Sterilization

2nd Prof

Pharm D

Pharmaceutical Microbiology

Misbah Hameed

MODULE

Microbiology

STERILISATION

Disinfection and sterilization are essential for ensuring that medical and surgical instruments do not transmit infectious pathogens to patients. Because sterilization of all patient-care items is not necessary, health-care policies must identify, primarily on the basis of the items' intended use, whether cleaning, disinfection, or sterilization is indicated.

After reading this lesson, you will be able to:

- define terms related to Sterilization and Disinfection
- classify items to be sterilised or disinfected
- discuss different Methods of sterilisation
- describe Evaluation and in Process Monitoring of Sterilization Procedures
- discuss Methods of disinfection
- describe the Testing of disinfectants

Sterilization: *Sterilization* describes a process that destroys or eliminates all forms of microbial life and is carried out in health-care facilities by physical or chemical methods.

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

INTEXT QUESTIONS 4.1

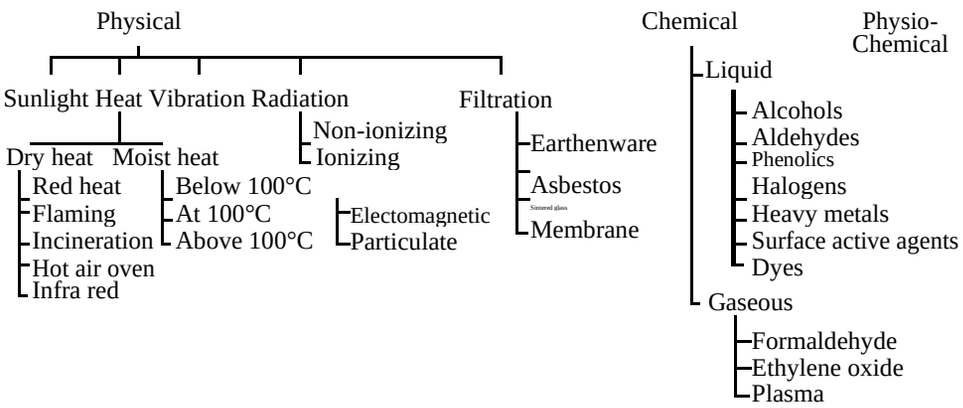
1. Sterilization	(a) Removal of visible soil
2. Disinfection	(b) Removal of Pathogenic Microorganisms
3. Cleaning	(c) Destroys all forms of Microbes
4. Decontamination	(d) Removal of Pathogenic Microorganism except bacteria spores

Notes

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



4.3.1 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.

Dry Heat Sterilization: Examples of Dry heat sterilization are:

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

Notes

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 thermo-stable 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.

Operation

(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

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).

Notes

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 4.1).

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

Notes

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 $(CH_2)_2O$ possess biocidal activity. Ethylene oxide is a colorless, odorless, and flammable gas.

The mechanism of antimicrobial action of the two gases 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

Notes

(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.

Notes

INTEXT QUESTIONS 4.2

Match the following

1. Dry heat Sterilisation	(a) Hydrogen peroxide Sterilizer
2. Moist heat	(b) Formaldehyde Sterilizer
3. Gas Sterilization	(c) Autoclave
4. Liquid Sterilisation	(d) Hot air Oven

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.

Notes

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 >0.3 micrometer in diameter. Air is first passed through prefilters to remove larger 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

Notes

(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.

Notes

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	A 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.

Notes

Indicator	Sterilization	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 impregnated into a carrier material.	Saturated steam
Biological	Moist heat	Temperature sensitive microbes	*Geobacillus stearother-mophilus*	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*

Notes

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.