



Sterilization

Sterilization is the killing or removal of all microorganisms, including bacterial spores which are highly resistant. Sterilization is an absolute term, i.e. the article must be sterile meaning the absence of all microorganisms.

Disinfection is the killing of many, but not all microorganisms. It is a process of reduction of number of contaminating organisms to a level that cannot cause infection, i.e. pathogens must be killed. Some organisms and bacterial spores may survive.

Antiseptics are mild forms of disinfectants that are used externally on living tissues to kill microorganisms, e.g. on the surface of skin and mucous membranes.

CLASSIFICATION OF METHODS

Sterilization and disinfection are done by :

(A). Physical Agents

1. Heat
2. Radiation
3. Filtration

(B). Chemical Agents

STERILIZATION BY HEAT

Heat is most effective and a rapid method of sterilization and disinfection. Excessive heat acts by coagulation of cell proteins. Less heat interferes metabolic reactions. Sterilization occurs by heating above 100°C which ensure lolling of bacterial spores. Sterilization by hot air in hot air oven and sterilization by autoclaving are the two most common method used in the laboratory.

Types of Heat :

- A. Sterilization by moist heat
- B. Sterilization by dry heat

A. Sterilization by Moist Heat

Moist heat acts by denaturation and coagulation of protein, breakage of DNA strands, and loss of functional integrity of cell membrane.

(I). Sterilization at 100°C

1. Boiling. Boiling at 100°C for 30 minutes is done in a water bath. Syringes, rubber goods and surgical instruments may be sterilized by this method. All bacteria and certain spores are killed. It leads to disinfection.

2. Steaming. Steam (100°C) is more effective than dry heat at the same temperature as: (a) Bacteria are more susceptible to moist heat, (b) Steam has more penetrating power, and (c) Steam has more sterilizing power as more heat is given up during condensation.

3. Tyndallization (Fractional Sterilization). Heat labile media like those containing sugar, milk, gelatin can be sterilized by this method. Steaming at 100°C is done in steam sterilizer for 20 minutes followed by incubation at 37°C overnight. This procedure is repeated for another 2 successive days. That is 'steaming' is done for 3 successive days. Spores, if any, germinate to vegetative bacteria during incubation and are destroyed during steaming on second and third day. It leads to sterilization.

II. Sterilization above 100°C: Autoclaving

Autoclaving is one of the most common methods of sterilization. Principle: In this method sterilization is done by steam under pressure. Steaming at temperature higher than 100°C is used in autoclaving. The temperature of boiling depends on the surrounding atmospheric pressure. A higher temperature of steaming is obtained by employing a higher pressure. When the autoclave is closed and made air-tight, and water starts boiling, the inside pressures increases and now the water boils above 100°C. At 15 lb per sq. inch pressure, 121°C temperatures is obtained. This is kept for 15 minutes for sterilization to kill spores. It works like a pressure cooker.

III. Sterilization below 100°C

1. Pasteurization. Pasteurization is heating of milk to such temperature and for such a period of time so as to kill pathogenic bacteria that may be present in milk without changing colour, flavour and nutritive value of the milk. *Mycobacterium bovis*, *Salmonella* species, *Escherichia coli* and *Brucella* species may be present in milk. It does not sterilize the milk as many living organisms including spores are not destroyed..

B. Sterilization by Dry Heat

Mechanisms. (1) Protein denaturation, (2) Oxidative damage, (3) Toxic effect of elevated electrolyte (in absence of water).

Dry heat at 160°C (holding temperature for one hour is required to kill the most resistant spores). The articles remain dry. It is unsuitable for clothing which may be spoiled.

1. Red Heat. Wire loops used in microbiology laboratory are sterilized by heating to 'red' in benzene burner or spirit lamp flame. Temperature is above 100°C. It leads to sterilization.

2. Flaming. The article is passed through flame without allowing it to become red hot, e.g. scalpel. Temperature is not high to cause sterilization.

3. Sterilization by Hot Air

Hot Air Oven (Sterilizer). It Is one of the most common method used for sterilization. Glass wares, swab sticks, all-glass syringes, powder and oily substances are sterilized in hot air oven. For sterilization, a temperature of 160°C is maintained (holding) for one hour. Spores are killed at this temperature. 160°C.

3. Incineration heat

4. Burning

Physical Antimicrobial Agents- Cooling, Refrigeration

Refrigeration- foods 5C , *Clostridium* spores produce lethal toxins in frig

Freezing- -20C, preserve foods on home and industry, slows rate of microbes so they do not spoil food.

Frozen foods should not be thawed and refroze.

Freeze-drying- lyophilization is drying from a frozen state to make instant coffee, to preserve cultures of microbes.

Physical Antimicrobial Agents- Radiation

UV-ultraviolet light-40-390nm 200nm is most effective wavelength for killing by DNA dimers, UV light for sewage treatment in some areas.

Ionizing radiation- X rays and gamma rays, .1-40nm very short

Microwave radiation-long wavelengths 1mm-1m.

Physical Antimicrobial Agents- other Methods

Sonic and Ultrasonic waves-

Filtration- passage of material through a filter, use millipore -membrane filters 25um

Microbes on filter can be transferred to agar (p346)

HEPA filters- clean air and capture microbes

Osmotic Pressure- plasmolysis or loss of water occurs with high concentration of salt, sugar used in jellies, syrup, pickles (p 347)

Specific Chemical Antimicrobial Agents

Soaps and detergents: remove microbes, oily deposits and dirt, detergent-cationic (+)(food utensils), or anionic (-) laundry and household cleaners.

Quats have amonium disinfectants: BAK, mouthwash.

Acids and Alkali: soap is a mild alkali, acid preservatives retard mold growth in breads, margarine, soft drinks.

Heavy Metals: selenium, mercury, copper, silver can inhibit bacterial growth such as silver nitrate and mercurochrome and merthiolate -tincture w/alcohol, selenium sulfide kills fungi and spores (dandruff shampoo).

Copper sulfate- kills algae in pools.

Halogens: Hypochlorous acid Cl, I, Br, Chloramine

Alcohols: denature proteins, skin antiseptics

Phenols: disrupt cell membranes

Oxidizing Agents:H₂O₂ disenfectant

Alkylating Agents: disrupt nucleic acids and protein structures, may cause cancer, formaldehyde, ethylene oxide, glutaraldehyde

Dyes: acridine and methylene blue, crystal violet

Other Agents: plant oils for thyme and clove, nitrates, sulfites, sodium nitrate (p 338)