Unit 5

M.Sc Microbiology/Applied Microbiology I Semester

Code –GM 101: Microbial Technique

(Dr. Preeti)

Basic principle and method of sterilization: Sterilization and Disinfection

Methods used for the destruction of microorganism vary with different organism and this has necessitated the development of methods that are different and specific. For example to eliminate sporulating organism we need a method which is more drastic compared to that required for non sporulating organism.

Disinfection: Disinfection describes a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects.

Cleaning: Cleaning is removal of visible soil (e.g., organic and inorganic material) from objects and surfaces. It is normally accomplished manually or mechanically using water with detergents or enzymatic products.

Decontamination: Decontamination removes pathogenic microorganisms from objects so they are safe to handle, use, or discard.

Classification of Materials to be Sterilised / Disinfected:

Earle H. Spaulding devised a rational approach to disinfection and sterilization of patient-care items and equipment. This has three categories

Critical Items : Critical items confer a high risk for infection if they are contaminated with any microorganism. Thus, objects that enter sterile tissue or the vascular system must be sterile because any microbial contamination could transmit disease. This category includes surgical instruments, cardiac and urinary catheters, implants, and ultrasound probes used in sterile body cavities etc.

Semi-critical Items: Semi-critical items contact mucous membranes or non-intact skin. This category includes respiratory therapy and anaesthesia equipment, some endoscopes, laryngoscope blades, esophageal manometry probes, cystoscopes, anorectal manometry catheters, and diaphragm fitting rings etc.

Noncritical Items: Noncritical items are those that come in contact with intact skin but not mucous membranes. Intact skin acts as an effective barrier to most microorganisms; therefore, the sterility of items coming in contact with intact skin is “not critical.” They can be Non-critical

patient care items: bedpans, blood pressure cuffs, crutches and computers Non-critical environmental surfaces.

METHOD OF STERILIZATION

The various methods of sterilization are:

1. Physical Method

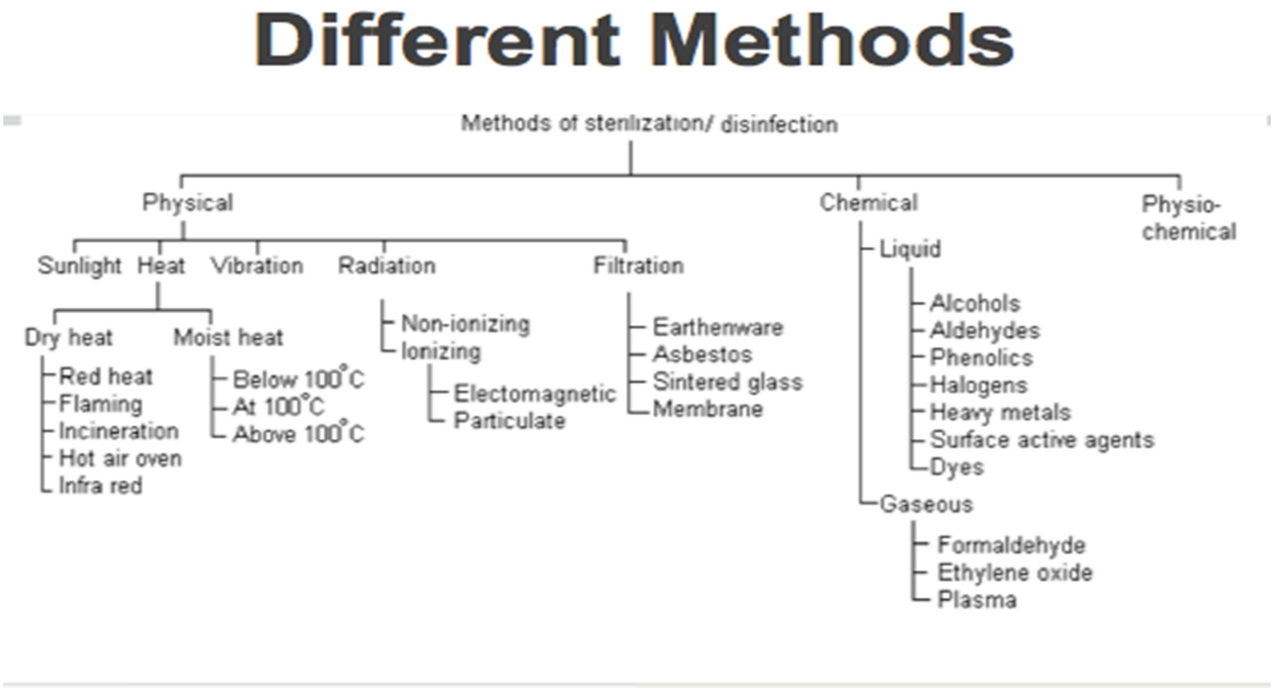
(a) Thermal (Heat) methods

(b) Radiation method

(c) Filtration method

2. Chemical Method

3. Gaseous method Methods of sterilization/disinfection



1. **Heat Sterilization**: Heat sterilization is the most widely used and reliable method of sterilization, involving destruction of enzymes and other essential cell constituents. The process is more effective in hydrated state where under conditions of high humidity, hydrolysis and denaturation occur, thus lower heat input is required. Under dry state, oxidative changes take place, and higher heat input is required. This method of sterilization can be applied only to the thermostable products, but it can be used for moisture-sensitive materials for which dry heat (160- 180°C) sterilization, and for moisture-resistant materials for which moist heat (121-134°C) sterilization is used. The efficiency with which heat is able to inactivate microorganisms is dependent upon the degree of heat, the exposure time and the presence of water. The action of heat will be due to induction of lethal chemical events mediated through the action of water and

oxygen. In the presence of water much lower temperature time exposures are required to kill microbe than in the absence of water. In this processes both dry and moist heat are used for sterilization.

2. **Dry Heat Sterilization**

Examples of Dry heat sterilization are:

1. Incineration
2. Red heat
3. Flaming
4. Hot air oven

It employs higher temperatures in the range of 160-180°C and requires exposures time up to 2 hours, depending upon the temperature employed. The benefit of dry heat includes good penetrability and non-corrosive nature which makes it applicable for sterilizing glass-wares and metal surgical instruments. It is also used for sterilizing non-aqueous thermo-stable liquids and thermostable powders. Dry heat destroys bacterial endotoxins (or pyrogens) which are difficult to eliminate by other means and this property makes it applicable for sterilizing glass bottles which are to be filled aseptically.

Hot-air oven: Dry heat sterilization is usually carried out in a hot air oven, which consists of the following: (i) An insulated chamber surrounded by an outer case containing electric heaters. (ii) A fan (iii) Shelves (iv) Thermocouples (v) Temperature sensor (vi) Door locking controls.

Operations :

(i) Articles to be sterilized are first wrapped or enclosed in containers of cardboard, paper or aluminium.
(ii) Then, the materials are arranged to ensure uninterrupted air flow.
(iii) Oven may be pre-heated for materials with poor heat conductivity.
(iv) The temperature is allowed to fall to 40°C, prior to removal of sterilized material. Moist Heat Sterilization: Moist heat may be used in three forms to achieve microbial inactivation

Moist Heat Sterilization: Moist heat may be used in three forms to achieve microbial inactivation

1. Dry saturated steam – Autoclaving 2. Boiling water/ steam at atmospheric pressure 3. Hot water below boiling point Moist heat sterilization involves the use of steam in the range of 121-134°C. Steam under pressure is used to generate high temperature needed for sterilization. Saturated steam acts as an effective sterilizing agent. Steam for sterilization

can be either wet saturated steam (containing entrained water droplets) or dry saturated steam (no entrained water droplets).

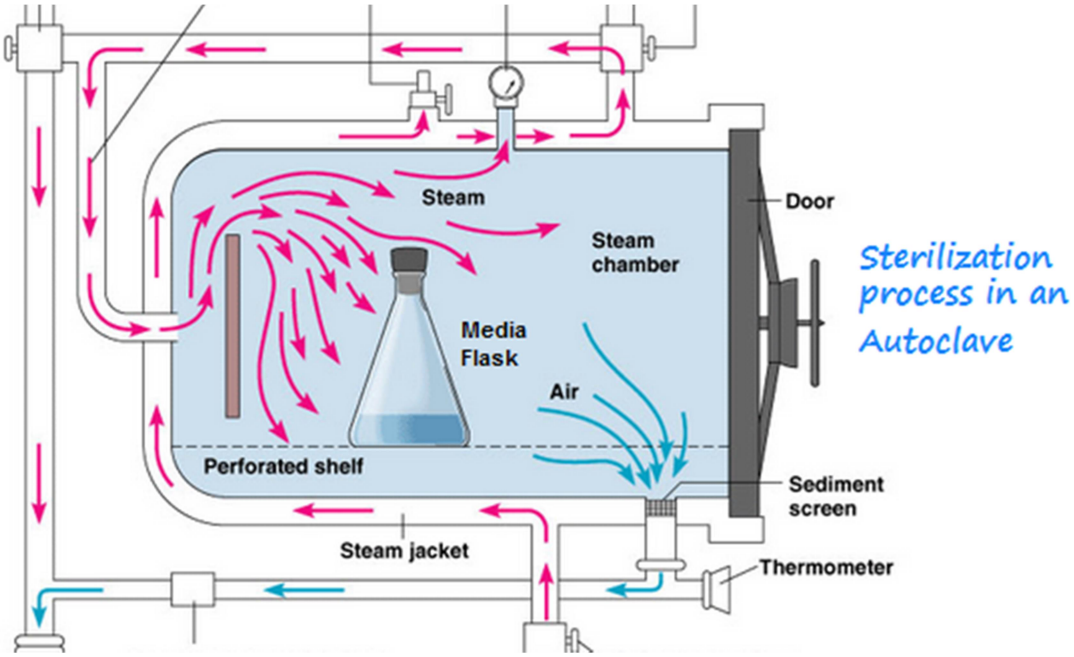


Fig 1

Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable systems available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation. Autoclaves should be tested periodically with biological indicators like spores of Bacillus stearothermophilus to ensure proper function. This method of sterilization works well for many metal and glass items but is not acceptable for rubber, plastics, and equipment that would be damaged by high temperatures (Figure 1).

Autoclaves, or steam sterilizers essentially consist of following:

1. A cylindrical or rectangular chamber, with capacities ranging from 400 to 800 litres.
2. Water heating system or steam generating system
3. Steam outlet and inlet valves
4. Single or double doors with locking mechanism.
5. Thermometer or temperature gauge 6. Pressure gauges

Operation :

For porous loads (dressings) sterilizers are generally operated at a minimum temperature of 134°C for one hour, and for bottled fluid, sterilizers employing a minimum temperature of 121°C are used. Ensure that there should be sufficient water in the autoclave to produce the steam. The

stages of operation of autoclaves include air removal, steam admission and sterilization cycle (includes heating up, holding/exposure, and cooling stages).

Gaseous Sterilization: The chemically reactive gases such as formaldehyde, (methanol, H.CHO) and ethylene oxide (CH2)2O possess biocidal activity. Ethylene oxide is a colorless, odorless, and flammable gas. The mechanism of antimicrobial action of the two gases is assumed to be through alkylations of sulphydryl, amino, hydroxyl and carboxyl groups on proteins and amino groups of nucleic acids. The concentration ranges (weight of gas per unit chamber volume) are usually in range of 800-1200 mg/L for ethylene oxide and 15-100 mg/L for formaldehyde with operating temperatures of 45-63°C and 70- 75°C respectively. Both of these gases being alkylating agents are potentially mutagenic and carcinogenic. They also produce acute toxicity including irritation of the skin, conjunctiva and nasal mucosa.

(a) **Ethylene oxide sterilizer**: An ethylene oxide sterilizer consists of a chamber of 100-300-Litre capacity and surrounded by a water jacket. Air is removed from sterilizer by evacuation, humidification and conditioning of the load is done by passing sub-atmospheric pressure steam, then evacuation is done again and preheated vaporized ethylene oxide is passed. After treatment, the gases are evacuated either directly to the outside atmosphere or through a special exhaust system. Ethylene oxide gas has been used widely to process heat-sensitive devices, but the aeration times needed at the end of the cycle to eliminate the gas made this method slow.

(b) Low temperature steam formaldehyde (LTSF) sterilizer: An LTSF sterilizer operates with sub atmospheric pressure steam. At first, air is removed by evacuation and steam is admitted to the chamber.

Liquid Sterilization

(a) Peracetic Acid liquid sterilization: Peracetic acid was found to be sporicidal at low concentrations. It was also found to be water soluble, and left no residue after rinsing. It was also shown to have no harmful health or environmental effects. It disrupts bonds in proteins and enzymes and may also interfere with cell membrane transportation through the rupture of cell walls and may oxidize essential enzymes and impair vital biochemical pathways. In a low-temperature liquid chemical sterile processing system, several steps must be followed for effective sterilization:

1. Pre-cleaning of the devices is necessary because many devices have small connected lumens.
2. Leak testing is done to ensure there are no leaks that could allow fluid to enter/leak the ampoules/vials and cause damage.
3. The appropriate tray/container must then be selected, and if the device has lumens, the appropriate connector attached.
4. The sterilant concentrate is provided in a sealed single- use cup and requires no pre-mixing or dilution.

The disadvantages of this method of sterilization are that the devices must be immersible, must fit in the appropriate tray, and must be able to withstand the 55°C temperature the process uses.

(b) **Hydrogen Peroxide Sterilization**: This method disperses a hydrogen peroxide solution in a vacuum chamber, creating a plasma cloud. This agent sterilizes by oxidizing key cellular

components, which inactivates the microorganisms. The plasma cloud exists only while the energy source is turned on. When the energy source is turned off, water vapor and oxygen are formed, resulting in no toxic residues and harmful emissions. The temperature of this sterilization method is maintained in the 40-50°C range, which makes it particularly well-suited for use with heat-sensitive and moisture-sensitive medical devices. The instruments are wrapped prior to sterilization, and can either be stored or used immediately. There are five phases of the hydrogen peroxide processing cycle:

1. A vacuum phase creates a vacuum in the chamber and the pressure drops to less than one pound per square inch. This phase lasts about 20 minutes.
2. In the injection phase, the aqueous hydrogen peroxide is introduced into the vacuum chamber and is vaporized into a gas, which creates a rise in pressure due to the increase of molecules.
3. During the diffusion phase the hydrogen peroxide vapor spreads throughout the chamber and the increased pressure drives the sterilant into the packs, exposing the instrument surfaces to the sterilant and killing the microorganisms.
4. During the plasma phase the radio frequency energy is applied, stripping the electrons from some of the molecules and producing a low-temperature plasma cloud. Following this reaction, the activated compounds lose their high energy and recombine to form oxygen and water.
5. The purpose of the venting phase is to introduce filtered air into the chamber and return the chamber to atmospheric pressure so that the door can be opened. It lasts about one minute.

RADIATION STERILIZATION

Many types of radiation are used for sterilization like electromagnetic radiation (e.g. gamma rays and UV light), particulate radiation (e.g. accelerated electrons).The major target for these radiation is microbial DNA. Gamma rays and electrons cause ionization and free radical production while UV light causes excitation. Radiation sterilization with high energy gamma rays or accelerated electrons has proven to be a useful method for the industrial sterilization of heat sensitive products. But some undesirable changes occur in irradiated products, an example is aqueous solution where radiolysis of water occurs. Radiation sterilization is generally applied to articles in the dry state; including surgical instruments, sutures, prostheses, unit dose ointments, plastic syringes.and dry pharmaceutical products. UV light, with its much lower energy, and poor penetrability finds uses in the sterilization of air, for surface sterilization of aseptic work areas, for treatment of manufacturing grade water, but is not suitable for sterilization of pharmaceutical dosage forms.

Gamma ray Sterilizer:

Gamma rays for sterilization are usually derived from cobalt-60 source, the isotope is held as pellets packed in metal rods, each rod carefully arranged within the source and containing 20 KCi of activity. This source is housed within a reinforced concrete building with 2 m thick walls. Articles being sterilized are passed through the irradiation chamber on a conveyor belt

and move around the raised source.

Ultraviolet Irradiation:

The optimum wavelength for UV sterilization is 260 nm. A mercury lamp giving peak emission at 254 nm is the suitable source of UV light in this region.

Electron Accelerator

There are two types of electron accelerator machines, the electrostatic accelerator which produces electrons with maximum energies of 5 MeV, and the microwave linear accelerator which produces electrons with maximum energies of 10 MeV. Higher energies cause better penetration into the product but there is a risk of induced radiation. A high energy electron beam is generated by accelerating electrons from a hot filament down an evacuated tube under high potential difference, and then additional energy is imparted to this beam in a pulsed manner by a synchronized traveling microwave. Articles to be sterilized are arranged on a horizontal conveyor belt and are irradiated from one or both sides.

Filtration Sterilization

Filtration process does not destroy but removes the microorganisms. It is used for both the clarification and sterilization of liquids and gases as it is capable of preventing the passage of both viable and non viable particles. The major mechanisms of filtration are sieving, adsorption and trapping within the matrix of the filter material. Sterilizing grade filters are used in the treatment of heat sensitive injections and ophthalmic solutions, biological products and air and other gases for supply to aseptic areas. They are also used in industry as part of the venting systems on fermentors, centrifuges, autoclaves and freeze driers. Membrane filters are used for sterility testing.

Application of filtration for sterilization of gases:

HEPA (High efficiency particulate air) filters can remove up to 99.97% of particles >0.3micrometer in diameter. Air is first passed through prefilters to remove large particles and then passed through HEPA filters. The performance of HEPA filter is monitored by pressure differential and airflow rate measurements.

There are two types of filters used in filtration sterilization

(a) Depth filters: Consist of fibrous or granular materials so packed as to form twisted channels of minute dimensions. They are made of diatomaceous earth, unglazed porcelain filter, sintered glass or asbestos.

(b) Membrane filters: These are porous membrane about 0.1 mm thick, made of cellulose acetate,cellulose nitrate, polycarbonate, and polyvinylidene fluoride, or some other synthetic material.The membranes are supported on a frame and held in special holders. Fluids are made to transverse membranes by positive or negative pressure or by centrifugation.

Application of filtration for sterilization of liquids: Membrane filters of 0.22 micrometer nominal pore diameter are generally used, but sintered filters are used for corrosive liquids, viscous fluids and organic solvents. The factors which affects the performance of filter is the titre

reduction value, which is the ratio of the number of organism challenging the filter under defined conditions to the number of organism penetrating it. The other factors are the depth of the membrane, its charge and the tortuosity of the channels.

Evaluation and In Process Monitoring of Sterilization Procedures

Dry Heat Sterilization

Physical indicator: In this process temperature record chart is made of each sterilization cycle with dry heat sterilization. This chart forms the batch documentation and is compared against a master temperature records. The temperature should be taken as the coolest part of the loaded sterilizer, further information on heat distribution and penetration within sterilizer can be gained by the use of thermocouple place at selected site in the chamber or injected into test packs or bottles.

Chemical indicator: It is based on the ability of heat to alter the chemical or physical characteristics of variety of chemical substances. This change should take place only when satisfactory condition for sterilization prevails. Thus conforming that sterilization cycle has been successfully completed. Chemical indicators generally undergo melting or colour change.

Biological indicator: The biological indicators are the standardized bacterial spore preparations which are usually in the form of suspension in water or culture medium or of spore dried on paper or plastic carriers, they are placed in sterilizer. After the sterilization process the aqueous suspension /spores are on carriers are aseptically transferred to an appropriate nutrient medium, which is then incubated and occasionally seen for the growth. Clostridium species is generally used for dry heat sterilization indicator.

Indicators	Sterilization Methods	Principle	Device	Parameter monitored
Physical	Dry heat	Temperature recording charts	Temperature recording charts	Temperature
Chemical	Dry heat	Temperature sensitive coloured solution	Browne's tube	Temperature time
		Temperature Sensitive chemical	Temperature sensitive white wax concealing a black marked	Temperature
Biological	Dry heat	Temperature Sensitive microbes	*Bacillus Subtilis*	D value

Moist Heat Sterilization

Physical Indicator: In this process temperature record chart is made of each sterilization cycle with dry heat sterilization. This chart of the batch documentation is compared against a master temperature records. The temperature should be taken as the coolest part of the loaded sterilizer, further information on heat distribution and penetration within sterilizer can be gained by the use of thermocouple place at selected site in the chamber or injected into test packs or bottles.

Chemical Indicator: It is based on the ability of heat to alter the chemical or physical characteristics of variety of chemical substances. This change should take place only when satisfactory condition for sterilization prevails. Thus conforming that sterilization cycle has been successfully completed chemical indicator generally undergoes melting or colour change.

Biological Indicator: Spores of G. steareothermophylus in sealed ampoules of culture medium are used for moist heat sterilization monitoring and these may be incubated directly at 55°C, thus may eliminate the need of aseptic transfer (Table 3). Aseptic transfer is also avoided by use of self-contained units where the spores strip and the nutrient medium are present in the same device ready for mixing after use.

The bacterial spores should have following qualities (i) It should be non-pathogenic (ii) Should possess above average resistant to the particular sterilization process.

Indicators	Sterilization Methods	Principle	Device	Parameter monitored
Physical	Moist heat	Temperature recording charts	Temperature recording charts	Temperature
Chemical	Moist heat	Temperature sensitive coloured solution	Browne's tube	Temperature time
		Steam Sensitive chemical	A device which is impregated into a carrier material	saturated steam
Biological	Moist heat	Temperature Sensitive microbes	*Geobacillus Sterothermophilus*	D value

Gaseous Sterilization

Physical Indicator: Gas concentration is measured independently of pressure rise, often by reference to weight of gas used.

Chemical Indicator: The chemical indicator used here are Royach Sacket, the indicator paper impregnated with reactive chemical which undergoes a distinct colour change on reaction. Chemical indicators are valuable monitors of the condition prevailing at the coolest of most in accessible part of a sterilizer.

Biological Indicator: As with chemical indicator they are usually packed in dummy packs located at strategic sites in the sterilizer. Alternatively for gaseous sterilization, these may also be placed in tubular helix device. The species of bacteria generally used for gaseous sterilization are B.subtilis var.niger and B.subtilis var.golbigii.

Radiation Sterilization

Physical Indicator: In radiation sterilization a plastic or perspex dosimeter which gradually darkens in proportion to the radiation it absorbs give an accurate measure of the radiation dose and is considered to be the best technique currently available for the radiation sterilization process.

Chemical Indicator: Chemical dosimeter acidified with cerric ammonium sulphate or cerric sulphate solution .These responds to irradiation by dose change in the applied density. Those are considered best and accurately measure relation dose.

Biological Indicator: These consist of standardized bacterial spore preparation which are usually in the form of suspension in water or culture medium or of spore dried on paper or plastic carriers, they are placed in sterilizer. After the sterilization process the aqueous suspension /spores are on carriers are aseptically transferred to an appropriate nutrient medium, which is then incubated and periodically observed for the growth. Clostridium species is generally used for dry heat sterilization indicator.

Filtration Sterilization

Physical Indicator: Sterilizing filters are subjected to a bubble point pressure test. This is a technique for determining the pore size of a filter, and may also be used to check the integrity of certain types of filters. The principle of the test is that the wetted filter in its assembled unit is subjected to an increasing air or nitrogen gas pressure difference. The pressure difference recorded when the first bubble of gas breaks away from the filter is related to maximum pore size. When the gas pressure is further increased slowly there is general eruption of bubble over the entire surface. The pressure difference here is related to the mean pore size. Pressure difference below the expected value would signify a damage or faulty filter.

Biological Indicator: Filtration sterilization requires a different approach from biological monitoring, the test effectively measure in the ability of a filter to produce a sterile filtrate from a culture of suitable organism S. marcesence, a small gram negative rod shape bacterium. B. diminuta used as a biological indicator having a dimension 0.5 micrometres and 0.3 micrometre respectively has been used for filters of 0.45 micrometre and 0.22 micrometre. The extent of the passage of this organism through membrane filter is enhanced by increasing the filtration pressure. Thus successful sterile filtration depends markedly on the challenge

condition. Such tests are used as the part of filter manufacture characterization and quality assurance process, and user's initial validation procedure.

CHEMICAL METHODS OF DISINFECTION

Disinfectants are those chemicals that destroy pathogenic bacteria from inanimate surfaces. Some chemicals when used at apropriate concentration for appropriate duration can be used for sterilization and are called sterilant liquids. Those chemicals that can be safely applied over skin and mucus membranes are called antiseptics.

An ideal antiseptic or disinfectant should have following properties:

1. Should have wide spectrum of activity
2. Should be able to destroy microbes within practical period of time
3. Should be active in the presence of organic matter
4. Should make effective contact and be wettable
5. Should be active in any pH
6. Should be stable
7. Should have long shelf life
8. Should be speedy
9. Should have high penetrating power
10. Should be non-toxic, non-allergenic, non-irritative or non-corrosive
11. Should not have bad odour
12. Should not leave non-volatile residue or stain
13. Efficacy should not be lost on reasonable dilution
14. 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 vapour)
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., Formaldehyde) (e) Damage to nucleic acids (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 40°C for 20 minutes is used to disinfect wool and 0.25% at 60oC 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 preoperative 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. 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. Melachite green is used in LJ medium for growth of Mycobacterium tuberculosis.

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.

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

Beta-propiolactone (BPL)

Mode of action: It is an alkylating agent and acts through alkylation of carboxyland 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.

Evaluation of microbial agent effectiveness

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.

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) The capacity test (Kelsey-Sykes) determine the appropriate use dilution of the disinfectants. The stability test (Maurer) determines the stability and long term. The capacity and stability test help to determine the choice of a disinfectant.

In-use test: The routine monitoring of disinfectant in use can be done by the 'in use' test (Kelsey & 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 37°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.

Principle and functioning of LAF

A laminar flow cabinet is an enclosed workbench which is used to create a contamination free work environment through installed HEPA filters that capture all the particles entering the

cabinet.A laminar flow hood is used for work with substances which are not hazardous for the personnel health.

Desktop laminar flow chambers, also known as laminar flow clean benches, are similar to biosafety cabinets in the sense that they are equipments used to clean up the ambient air completely through a filtration process.

A laminar flow unit has an industry-wide usage and can be applied in quite a lot of industries such as medical, research, pharmacy, educational, and also in electronics, optics, micromechanics, plastic industries, etc. since they carry out processes that require a clean and sterile environment. A laminar hood is used for work with substances which are not hazardous for the personnel health, and it does not provide personal protection. The best usage of a clean bench is for working on certainly specialized experiments in the labs that require a clean environment to design products that are nontoxic.

How does a laminar air flow unit work?

A laminar flow unit creates dust free a bacterial air environment.

Air from the room passes through the HEPA (High Efficiency Particulate Absorbing) filters and is fed into the working chamber by a unidirectional vertical descending flow. From the working area the air is moved back to the environment in the following way: partly – through the perforation in the bottom rear area of the cabinet, but most air – through the space between the working surface of the table and the protecting glass.

Laminar flow hood involve a unidirectional exhaustion of air to the workplace and personnel whereby filtered air is discharged with a regular and fixed velocity.

Some of the basic components of a laminar flow chamber include UV light, glass shield, an air intake fan, a protection plate, windows, etc.

Types of laminar flow hoods

The direction in which the air moves is based on the specific laminar cabinet that is being used. The type of cell culture hood needed depends very much on the requirements of the laboratory, the kind of airflow needed, working principle, and the type of operation. Two main types of laminar flow hoods differ according to the functions they can perform: horizontal airflow hood and vertical airflow hood.

Horizontal airflow hood

The ambient air comes from the behind the laminar air flow bench, then it is projected through a blower towards the HEPA filter, and the filtered air is exhausted in a horizontal direction to the workplace environment.

Airflow that is parallel to the workplace cleanses the environment with a constant velocity. Horizontal laminar chamber needs a larger operational space and more depth to provide a germ-free environment and hence is more difficult to handle.

Vertical airflow hood

Our company specializes in the production of vertical airflow laminar cabinets, it is the most demanded product because of the advanced technology combined to remove all the impurities from the environment.

- The illumination block is taken out of the working chamber and does not initiate air flow turbulence.
- Coloring of the cabinet is made with the powder enamel resistant to disinfectant solutions.
- The control unit with LCD indicates the switching of the systems, their possible malfunctions, the operation mode chosen and devices technological timer.
- HEPA filter is held by springs providing filter leak tightness for the whole lifetime.
- The electronic shield panel provides easy operation and disinfection.
- The UV light module is used for disinfection of the working chamber and its surfaces.
- The working principle of a vertical laminar unit is quite different from a horizontal airflow hood because it consists of a fan which is placed on the ceiling of the cabinet and the contaminated air is taken in through that fan and directed from the bench top downwards in a vertical direction with a positive pressure.

  Usually, vertical airflow benches have a width ranging 0,9-2,4 m. With all these excellent machines, technologies and an economical system, vertical airflow laminar hood is state-of-the-art. It provides greater protection from harm and uses less operational and floor space.

  Horizontal versus vertical chamber

  Since we are the largest suppliers of laminar airflow chambers, it is our responsibility to guide you to the best type of product to use by comparing both of them. A vertical airflow clean bench does not require as much depth and floor space as a horizontal airflow hood which makes it more manageable and decreases the chances of airflow obstruction or collection of contaminated air downstream.

  Moreover, the safety level of the vertical laminar hood is comparatively greater because it does not blow the air directly towards the person carrying out the experiments. The front sash installed acts as a barrier against the fumes by preventing a direct attack on the person's face. Also, in a horizontal airflow hood filter replacement needs moving of hoods and cabinets but in the vertical airflow chamber, filter replacement is much more rapid and easier, since the filter is placed at the top of the vertical module.

  Lastly, vertical airflow laminar units have more unidirectional and less turbulent effects from the air streams projected through them on the products being processed. After knowing all these extra benefits, you have quite a lot of compelling reasons to choose a vertical airflow laminar unit.

  Recommendations

  When choosing to buy a laminar airflow workstation, there are certain things that need to be kept in mind. The standards of each type of laminar airflow cabinet are different according to the specific purposes they have. Whichever type you buy, the SOPs need to be considered. Some of them are discussed below:
- It is the responsibility of the operator to be aware of how to use this equipment, and to be familiar with the complete kit of laminar flow chambers.
- The operator should wear a lab coat and long gloves.

- Before operating the cabinet and upon completion of work, sterilize the laminar flow cabinet with UV irradiation.
- Walls, tabletop and glass shields must be sterilized before start and completion of the work.

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