

L4: Sterilization & Disinfection

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- **Sterilization** is the killing of all microorganisms, including bacterial spores, which are highly resistant. Sterilization is usually carried out by there are three methods: Physical, Chemical, & Mechanical methods.

Disinfections: is the removing of MO not all microorganisms. It is carried out by using of disinfectants: chemical agents that have bacteriocidal or bacteriostatic effects.

- **Bacteriocidal:** is killing growth of the MO.
- **Bacteriostatic:** is inhibiting growth of the MO.

Methods of Sterilization

1-Chemical methods

There are two terms in these methods: Antiseptic & Disinfectant.

- **Antiseptic:** is the chemical agent that used in sterilization of **biological surface** (skin).
- **Disinfectant:** is the chemical agent that used in sterilization of **non biological surface** such as bench.
- Alcohol is effective in reducing the number of MO on skin, may be used disinfection of contaminated objects. For adequate disinfection, pathogens must be killed, but some organisms and bacterial spores may survive. Disinfectants vary in their tissue-damaging properties from the phenol-containing compounds, which should be used only on inanimate objects, to less toxic materials such as ethanol and iodine, which can be used on skin surfaces.

Rate of killing of microorganisms

- Death of microorganisms occurs at a certain rate dependent primarily upon two variables: the concentration of the killing agent and the length of time the agent are applied. The rate of killing is defined by the relationship which shows that the number of survivors.
- The relationship is usually stated in terms of survivors, because they are easily measured by colony formation. Death is defined as the inability to reproduce. In certain circumstances, the physical remains of dead bacteria can still cause problems.

Chemicals Agents

- Chemicals vary greatly in their ability to kill microorganisms. A quantitative measure of this variation is expressed as the phenol coefficient, which is the ratio of the concentration of phenol to the concentration of the agent required to cause the same amount of killing under the standard conditions of the test. **Chemical agents act primarily by one of the three, mechanisms:**
 - (1) **Disruption of the lipid containing cell membrane,**
 - (2) **modification of proteins**
 - (3) **Modification of DNA.** Each of the following chemical agents has been classified into one of the three types, but some of the chemicals act by more than one mechanism.

Disruption of cell membranes

- **Alcohol**

Ethanol is widely used to clean the skin before immunization or venipuncture. It acts mainly by the lipid structure in membranes, but it denatures proteins as well. Ethanol requires the presence of water for maximal activity; i.e., it is far **more effective at 70%** than at 100%. It is often used as an antiseptic to clean the skin prior to venipuncture. However, because it is not as effective as iodine-containing compounds, the latter should be used prior to obtaining a blood culture and installing intravenous catheters.

- **Alcohol + phenol action on (cell membranes and Proteins)**

• Detergents

Detergents are “surface active” agents composed of long-chain, **lipid-soluble**, hydrophobic portion and a polar hydrophilic group, which can be a cation, an anion, or a nonionic group. These surfactants interact with the lipid in the cell membrane through their hydrophobic chain and with the surrounding water through their polar group and thus disrupt the membrane. Quaternary ammonium compounds, e.g., benzalkonium chloride, are cationic detergents widely used for skin antiseptics.

• Phenols

Phenol was the first disinfectant used in the operating room (by Lister in the 1860s), but it is rarely used as a disinfectant today because it is too caustic. Hexachlorophene, which is a biphenol with six chlorine atoms, is used in germicidal soaps, but concern over possible neurotoxicity has limited its use. Another phenol derivative is cresol (methylphenol), the active ingredient in Lysol. **Phenols not only damage membranes but also denature proteins.**

Modification of proteins

- **Chlorine**

Chlorine is used as a disinfectant to purify the water supply and to treat swimming pools. It is also the active component of hypochlorite, which is used as a disinfectant in the home and in hospitals. Chlorine is a powerful oxidizing agent that kills by cross-linking essential sulfhydryl groups in enzymes to form the inactive disulfide.

• Iodine

Iodine is the most effective skin antiseptic used in medical practice and should be used prior to obtaining a blood culture and installing intravenous catheters because contamination with skin flora such as *Staphylococcus epidermidis* can be a problem. Iodine is supplied in two forms:

- (1) Tincture of iodine (2% solution of iodine and potassium iodide in ethanol) is used to prepare the skin prior to blood culture. Because tincture of iodine can be irritating to the skin, it should be removed with alcohol.
- (2) Iodophors are complexes of iodine with detergents that are frequently used to prepare the skin prior to surgery because they are less irritating than tincture of iodine. Iodine, like chlorine, is an oxidant that inactivates sulfhydryl-containing enzymes. It also binds specifically to tyrosine residues in proteins. **Action of Iodine on denature proteins.**

- **Heavy Metals**

Mercury and silver have the greatest antibacterial activity of the heavy metals and are the most widely used in medicine. They act by binding to sulfhydryl groups, thereby blocking enzymatic activity. A Thimerosal (Merthiolate) and merbromin (Mercurochrome), which contain mercury, are used as skin antiseptics. **Silver nitrate drops are useful in preventing gonococcal ophthalmia neonatorum.** Silver sulfadiazine is used to prevent infection of burn wounds.

- **Hydrogen Peroxide**

Hydrogen peroxide is used antiseptic to clean wounds and to disinfect contact lenses. **Its effectiveness is limited by the organism's ability to produce catalase**, an enzyme that degrades H_2O_2 . (The bubbles produced when peroxide is used on wounds are formed by oxygen arising from the breakdown of H_2O_2 ; by tissue catalase.) **Hydrogen peroxide is an oxidizing agent that attacks sulfhydryl groups, thereby inhibiting enzymatic activity.**

- **Formaldehyde & Glutaraldehyde**

Formaldehyde, which is available as a 37% solution in water (Formalin), **denatures proteins and nucleic acids**. Both proteins and nucleic acids contain essential -NH₂ and —OH groups, which are the main sites of alkylation by the hydroxymethyl group of formaldehyde. Glutaraldehyde, which has two reactive aldehyde groups, is 10 times more effective than formaldehyde and is less toxic. In hospitals, it is used to sterilize respiratory therapy equipment.

- **Ethylene Oxide**

Ethylene oxide gas is used extensively in hospitals for the sterilization of heat-sensitive materials such as surgical instruments and plastics. **It kills by alkylating both proteins and nucleic acids**; the hydroxyethyl group attacks the reactive hydrogen atoms on essential amino and hydroxyl groups.

- **Acids & Alkalis**

Strong acids and alkalis **kill by denaturing proteins**. Although most bacteria are susceptible, it is important to note that *Mycobacterium tuberculosis* are relatively resistant to 2% NaOH, which is used in the clinical laboratory to liquefy sputum prior to culturing the organism. Weak acids, such as benzoic, propionic, and citric acids, are frequently used as food preservatives because they are bacteriostatic.

- **Modifications Nucleic acids**

A variety of dyes not only stains microorganisms but also inhibit their growth. One of these is crystal violet, which is used as a skin antiseptic. **Its action is based on binding of the positively charged dye molecule to the negatively charged phosphate groups of the nucleic acids. Malachite green, dye-like crystal violet**, is a component of Lowenstein Jensen's medium, which is used to grow *M. tuberculosis*. The dye inhibits the growth of unwanted organisms in the sputum during the 6—week incubation period.

2-Physical methods (heat or radiation)

A-HEAT

Heat energy can be applied in three ways: in the Form of moist heat (either boiling or autoclaving) or dry heat or by pasteurization. In general, **heat kills by denaturing proteins, but membrane damage and enzymatic cleavage of DNA may also be involved.** Moist heat sterilizes at a lower temperature than dry heat, because water aids in the disruption of non covalent bonds, e.g., hydrogen bonds, which hold protein chains together in their secondary and tertiary structures.

Moist-heat sterilization, usually autoclaving, is the most frequently used method of sterilization. Because bacterial spores are resistant to boiling, they must be exposed to a higher temperature; this cannot be achieved unless the pressure is increased. For this purpose, an autoclave chamber is used in which steam, at a pressure of 15 lb/in², reaches a temperature of 121°C and is held for 15-20 minutes. This kills even the highly heat resistant spores of *Clostridium*, with a margin of safety.

- Sterilization by dry heat, on the other hand, requires temperatures in the range of 180°C for 2 hours. This process is used primarily for glassware and is used less frequently than autoclaving. **Pasteurization**, which is used primarily for milk, consists of heating the milk to 62°C for 30 minutes followed by rapid cooling. (“Flash” pasteurization at 72°C for 15 seconds is often used.) This is sufficient to kill the vegetative cells of the milk-borne pathogens, e.g., *Mycobacterium bovis*, *Salmonella*, *Streptococcus*, *Listeria*, and *Brucella*, but not to sterilize the milk.

B-RADIATION

• Radiation, such as ultraviolet light, gamma and x-radiation, is often used to sterilize heat-sensitive items. Ultraviolet light and x-radiation **kill microorganisms by damaging DNA**. The greatest antimicrobial activity of UV light occurs at 250-260 nm which is the wavelength region of maximum **absorption by the purine and pyrimidine bases of DNA**, most significant **lesion caused by UV irradiation is the formation of thymine dimers (T=T) rather than (T=A)**, but addition of hydroxyl groups to the bases also occurs. As a result, DNA replication is inhibited and the organism cannot grow. Because UV radiation can damage the cornea and skin, the use of UV irradiation in medicine is limited. However, it is used in hospitals to kill airborne organisms, especially in operating rooms when they are not in use. Bacterial spores are quite resistant and require a dose up to 10 times greater than do the vegetative bacteria.

X-rays have higher energy and penetrating power than UV radiation and **kill mainly by the production of free radicals OH, e.g., production of hydroxyl radicals by the hydrolysis of water. These highly reactive radicals can break covalent bonds in DNA&DNA breakage, thereby killing the organism.**

X-rays kill vegetative cells readily, but spores are remarkably resistant, probably because of their lower water content. X-rays are used in medicine for sterilization of heat-sensitive items, such as sutures and surgical gloves, and plastic items, such as syringes.

3-Mechanical methods (filtration)

These methods using **for sterilization the biological fluids; blood, enzyme, antibiotics, these solutions are spoiled in high temperature;** Filtration can sterilize liquids if the pore size of the filter is small enough to retain all bacteria and spores. The most commonly used filter is composed of nitrocellulose and has a pore size of 0.22 μm . This size will retain all bacteria and spores. That depending on:

- Size of substances that contaminated the liquid.
- Nature of liquid.
- Diameter of filter pits.
- Electric charge of filter.
- Electric charge of MO that found in the liquid.