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Sterilization and Disinfection

Sterilization is defined as the process where all the living micro - organisms, including bacterial spores are killed. Sterilization can be achieved by physical, chemical and physiochemical means. Chemicals used as sterilizing agents are called chemisterilants.

Disinfection is the process of elimination of most pathogenic micro-organisms (excluding bacterial spores) on inanimate objects. Disinfection can be achieved by physical or chemical methods. Chemicals used in disinfection are called disinfectants. Different disinfectants have different target ranges, not all disinfectants can kill all microorganisms. Some methods of disinfection such as filtration do not kill bacteria, they separate them out. Sterilization is an absolute condition while disinfection is not. The two are not synonymous. Decontamination is the process of removal of contaminating pathogenic microorganisms from the articles by a process of sterilization or disinfection. It is the use of physical or chemical means to remove, inactivate, or destroy living organisms on a surface so that the organisms are no longer infectious.

Sanitization is the process of chemical or mechanical cleansing,applicable in public health systems. Usually used by the food industry. It reduces microbes on eating utensils to safe, acceptable levels for public health.

Asepsis is the employment of techniques (such as usage of gloves, air filters, uv rays etc) to achieve microbe-free environment.

Antisepsis is the use of chemicals (antiseptics) to make skin or mucus membranes devoid of pathogenic microorganisms.

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

Bactericidal 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 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.

PHYSICAL METHODS OF STERILIZATION:

Sunlight: The microbicidal activity of sunlight is mainly 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 © Sridhar Rao P.N (www.microrao.com) 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. Sunlight is not sporicidal, hence it does not sterilize.

Heat:

Heat is considered to be most reliable method of sterilization of articles that can withstand 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.

Factors affecting sterilization by heat are:

o Nature of heat: Moist heat is more effective than dry heat

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

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

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

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

o Presence of organic material: Organic materials such as protein, sugars, oils and fats increase the time required.

Action of heat:

Dry heat acts by protein denaturation, oxidative damage and toxic effects of elevated levels of electrolytes. The moist heat acts by coagulation and denaturation of proteins. Moist heat is superior to dry heat in action. Temperature required to kill microbe by dry heat is more than the moist heat. Thermal death time is the minimum time required to kill a suspension of organisms at a predetermined temperature in a specified environment.

DRY HEAT:

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.

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.

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.

Hot air oven: This method was introduced by Louis Pasteur. Articles to be sterilized are exposed to high temperature (160o 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.

Articles sterilized: Metallic instruments (like forceps, scalpels, scissors), glasswares (such as petri-dishes, pipettes, flasks, all-glass syringes), swabs, oils, grease, petroleum jelly and some pharmaceutical products.

Sterilization process: Articles to be sterilized must be perfectly dry before placing them inside to avoid breakage. Articles must be placed at sufficient distance so as to allow free circulation of air in between. Mouths of flasks, test tubes and both ends of pipettes must be plugged with cotton wool. Articles such as petri dishes and pipettes may be arranged inside metal canisters and then placed. Individual glass articles must be wrapped in kraft paper or aluminum foils.

Sterilization cycle: This takes into consideration the time taken for the articles to reach the sterilizing temperature, maintenance of the sterilizing temperature for a defined period (holding time) and the time taken for the articles to cool down. Different temperature-time relations for holding time are

60 minutes at 160o

C, 40 minutes at 170oC and

20 minutes at 180o C

Increasing temperature by 10 degrees shortens the sterilizing time by 50 percent. The hot air oven must not be opened until the temperature inside has fallen below 60o C to prevent breakage of glasswares. Sterilization control: Three methods exist to check the efficacy of sterilization process, namely physical, chemical and biological

Physical:

Temperature chart recorder and thermocouple.

Chemical: Browne's tube No.3 (green sp Chemical: Browne's tube No.3 (green spot, color changes from red to green)

Biological: 106 spores of Bacillus subtilis var niger or Clostridium tetani on paper strips are placed inside envelopes and then placed inside the hot air oven. Upon completion of sterilization cycle, the strips are removed and inoculated into thioglycollate broth or cooked meat medium and incubated at 37o C for 3-5 days. Proper sterilization should kill the spores and there should not be any growth.

Advantages: It is an effective method of sterilization of heat stable articles. The articles remain dry after sterilization.

This is the only method of sterilizing oils and powders.

Disadvantages: Since air is poor conductor of heat, hot air has poor penetration. ƒ Cotton wool and paper may get slightly charred. Glasses may become smoky.

Takes longer time compared to autoclave.

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 180o 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 equipments, hence is not applicable in diagnostic laboratory. Efficiency can be checked using Browne's tube No.4 (blue spot).

MOIST HEAT: Moist heat acts by coagulation and denaturation of proteins.

At temperature below 100o C:

Pasteurization: This process was originally employed by Louis Pasteur. Currently this procedure is employed in food and dairy industry. There are two methods of pasteurization, the holder method (heated at 63o C for 30 minutes) and flash method (heated at 72o C for 15 seconds) followed by quickly cooling to 13o C. Other pasteurization methods include Ultra-High Temperature (UHT), 140o C for 15 sec and 149o C for 0.5 sec. 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 60o 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 56o 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 80-85o 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.

At temperature 100o C:

Boiling: Boiling water (100o 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 glasswares 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 100o C: Instead of keeping the articles in boiling water, they are subjected to free steam at 100o C.

Traditionally Arnold's and Koch's steamers were used. An autoclave (with discharge tap open) 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 (after John Tyndall) 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.

At temperature above 100oC autoclave: Sterilization

Autoclave: Sterilization can be effectively achieved at a temperature above 100o C using an autoclave. Water boils at 100o 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 121o 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; 135o C or 121o 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 and high pressure pre-vacuum autoclave .

Construction And Operation Of 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 121o 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.

RADIATION:

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

Ionizing rays:

Ionizing rays are of two types, particulate and electromagnetic rays. Electron beams are particulatee in nature while gamma rays are electromagnetic in nature .High speed electrons are produced by a linear accelerator from a heated cathode 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 beswitched off. Disadvantage includes poor penetrative power and requirement of sophisticated equipment.

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 megarads 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

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:

1. 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:

a. Pasteur-Chamberland filter: These candle filters are from France and are made up of porcelain (sand and kaolin). Similar filter from Britain is Doulton. Chamberland filters are made with various porosities, which are graded as L1, L1a, L2, L3, L5, L7, L9 and L11. Doulton filters are P2, P5 and P11.

b. Berkefeld filter: These are made of Kieselguhr, a fossilized diato - maceous earth found in Germany.

They are available in three grades depending on their porosity (pore size); they are V (veil), N (normal) and W (wenig). Quality of V grade filter is checked using culture suspension of Serrtia marcescens (0.75 µm).

c. Mandler filter: This filter from America is made of kieselguhr, asbestos and plaster of Paris.

2. 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. They are available in following grades; HP/PYR (for removal of pyrogens), HP/EKS (for absolute sterility) and HP/EK (for claryfying).

3. 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.

4. 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. Membrane filters are made in two ways, the capillary pore membranes have pores produced by radiation while the labyrinthine pore membranes are produced by forced evaporation of solvents fromcellulose esters.

The disadvantages of depth filters are migration of filter material into the filtrate, absorption or retention of certain volume of liquid by the filters, pore sizes are not definite and viruses and mycoplasma could pass through. The advantages of membrane filters are known porosity, no retention of fluids, reusable after autoclaving and compatible with many chemicals. However, membrane filters have little loading capacity and are fragile.

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 severely neutropenic patients and those operating rooms designated for orthopedic implant procedures. HEPA filter efficiency is monit

SONIC AND ULTRASONIC VIBRATIONS:

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.

CHEMICAL METHODS OF DISINFECTION:

Disinfectants are those chemicals that destroy pathogenic bacteria from inanimate surfaces. Some chemical have very narrow spectrum of activity and some have very wide. 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 ƒ 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)

2. Based on spectrum of activity

a. High level

b. Intermediate level

c. Low level

3. 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. It is used to disinfect clinical thermometers. Methyl alcohol kills fungal spores, hence is useful in disinfecting inoculation hoods.

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. 2% formaldehyde at 40o C for 20 minutes is used to disinfect wool and 0.25% at 60o C for six hours to disinfect animal hair and bristles.

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 wettable 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: Joseph Lister used it to prevent infection of surgical wounds. 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 (Crede's method). 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, equipments 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 OXIDE (EO):

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 dichloro -difluoromethane. 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 (BPL):

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.

PHYSIO-CHEMICAL METHOD:

Mode of action: A physio-chemical methodadopts both physical and chemical method. formaldehyde is a physio-chemical method of sterilizationwhich takes into account action of steam as well as that of formaldehydeSaturated steam at a pressure of 263 mm has a temperature of 70o 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 llarge volume of formaldehyde wets the steam resulting in loss of latent heat. Sterilization control: using paper strips containing 106 spores of G.stearothermophilus.

TESTING OF DISINFECTANTS:

A disinfectant must be tested to know the required effective dilution, the time taken to effect disinfection and to periodically monitor its activity. As disinfectants are known to lose their activity on standing as well as in the presence of organic matter, their activity must be periodically tested.

Different methods are:

1. Koch's method
2. Rideal Walker Method
3. Chick Martin test
4. Capacity use dilution test (Kelsey-Sykes test)
5. In-use test

Koch's method:

Spores of Bacillus anthracis were dried on silk thread and were subjected to action of disinfectants. Later, it was washed and transferred to solid medium.

Rideal Walker method:

This method relies on the estimation of phenol coefficient. Phenol coefficient of a disinfectant is calculated by dividing the dilution of test disinfectant by the dilution of phenol that disinfects under predetermined conditions. Both the phenol and the test disinfectant are diluted from 1/95 to 1/115 and their bactericidal activity is deter -mined against Salmonella typhi suspension. Subcultures are per -formed from both the test and phenol at intervals of 2.5, 5, 7.5 and 10 minutes. The plates are incubated for 48-72 hours at 37°C. That dilution of disinfectant which disinfects

the suspension in a given time is divided by that dilution of phenol which disinfects the suspension in same time gives its phenol coefficient.

Disadvantages of the Rideal-Walker test are: No organic matter is included; the microorganism Salmonella typhi may not be appropriate; the time allowed for disinfection is short; it should be used to evaluate phenolic type disinfectants only.

Chick Martin test:

This test also determines the phenol coefficient of the test disinfectant. Unlike in Rideal Walker method where the test is carried out in water, the disinfectants are made to act in the presence of yeast suspension (or 3% dried human feces). Time for subculture is fixed at 30 minutes and the organism used to test efficacy is S.typhi as well as S.aureus. The phenol coefficient is lower than that given by Rideal Walker method. Capacity use dilution test (Kelsey-Sykes test):

Inoculum of four different test organisms, namely Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Proteus vulgaris are added to the disinfectant in three successive. Dried yeast is included to simulate presence of organic matter. The method can be carried out under 'clean' or 'dirty' conditions. The dilutions of the disinfectant are made in hard water for clean conditions and in yeast suspension for dirty conditions. Test organism alone or with yeast is added at 0,10 and 20 minutes interval. The contact time of disinfectant and test organism is 8 min. The disinfectant is evaluated on its ability to kill microorganisms or lack of it and the result is reported as a pass or a fail and not as a coefficient. The capacity test of Kelsey and Sykes gives a good guideline for the dilution of the preparation to be used. Disadvantage of this test is the fact that it is rather complicated.

In-use test:

The routine monitoring of disinfectant in use can be done by the ‘in use’ test of Maurer. This test is intended to estimate the number of living organism in a vessel of disinfectant in actual use.

The disinfectant that is already in use is diluted 1 in 10 by mixing 1 ml of the disinfectant with 9 ml of sterile nutrient broth. Ten drops of the

diluted disinfectant (each 0.02 ml) is placed on two nutrient agar plates. One plate is incubated at 37o C for 3 days while the other is held at room temperature for 7 days. The number of drops that . yielded growth is counted after incubation. If there growth in more than five drops on either plate, it represents failure of disinfectant