dry air, it moistens the spores (moisture is essential for coagulation of proteins), condensation of steam on cooler surface releases latent heat, condensation of steam draws in fresh steam.

Different types of autoclave:

* Simple “pressure-cooker type” laboratory autoclave
* Steam jacketed downward displacement laboratory autoclave
* high pressure pre-vacuum autoclave

1. **DRY HEAT:**
2. **Red heat:**

Articles such as bacteriological loops, straight wires, tips of forceps and searing spatulas are sterilized by holding them in Bunsen flame till they become red hot. This is a simple method for effective sterilization of such articles, but is limited to those articles that can be heated to redness in flame.

1. **Flaming:**

This is a method of passing the article over a Bunsen flame, but not heating it to redness. Articles such as scalpels, mouth of test tubes, flasks, glass slides and cover slips are passed through the flame a few times. Even though most vegetative cells are killed, there is no guarantee that spores too would die on such short exposure. This method too is limited to those articles that can be exposed to flame. Cracking of the glassware may occur.

1. **Incineration:**

This is a method of destroying contaminated material by burning them in incinerator. Articles such as soiled dressings; animal carcasses, pathological material and bedding etc should be subjected to incineration. This technique results in the loss of the article, hence is suitable only for those articles that have to be disposed. Burning of polystyrene materials emits dense smoke, and hence they should not be incinerated.

1. **Hot air oven:**

This method was introduced by Louis Pasteur. Articles to be sterilized are exposed to high temperature (160°C) for duration of one hour in an electrically heated oven. Since air is poor conductor of heat, even distribution of heat throughout the chamber is achieved by a fan. The heat is transferred to the article by radiation, conduction and convection. The oven should be fitted with a thermostat control, temperature indicator, meshed shelves and must have adequate insulation.

1. **Infra-red rays:**

Infrared rays bring about sterilization by generation of heat. Articles to be sterilized are placed in a moving conveyer belt and passed through a tunnel that is heated by infrared radiators to a temperature of 180°C.The articles are exposed to that temperature for a period of 7.5 minutes. Articles sterilized included metallic instruments and glassware. It is mainly used in central sterile supply department. It requires special equipment, hence is not applicable in diagnostic laboratory. Efficiency can be checked using Browne’s tube No.4 (blue spot).

1. **Vibrations:**

Sonic and ultra-sonic vibrations are used. Sound waves of frequency >20,000 cycle/second kills bacteria and some viruses on exposing for one hour. Microwaves are not particularly antimicrobial in themselves, rather the killing effect of microwaves are largely due to the heat that they generate. High frequency sound waves disrupt cells. They are used to clean and disinfect instruments as well as to reduce microbial load. This method is not reliable since many viruses and phages are not affected by these waves.

1. **RADIATION:**

Two types of radiation are used, ionizing and non-ionizing.

1. Non-ionizing rays are low energy rays with poor penetrative power while ionizing rays are high-energy rays with good penetrative power. Since radiation does not generate heat, it is termed "cold sterilization". In some parts of Europe, fruits and vegetables are irradiated to increase their shelf life up to 500 percent. Non-ionizing rays: Rays of wavelength longer than the visible light are non-ionizing. Microbicidal wavelength of UV rays lie in the range of 200-280 nm, with 260 nm being most effective. UV rays are generated using a high-pressure mercury vapor lamp. It is at this wavelength that the absorption by the microorganisms is at its maximum, which results in the germicidal effect. UV rays induce formation of thymine-thymine dimers, which ultimately inhibits DNA replication. UV readily induces mutations in cells irradiated with a non-lethal dose. Microorganisms such as bacteria, viruses, yeast, etc. that are exposed to the effective UV radiation are inactivated within seconds. Since UV rays don’t kill spores, they are considered to be of use in surface disinfection. UV rays are employed to disinfect hospital wards, operation theatres, virus laboratories, corridors, etc. Disadvantages of using uv rays include low penetrative power, limited life of the uv bulb, some bacteria have DNA repair enzymes that can overcome damage caused by uv rays, organic matter and dust prevents its reach, rays are harmful to skin and eyes. It doesn't penetrate glass, paper or plastic.
2. Ionizing rays are of two types, particulate and electromagnetic rays.
3. Electron beams are particulate in nature while gamma rays are electromagnetic in nature. High-speed electrons are produced by a linear accelerator from a heated cathode. Electron beams are employed to sterilize articles like syringes, gloves, dressing packs, foods and pharmaceuticals. Sterilization is accomplished in few seconds. Unlike electromagnetic rays, the instruments can be switched off. Disadvantage includes poor penetrative power and requirement of sophisticated equipment.
4. Electromagnetic rays such as gamma rays emanate from nuclear disintegration of certain radioactive isotopes (Co60, Cs137). They have more penetrative power than electron beam but require longer time of exposure. These high-energy radiations damage the nucleic acid of the microorganism. A dosage of 2.5 mega rads kills all bacteria, fungi, viruses and spores. It is used commercially to sterilize disposable petri dishes, plastic syringes, antibiotics, vitamins, hormones, glasswares and fabrics. Disadvantages include; unlike electron beams, they can’t be switched off, glasswares tend to become brownish, loss of tensile strength in fabric. Gamma irradiation impairs the flavour of certain foods. Bacillus pumilus E601 is used to evaluate sterilization process.
5. **FILTRATION:**

Filtration does not kill microbes, it separates them out. Membrane filters with pore sizes between 0.2-0.45 μm are commonly used to remove particles from solutions that can't be autoclaved. It is used to remove microbes from heat labile liquids such as serum, antibiotic solutions, sugar solutions, urea solution. Various applications of filtration include removing bacteria from ingredients of culture media, preparing suspensions of viruses and phages free of bacteria, measuring sizes of viruses, separating toxins from culture filtrates, counting bacteria, clarifying fluids and purifying hydatid fluid. Filtration is aided by using either positive or negative pressure using vacuum pumps. The older filters made of earthenware or asbestos are called depth filters.

**Different types of filters are:**

* **Earthenware filters:**

These filters are made up of diatomaceous earth or porcelain. They are usually baked into the shape of candle. Different types of earthenware filters are:

* Pasteur-Chamberland
* Berkefeld filter
* Mandler filter
* **Asbestos filters:**

These filters are made from chrysotile type of asbestos, chemically composed of magnesium silicate. They are pressed to form disc, which are to be used only once. The disc is held inside a metal mount, which is sterilized by autoclaving.

* **Sintered glass filters:**

These are made from finely ground glass that are fused sufficiently to make small particles adhere to each other. They are usually available in the form of disc fused into a glass funnel.Filters of Grade 5 have average pore diameter of 1-1.5 μm. They are washed in running water in reverse direction and cleaned with warm concentrated H2SO4 and sterilized by autoclaving.

* **Membrane filters:**

These filters are made from a variety of polymeric materials such as cellulose nitrate,cellulose diacetate, polycarbonate and polyester. The older type of membrane, called gradocol (graded colloidion) membrane was composed of cellulose nitrate. Gradocol membranes have average pore diameter of 3-10 μm. The newer ones are composed of cellulose diacetate. These membranes have a pore diameter ranging from 0.015 μm to 12 μm. These filters are sterilized by autoclaving.

* **Air Filters:**

Air can be filtered using HEPA (High Efficiency Particle Air) filters. They are usually used in biological safety cabinets.

HEPA filters are at least 99.97% efficient for removing particles >0.3 μm in diameter. Examples of areas where HEPA filters are used include rooms housing severelyneutropenic patients and those operating rooms designated for orthopedic implant procedures. HEPA filter efficiency is monitored with the dioctylphthalate (DOP) particle test using particles that are 0.3 μm in diameter.

1. **Chemical methods of Sterilization:**

Those chemicals that can sterilize are called chemisterilants. Those chemicals that can be safely applied over skin and mucus membranes are called antiseptics.

An ideal antiseptic or disinfectant should have following properties:

* Should have wide spectrum of activity
* Should be able to destroy microbes within practical period of time
* Should be active in the presence of organic matter
* Should make effective contact and be wettable
* Should be active in any pH
* Should be stable
* Should have long shelf life
* Should be speedy
* Should have high penetrating power
* Should be non-toxic, non-allergenic, non-irritative or non-corrosive
* Should not have bad odour
* Should not leave non-volatile residue or stain
* Efficacy should not be lost on reasonable dilution
* Should not be expensive and must be available easily

Such an ideal disinfectant is not yet available. The level of disinfection achieved depends on contact time, temperature, type and concentration of the active ingredient, the presence of organic matter, the type and quantum of microbial load. The chemical disinfectants at working concentrations rapidly lose their strength on standing.

Classification of disinfectants:

1. Based on consistency

a. Liquid (E.g., Alcohols, Phenols)

b. Gaseous (Formaldehyde vapor, Ethylene oxide)

1. Based on spectrum of activity

a. High level

b. Intermediate level

c. Low level

1. Based on mechanism of action

a. Action on membrane (E.g., Alcohol, detergent)

b. Denaturation of cellular proteins (E.g., Alcohol, Phenol)

c. Oxidation of essential sulphydryl groups of enzymes (E.g., H2O2, Halogens)

d. Alkylation of amino-, carboxyl- and hydroxyl group (E.g., Ethylene Oxide, Formaldehyde)

e. Damage to nucleic acids (Ethylene Oxide, Formaldehyde)

* **Alcohols**

**Mode of action:**

Alcohols dehydrate cells, disrupt membranes and cause coagulation of protein.

**Examples:**

Ethyl alcohol, isopropyl alcohol and methyl alcohol

**Application:** A 70% aqueous solution is more effective at killing microbes than absolute alcohols. 70% ethyl alcohol (spirit) is used as antiseptic on skin. Isopropyl alcohol is preferred to ethanol. It can also be used to disinfect surfaces.

**Disadvantages:** Skin irritant, volatile (evaporates rapidly), inflammable

* **Aldehydes**

**Mode of action:**

Acts through alkylation of amino-, carboxyl- or hydroxyl group, and probably damages nucleic acids. It kills all microorganisms, including spores.

**Examples:**

Formaldehyde, Gluteraldehyde

**Application:**

40% Formaldehyde (formalin) is used for surface disinfection and fumigation of rooms, chambers, operation theatres, biological safety cabinets, wards, sick rooms etc. Fumigation is achieved by boiling formalin, heating paraformaldehyde or treating formalin with potassium permanganate. It also sterilizes bedding, furniture and books. 10% formalin with 0.5% tetraborate sterilizes clean metal instruments. 2% gluteraldehyde is used to sterilize thermometers, cystoscopes, bronchoscopes, centrifuges, anasethetic equipments etc. An exposure of at least 3 hours at alkaline pH is required for action by gluteraldehyde.

**Disadvantages:**

Vapors are irritating (must be neutralized by ammonia), has poor penetration, leaves non-volatile residue, activity is reduced in the presence of protein. Gluteraldehyde requires alkaline pH and only those articles that are wet-able can be sterilized.

* **Phenol**

**Mode of action:**

Act by disruption of membranes, precipitation of proteins and inactivation of enzymes.

**Examples:**

5% phenol, 1-5% Cresol, 5% Lysol (a saponified cresol), hexachlorophene, chlorhexidine, chloroxylenol (Dettol)

**Applications:**

Phenols are coal-tar derivatives. They act as disinfectants at high concentration and as antiseptics at low concentrations. They are bactericidal, fungicidal, mycobactericidal but are inactive against spores and most viruses. They are not readily inactivated by organic matter. The corrosive phenolics are used for disinfection of ward floors, in discarding jars in laboratories and disinfection of bedpans. Chlorhexidine can be used in an isopropanol solution for skin disinfection, or as an aqueous solution for wound irrigation. It is often used as an antiseptic hand wash. 20% Chlorhexidine gluconate solution is used for pre-operative hand and skin preparation and for general skin disinfection. Chlorhexidine gluconate is also mixed with quaternary ammonium compounds such as cetrimide to get stronger and broader antimicrobial effects (eg. Savlon). Chloroxylenols are less irritant and can be used for topical purposes and are more effective against gram positive bacteria than gram negative bacteria. Hexachlorophene is chlorinated diphenyl and is much less irritant. It has marked effect over gram positive bacteria but poor effect over gram negative bacteria, mycobacteria, fungi and viruses. Triclosan is an organic phenyl ether with good activity against gram positive bacteria and effective to some extent against many gram negative bacteria including Pseudomonas. It also has fair activity on fungi and viruses.

**Disadvantages:**

It is toxic, corrosive and skin irritant. Chlorhexidine is inactivated by anionic soaps. Chloroxylenol is inactivated by hard water.

* **Halogens**

**Mode of action:**

They are oxidizing agents and cause damage by oxidation of essential sulfydryl groups of enzymes. Chlorine reacts with water to form hypochlorous acid, which is microbicidal.

**Examples:**

Chlorine compounds (chlorine, bleach, hypochlorite) and iodine compounds (tincture iodine, iodophores)

**Applications:**

Tincture of iodine (2% iodine in 70% alcohol) is an antiseptic. Iodine can be combined with neutral carrier polymers such as polyvinylpyrrolidone to prepare iodophores such as povidone-iodine. Iodophores permit slow release and reduce the irritation of the antiseptic. For hand washing iodophores are diluted in 50% alcohol. 10% Povidone Iodine is used undiluted in pre and postoperative skin disinfection. Chlorine gas is used to bleach water. Household bleach can be used to disinfect floors. Household bleach used in a stock dilution of 1:10. In higher concentrations chlorine is used to disinfect swimming pools. 0.5% sodium hypochlorite is used in serology and virology. Used at a dilution of 1:10 in decontamination of spillage of infectious material. Mercuric chloride is used as a disinfectant.

**Disadvantages:**

They are rapidly inactivated in the presence of organic matter. Iodine is corrosive and staining. Bleach solution is corrosive and will corrode stainless steel surfaces.

* **Heavy Metals**

**Mode of action:**

Act by precipitation of proteins and oxidation of sulfydryl groups. They are bacteriostatic.

**Examples:**

Mercuric chloride, silver nitrate, copper sulfate, organic mercury salts (e.g., mercurochrome, merthiolate)

**Applications:** 1% silver nitrate solution can be applied on eyes as treatment for opthalmia neonatorum.This procedure is no longer followed. Silver sulphadiazine is used topically to help to prevent colonization and infection of burn tissues. Mercurials are active against viruses at dilution of 1:500 to 1:1000. Merthiolate at a concentration of 1:10000 is used in preservation of serum. Copper salts are used as a fungicide.

**Disadvantages:**

Mercuric chloride is highly toxic, are readily inactivated by organic matter.

* **Surface Active Agents**

**Mode of actions:**

They have the property of concentrating at interfaces between lipid containing membrane of bacterial cell and surrounding aqueous medium. These compounds have long chain hydrocarbons that are fat soluble and charged ions that are water-soluble. Since they contain both of these, they concentrate on the surface of membranes. They disrupt membrane resulting in leakage of cell constituents.

**Examples:**

These are soaps or detergents. Detergents can be anionic or cationic. Detergents containing negatively charged long chain hydrocarbon are called anionic detergents. These include soaps and bile salts. If the fat-soluble part is made to have a positive charge by combining with a quaternary nitrogen atom, it is called cationic detergents. Cationic detergents are known as quaternary ammonium compounds (or quat). Cetrimide and benzalkonium chloride act as cationic detergents.

**Application:**

They are active against vegetative cells, Mycobacteria and enveloped viruses. They are widely used as disinfectants at dilution of 1-2% for domestic use and in hospitals.

**Disadvantages:**

Their activity is reduced by hard water, anionic detergents and organic matter. Pseudomonas can metabolise cetrimide, using them as a carbon, nitrogen and energy source.

* **Dyes**

**Mode of action:**

Acridine dyes are bactericidal because of their interaction with bacterial nucleic acids.

**Examples:**

Aniline dyes such as crystal violet, malachite green and brilliant green. Acridine dyes such as acriflavin and aminacrine. Acriflavine is a mixture of proflavine and euflavine. Only euflavine has effective antimicrobial properties. A related dye, ethidium bromide, is also germicidal. It intercalates between base pairs in DNA. They are more effective against gram positive bacteria than gram negative bacteria and are more bacteriostatic in action.

**Applications:**

They may be used topically as antiseptics to treat mild burns. They are used as paint on the skin to treat bacterial skin infections. The dyes are used as selective agents in certain selective media.

* **Hydrogen Peroxide**

**Mode of action:**

It acts on the microorganisms through its release of nascent oxygen. Hydrogen peroxide produces hydroxyl-free radical that damages proteins and DNA.

**Application:**

It is used at 6% concentration to decontaminate the instruments, equipment such as ventilators. 3% Hydrogen Peroxide Solution is used for skin disinfection and deodorising wounds and ulcers. Strong solutions are sporicidal.

**Disadvantages:**

Decomposes in light, broken down by catalase, proteinaceous organic matter drastically reduces its activity.

* **Ethylene Dioxide**

**Mode of action:**

It is an alkylating agent. It acts by alkylating sulfydryl-, amino-, carboxyl- and hydroxyl- groups.

**Properties:** It is a cyclic molecule, which is a colorless liquid at room temperature. It has a sweet ethereal odor, readily polymerizes and is flammable.

**Application:**

It is a highly effective chemisterilant, capable of killing spores rapidly. Since it is highly flammable, it is usually combined with CO2 (10% CO2+ 90% EO) or dichlorodifluoromethane. It requires presence of humidity. It has good penetration and is well absorbed by porous material. It is used to sterilize heat labile articles such as bedding, textiles, rubber, plastics, syringes, disposable petri dishes, complex apparatus like heart-lung machine, respiratory and dental equipments. Efficiency testing is done using Bacillus subtilis var niger.

**Disadvantages:**

It is highly toxic, irritating to eyes, skin, highly flammable, mutagenic and carcinogenic.

* **Beta Propiolactone:**

**Mode of action:**

It is an alkylating agent and acts through alkylation of carboxyl- and hydroxyl- groups.

**Properties:**

It is a colorless liquid with pungent to slightly sweetish smell. It is a condensation product of ketane with

formaldehyde.

**Application:**

It is an effective sporicidal agent, and has broad-spectrum activity. 0.2% is used to sterilize biological

products. It is more efficient in fumigation that formaldehyde. It is used to sterilize vaccines, tissue grafts, surgical

instruments and enzymes

**Disadvantages:**

It has poor penetrating power and is a carcinogen.

1. **Physio-Chemical Method:**

**Mode of action:**

A physio-chemical method adopts both physical and chemical method. Use of steam-

formaldehyde is a physio-chemical method of sterilization, which takes into account action of steam as well as that of formaldehyde. Saturated steam at a pressure of 263 mm has a temperature of 70 °C. The air is removed from the

autoclave chamber and saturated steam at sub-atmospheric pressure is flushed in. Formaldehyde is then injected with steam in a series of pulses, each of 5-10 minutes. The articles are held at this holding temperature for one hour. Formaldehyde is then flushed by inflow of steam.

**Disadvantages:**

Condensation of formaldehyde occurs and induction of large volume of formaldehyde wets the steam resulting in loss of latent heat.

**Conditions of Sterilization:**

Equipment usually used for sterilization is called autoclave. A simple autoclave has vertical or horizontal cylindrical body with a heating element, a perforated try to keep the articles, a lid that can be fastened by screw clamps, a pressure gauge, a safety valve and a discharge tap. The articles to be sterilized must not be tightly packed. The screw caps and cotton plugs must be loosely fitted. The lid is closed but the discharge tap is kept open and the water is heated. As the water starts boiling, the steam drives air out of the discharge tap. When all the air is displaced and steam start appearing through the discharge tap, the tap is closed. The pressure inside is allowed to rise upto 15 lbs per square inch. At this pressure the articles are held for 15 minutes, after which the heating is stopped and the autoclave is allowed to cool. Once the pressure gauge shows the pressure equal to atmospheric pressure, the discharge tap is opened to let the air in. The lid is then opened and articles removed.

**Articles sterilized:**

Culture media, dressings, certain equipment, linen etc.

**Precautions:**

Articles should not be tightly packed, the autoclave must not be overloaded, air discharge must be complete and there should not be any residual air trapped inside, caps of bottles and flasks should not be tight, autoclave must not be opened until the pressure has fallen or else the contents will boil over, articles must be wrapped in paper to prevent drenching, bottles must not be overfilled.

**Advantage:**

Very effective way of sterilization, quicker than hot air oven.

**Disadvantages:**

Drenching and wetting or articles may occur, trapped air may reduce the efficacy, takes long time to cool.

**Sterilization control:**

Physical method includes automatic process control, thermocouple and temperature chart recorder. Chemical method includes Browne’s tube No.1 (black spot) and succinic acid (whose melting point is 121 °C) and Bowie Dick tape. Bowie Dick tape is applied to articles being autoclaved. If the process has been satisfactory, dark brown stripes will appear across the tape. Biological method includes a paper strip containing 106 spores of Geobacillus stearothermophilus.

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**Factors affecting Sterilization:**

* **Nature of heat:**

Moist heat is more effective than dry heat

* **Temperature and time:**

Temperature and time are inversely proportional. As temperature increases the time taken decreases.

* **Number of microorganisms:**

More the number of microorganisms, higher the temperature or longer the duration required.

* **Nature of microorganism:**

Depends on species and strain of microorganism, sensitivity to heat may vary. Spores are highly resistant to heat.

* **Type of material:**

Articles that are heavily contaminated require higher temperature or prolonged exposure. Certain heat sensitive articles must be sterilized at lower temperature.

* **Presence of organic material:**

Organic materials such as protein, sugars, oils and fats increase the time required.

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