College Of Veterinary Medicine .

3rd class First Semester

MICROBIOLOGY- BACTERIOLOGY *The Sixth – Lecture*

Sterilization And Disinfection :-

Sterilization: is defined as the process where all the living microorganisms, 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 microorganisms (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.

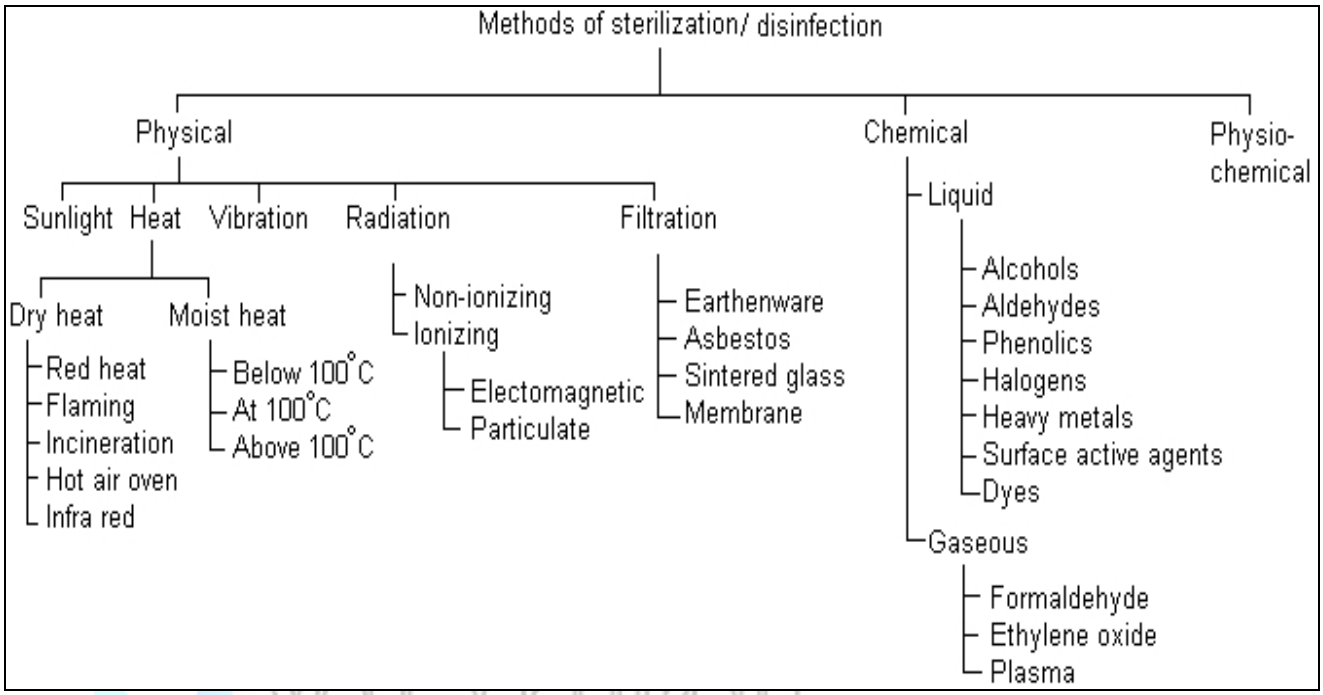
Asepsis : is the employment of techniques (such as usage of gloves, air filters, uv rays etc) to achieve microbe-free environment.

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:-

1- Sunlight: The microbial activity of sunlight is mainly due to the presence of ultra violet rays in it. It is responsible In tropical countries, the sunlight is more effective in Killing germs.

Due to combination of ultraviolet rays and heat. By killing bacteria suspended in water, sunlight provides natural method of disinfection of water bodies such as tanks and lakes. Sunlight is not sporicidal, hence it does not sterilize.

2- 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:

1- Nature of heat:

Moist heat is more effective than dry heat.

2- Temperature and time:

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

3- Number of microorganisms:

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

4- Nature of microorganism:

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

5- Type of material:

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

6- 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:

1- 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.

2- 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.

3- Incineration.

4- Hot air oven.

5-Infra red rays.

MOIST HEAT:

Moist heat acts by coagulation and denaturation of proteins.

At temperature below 100C:

- Pasteurization:
- Vaccine bath:
- Serum bath:
- Inspissation:

At temperature 100C :

- Boiling:
- Steam at 100C:

At temperature above 100C:

Autoclave: Sterilization can be effectively achieved at a temperature above 100C using an autoclave. Water boils at 100C at atmospheric pressure, but if pressure is In an autoclave the .raised, the temperature at which the water boils also increases 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 121C. 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; 135C or 121C for at least one hour are recommended .

3-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 toincrease their shelf life up to 500 percent.

1. **Non-ionizing rays:**
2. **Ionizing rays:**

4-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.

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:

ALCOHOLS:

ALDEHYDES:

PHENOL:

HALOGENS:

HEAVY METALS:

SURFACE ACTIVE AGENTS

DYES:

HYDROGEN PEROXIDE:

ETHYLENE OXIDE (EO):

BETA-PROPIOLACTONE (BPL):

PHYSIO-CHEMICAL METHOD:

Mode of action: A physio-chemical method adopts both physical and chemical method. Use of steamformaldehyde is a physio-chemical method of sterilization, which takes into account action of steam as well as that of

formaldehyde. Saturated steam at a pressure of 263 mm has a temperature of 70C. 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.