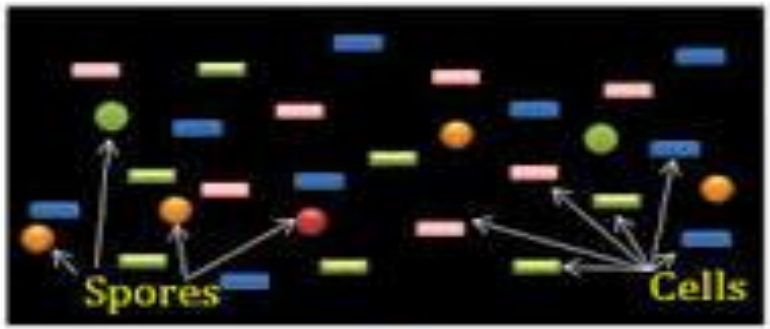
STERILIZATION AND DISINFECTION

- **Sterilization** is defined as the process by which an article, surface or medium is freed of all living **microorganisms either in the vegetative or spores state.**

- **Disinfection**- is the destruction of all pathogenic organisms.

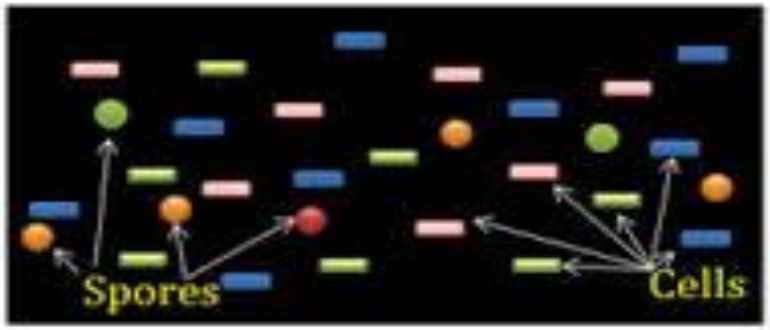
Sterilization vs Disinfection



Treatment with Sterilant

Sterilization

Absolute killing of Microbial vegetative cells and spores



Treatment with Disinfectant

Disinfection

Adequate killing of Microbial vegetative cells, NOT the spores

- **Antiseptics** : are agents that can be safely applied on the skin or mucous membrane to ptevent infection by inhibiting the growth of bacteria.
- **Bactericidal agents** : are substances that can kill bacteria
- **Bacteriostatic agents** : A chemical which is bactericidal at a particular concentration may become bacteriostatic at a higher dilution
- **Decontamination** : a process of rendering an article or area free of contaminants, including

Classification of sterilization methods - Physical agents

Dry heat :

1. Flaming
2. Incineration
3. Hot Air Oven

Moist heat:

1. Temperatures below 100°C
2. Temperatures at 100°C
3. Temperatures above100°C (steam under pressure)

Filtration :

1. Candles
2. Sintered glass filters
3. Asbestos pads
4. Membranes

Chemical Agents

- Alcohols:

  ethyl, isopropyl, trichorobutanol
- Aldehydes : formaldehyde,glutaradehyde,Orthopthalaldehyde
- Peracetic acid
- Hydrogen peroxide
- Hypochlorous acid
- Dyes
- Halogens
- Phenols
- Surface active agents
- Metallic Salts
- Gases : ethylene oxide, formaldehyde,

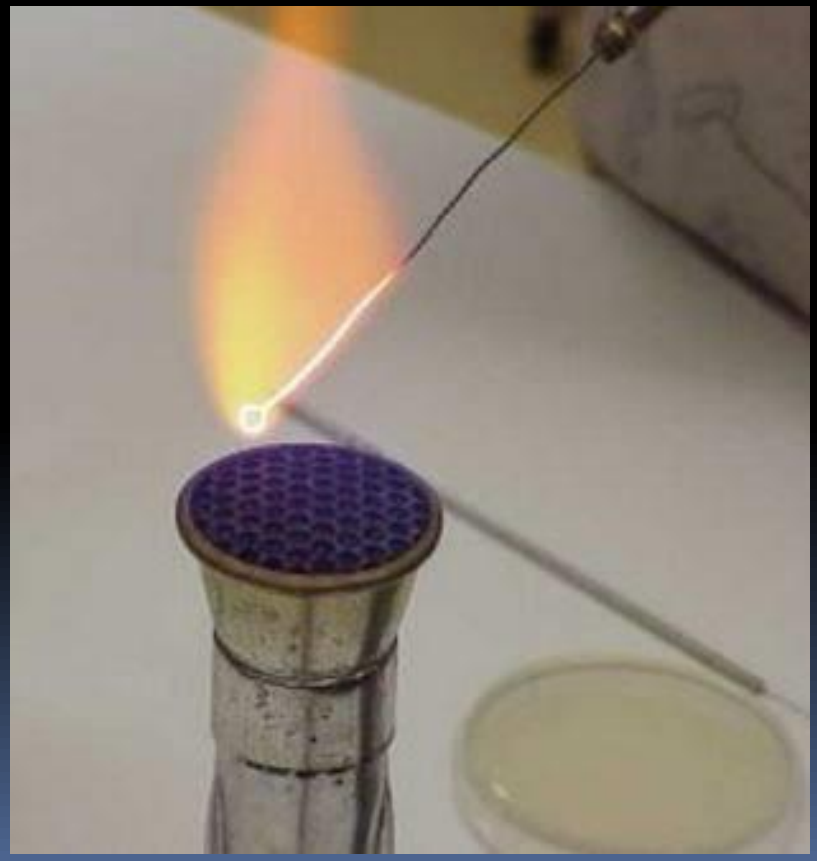
Classification of sterilization methods - Physical agents

A. Dry Heat

- **Principle :**The killing effect of dry heat is due to protein denaturation, oxidative damage and toxic effects of elevated level of electrolytes.

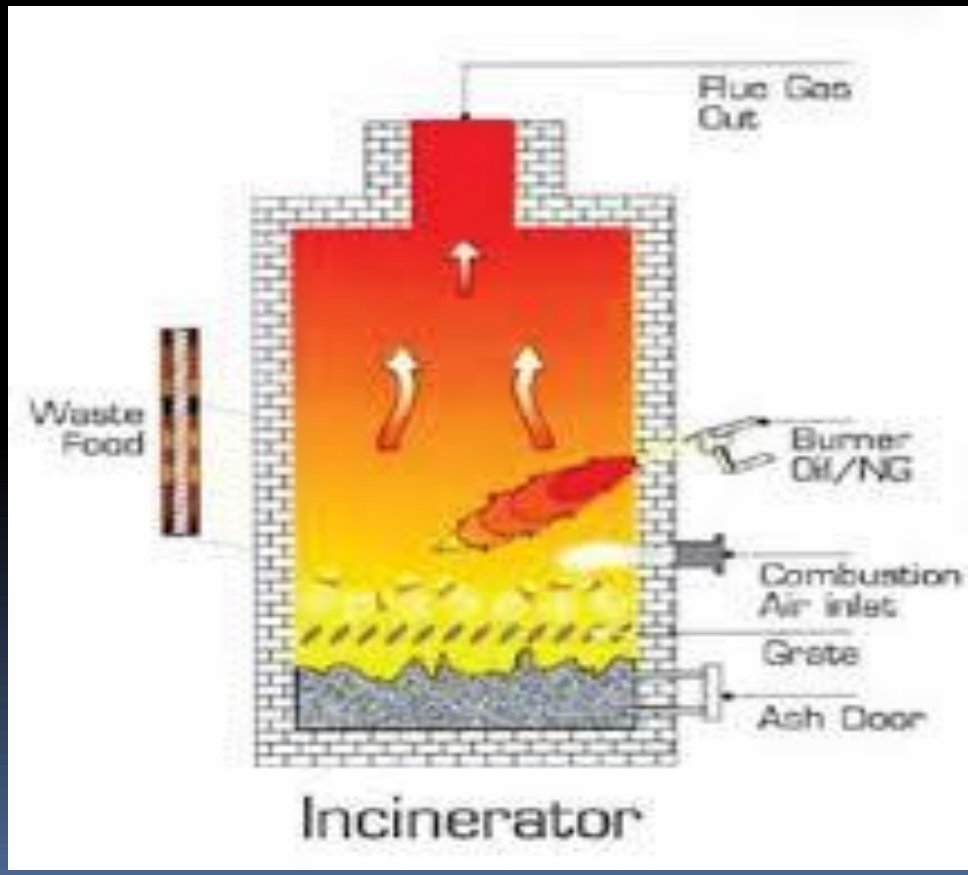
Classification of sterilization methods - Physical agents

- **Flaming** :
- Items are held in the flame of a Bunsen burner either for long time or short time.
  - Longer time exposure - for inoculating wires or loops, tips of forceps.
  - For shorter period - mouth of test tubes.



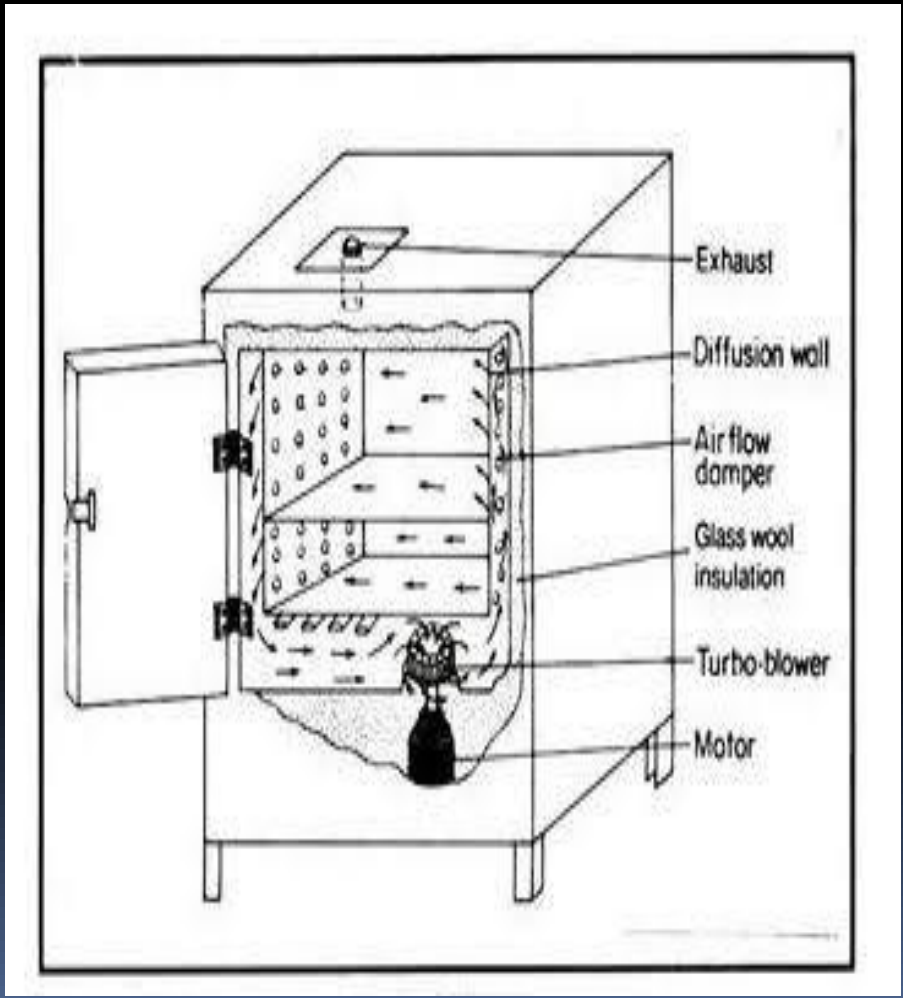
Incineration

- Used for the disposal of waste materials.
  - It burns (sterilizes) the anatomical waste and microbiology waste by providing a very high temperature 870 to 1,200°C - converting the waste into ash, flue gas, and heat.



Hot Air Oven

- Most widely used method of sterilization by dry heat.
- It is electrically heated and is fitted with a fan to ensure adequate and even distribution of hot air in the chamber.
- It is also fitted with a thermostat which maintains the chamber air at a chosen temperature.
- **Holding temperature and time of 160°C** for 2 hours.



- **Materials sterilized:**
  - Glassware - syringes, petri dishes, flasks, pipettes and test tubes.
  - Surgical instruments - scalpels, forceps, etc.
  - Chemicals such as liquid paraffin, fats, glycerol, oil, and glove powder, etc.

- Sterilization control:
- Physical : temperature monitoring by thermocouples
- Chemical: Browne's tube (green spot)
- Biological : Heat resistant spores of a non-toxicgenic strain of Clostridium tetani

- **Precautions :**
- Overloading of hot air oven should be avoided.
- Material should be arranged in a manner so that free circulation of air is maintained.
- Material to be sterilized should be dried completely.
- Cotton plugs should be used to close the mouths of test tubes, flasks, etc.
- Paper wrapping of the items should be done.
- Any inflammable material like rubber (except silicone rubber) should not be kept inside the oven.
- Oven must be allowed to cool for two hours before opening the doors, since the glassware may crack by sudden cooling.

B. Moist Heat :

Principle: The killing effect of moist heat is due to protein denaturation and coagulation – a process that requires participation of water.

I) **Temperature below 100°C –**

Pasteurization – For sterilization of milk.

Holder method – (63°C for 30 minutes)

Flash method – (72°C for 15-20 seconds followed by rapid cooling to 13°C or lower)

- All non sporing pathogens are killed except Coxiella burnetti which may survive in the holder method.

Spores may not destroy.

- **Inspissation :**

- Process of heating an article on 3 successive days at **80 -85°C for 30 minutes.**
- Working principle:
  - first exposure - kills all the vegetative forms.
  - Intervals between the heating the remaining spores germinate into vegetative forms which are then killed on subsequent heating.
- **Water bath**:
  - For sterilization of serum or body fluid containing coagulable protein
  - Heating for 1 hour at 56°C on several successive days
- **Vaccine bath** :
  - For inactivation of non sporing bacteria for preparation of vaccines
  - Heating for 1 hour at 60°C

2) **Temperature at 100°C –**

- **Boiling** - 100°C for 10-30 minutes kills almost all vegetative bacteria. Spore forming bacteria may not be eliminated completely
- Steaming- used for sterilization of culture media containing sugar & gelatin, which may be damaged at higher temperature.

Tyndallisation – (Intermittent sterilization):

- Done at 100°C for 30 minutes on three successive days
- USE – Sterilize the media containing carbohydrates and gelatin
- Steam at atmospheric pressure(100°C)- Koch or Arnold steamer

3) **Temperature above 100 ° C - Steam under pressure above 100°C –**

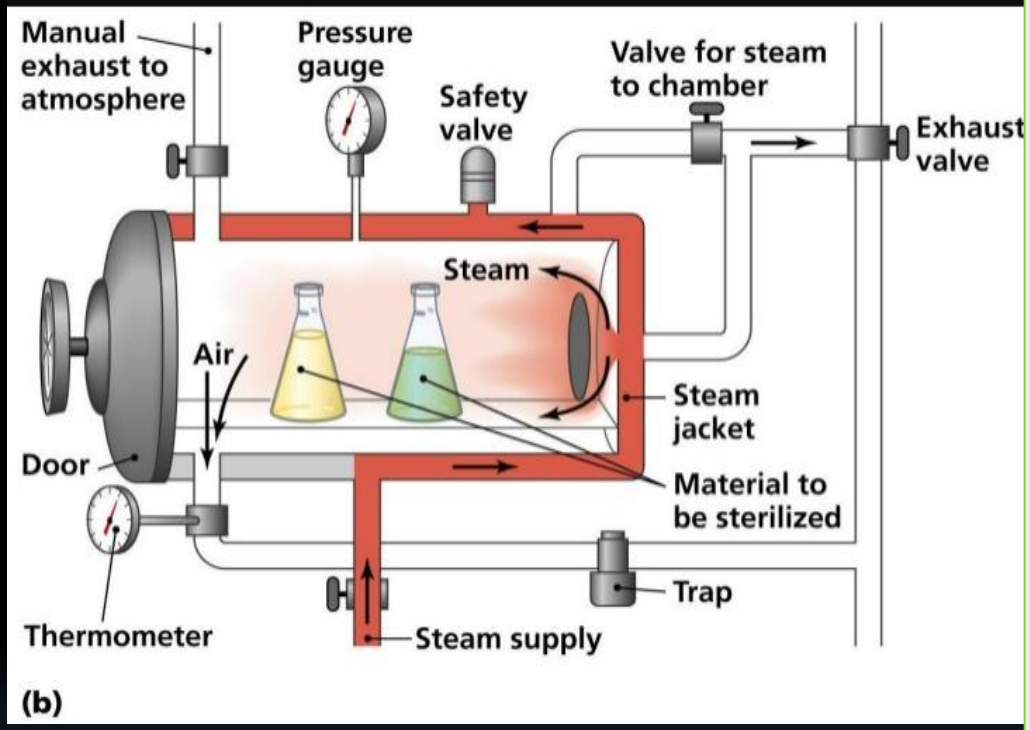
- **AUTOCLAVE**

Principle : Water boils when its vapour pressure equals that of surrounding atmosphere. When pressure inside the closed vessel increases, the temperature at which water boils also increases. When steam comes into contact with cooler surface, it condenses to water and gives up its latent heat to that surface. The condensed water ensures moist condition for killing the microbes present.

- Sterilisation by steam under pressure is carried out between 108°C (above atmorspheric pressure) and 147°C (30 lbs / inch^2)

  Use -To sterilize media, containers, instruments, glassware., lab coat, dressing material, distilled water, saline etc. which ensure complete killing of microorganism including spores.
- Autoclave can be set to provide higher temperatures by adjusting the pressure provided to the vessel.
  - 121°C for 15 minutes at pressure of 15 lbs psi.
  - 126 °C for 10 minutes at pressure of 20 psi.
  - 133 °C for 3 minutes at pressure of 30 psi.

AUTOCLAVE



Gravity displacement type autoclave: most commonly used.

Positive pressure displacement type autoclave

Negative pressure (vacuum) displacement type.

Laboratory autoclave

Hospital dressing sterilisers

Instrument sterilisers

Rapid cooling sterilisers

Precautions to be taken :

- Materials are loaded in such a way that it allows efficient steam penetration (do not overfill the chamber)
- Clean items and the wastes should be autoclaved separately
- Polyethylene trays should not be used as they may melt and cause damage to the autoclave
- If lid opened after the pressure inside has fallen below atmospheric pressure otherwise liquid media boils violently spill from the container & may explode

Sterilisation control :

- **Biological indicator:** Spores of *Geobacillus stearothermophilus* (formerly called *Bacillus stearothermophilus). S*pores are killed in 12 minutes at 121°C
- **Chemical indicators**
  - Autoclave tape
  - Bowie-Dick test
  - Physical control: For example, digital displays on the equipment displaying temperature, time and pressure.

Radiation

A. Nonionising *radiation :*

Infrared and UV rays (Low energy type)

- Effective wavelength is between 240 nm and 280 nm ; 254 nm is most effective wave length.
- Action: These are mutagenic and produce lethal photochemical changes in enzymes and cell constituents
- Use - Infrared radiation for rapid mass sterilization of pre packed items
- -UV radiation for disinfecting enclosed area
- -Preparation of bacterial and viral vaccines

Cold Sterilisation :

Process carried out at low temperature with help of chemicals,radiation, membranes(filters) & all other means excluding high temperature

B. Ionizing radiation -

Gamma rays, X rays, cosmic rays and high energy

electron (High energy type)

Action :Lethal to DNA

High penetrative power

Consider as a cold sterilization

Use: for sterilizing items like syringe, swabs, catheter, oil, greases etc.

Filtration :

- Helps to remove bacteria from heat labile liquid.
- Can be used to obtain bacteria-free filtrates of clinical samples for virus isolation.
- Bacterial toxin can be obtained by passing culture through filters.

A)Candle filters: for purification of water : Two types:

1) Unglazed ceramic filter eg. **Chamberland and Doulton filter**

2) Diatomaceous earth filters eg. **Berkefeld filter**

B) Asbestos filters: Have high adsorbing capacity and tend to alkalinize filtered liquid. It is potentially carcinogenic. eg. **Seitz filter**

C) **Membrane filters:** made of cellulose ester and are called as Millipore or Polypore filter.

Use: water purification, sterility testing, and for the preparation of solutions for parenteral use. Average pore diameters – 0.22mm

D) Sintered glass filters: composed of finely powdered glass particles of different sizes. They have low adsorbing capacity, can be cleaned easily but expensive and being glass, fragile.

Sintered Glass

Membrane filter

Other Physical Methods of Microbial Control:

Filtration: Removal of microbes by passage of a liquid or gas through a screen like material with small pores. Used to sterilize heat sensitive materials like vaccines, enzymes, antibiotics, and some culture media.

- **High Efficiency Particulate Air Filters (HEPA):** Used in operating rooms and burn units to remove bacteria from air.
- **Membrane Filters**: Uniform pore size. Used in industry and research. Different sizes:
  - **0.22 and 0.45um Pores**: Used to filter most bacteria. Don't retain spirochetes, mycoplasmas and viruses.
  - **0.01 um Pores**: Retain all viruses and some large proteins.

- High Efficiency Particulate Air filters (HEPA)
- Used in operating rooms & burns units to remove bacteria from air
- Membrane filters :
- Uniform pore sizes. Used in industry & research. Different sizes
- 0.45 & 0.22 µm pores : used to filter most bacteria . Don't retain Spirochetes, mycoplasma & viruses
- 0.01 µm pores :Retain all viruses & some large proteins

Disinfection means the destruction or removal of **all pathogenic organism**, or organism capable of giving rise to infection.

Mode of action :

- Damage to cell wall
- Alter permeability of cell membrane
- Alter proteins – form protein salts or cause protein coagulation
- Inhibit enzyme action and interfere with metabolism
- Inhibit nucleic acid synthesis or alter nucleic acid metabolism
- Disruption of cell membrane
- Cause oxidation or hydrolysis

CHEMICAL METHODS OF MICROBIAL CONTROL

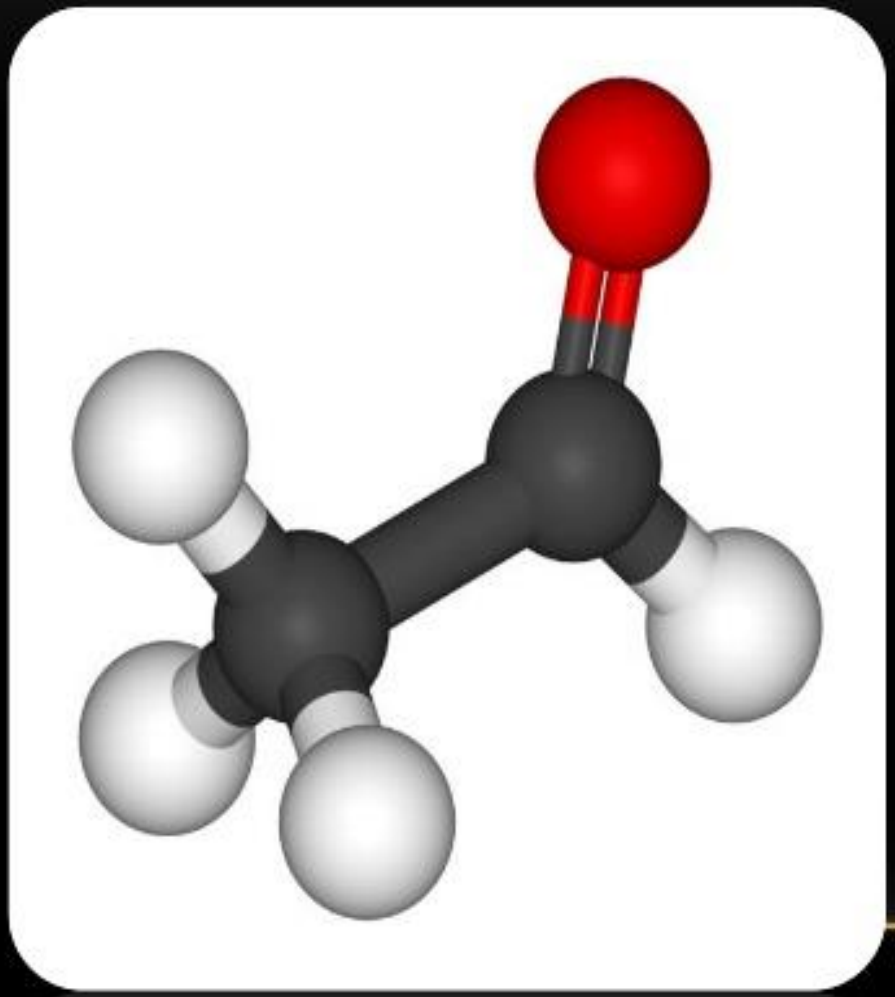
- **Major Categories**
  - Phenols
  - Alcohols
  - Halogens
  - Oxidizing agents
  - Surfactants
  - Heavy Metals
  - Aldehydes
  - Gaseous Agents
  - Antimicrobics

ISOPROPYL ALCOHOL (70%)

ISOPROPYL ALCOHOL (70%)

- Powerful disinfectant and antiseptic
- Mode of action: denatures proteins, dissolves lipids and can lead to cell membrane disintegration
- Effectively kills bacteria and fungi
  - **But <u>does not</u> inactivate spores!**

ALDEHYDES



- Denature proteins and inactivate nucleic acids
- Glutaraldehyde both disinfects (short exposure) and sterilizes (long exposure)
- Formalin used in embalming and disinfection of rooms and instruments

PHENOL AND PHENOLICS

- Intermediate- to low-level disinfectants
- Denature proteins and disrupt cell membranes
- Effective in presence of organic matter and remain active for prolonged time
- Commonly used in health care settings, labs, and homes (Lysol, triclosan)
- Have disagreeable odor and possible side effects

HALOGENS

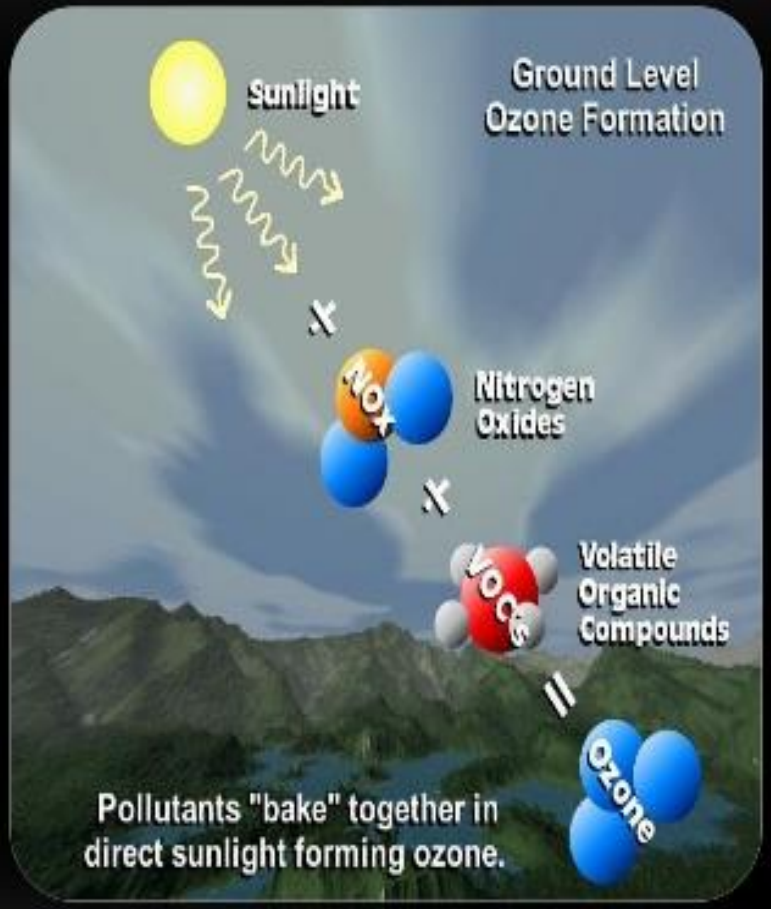
- Intermediate-level antimicrobial chemicals
- Believed that they damage enzymes via oxidation or by denaturing them
- Iodine tablets, iodophores (Betadine®), chlorine treatment of drinking water, bleach, chloramines in wound dressings, and bromine disinfection of hot tubs

GASEOUS AGENTS

- Ethylene oxide, propylene oxide, and beta-propiolactone used in closed chambers to sterilize items
- Denature proteins and DNA by cross-linking functional groups
- Used in hospitals and dental offices
- Can be hazardous to people, often highly explosive, extremely poisonous, and are potentially carcinogenic

OXIDIZING AGENTS

- Peroxides, ozone, and per acetic acid kill by oxidation of microbial enzymes
- High-level disinfectants and antiseptics
- Hydrogen peroxide can disinfect and sterilize surfaces of objects
- **Ozone treatment of drinking water**
- Per acetic acid – effective sporocide used to sterilize equipment



SURFACTANTS

- Surface active" chemicals that reduce surface tension of solvents to make them more effective at dissolving solutes
- Soaps and detergents
  - Soaps have hydrophilic and hydrophobic ends; good degerming agents but not antimicrobial
  - Detergents are positively charged organic surfactants
- Quats – colorless, tasteless, harmless to humans, and antimicrobial; ideal for many medical and industrial application
  - Low-level disinfectants

SPORICIDAL AGENTS

- Glutaraldehyde
- Formaldehyde
- Other aldehydes
- Chlorine-releasing agents
- Iodine and iodophores
- Peroxygens
- Ethylene oxide
- P-Propiolactone

- A. D. RUSSELL, 1999. Bacterial Spores and Chemical Sporicidal Agents. CLINICAL MICROBIOLOGY REVIEWS Vol. 3, No. 2 p. 99-119 .

- **Testing of Disinfectants**
- 1. Rideal- Walker test
- 2. Chick-Martin test
- 3. Kelsey-Sykes or in –use test
- **Spaulding's classification**
- 1.Critical items
- 2. Semi-critical items
- 3.Non-critical items
- **New method of sterilisation of heat-sensitive articles :**

- Plasma sterilisation :
- Fourth state of matter
- Consists of ions,electrons or neutral particles
- Radiofrequency energy is applied to create an elecromagnatic field
- H_2O_2 vapours generates a state of plasma contains free radicals of hydrogen & oxygen.
- MOA : denature of all microorganisms
- Use : Arthroscopes, urethroscopes etc.