Control of Microbial Growth

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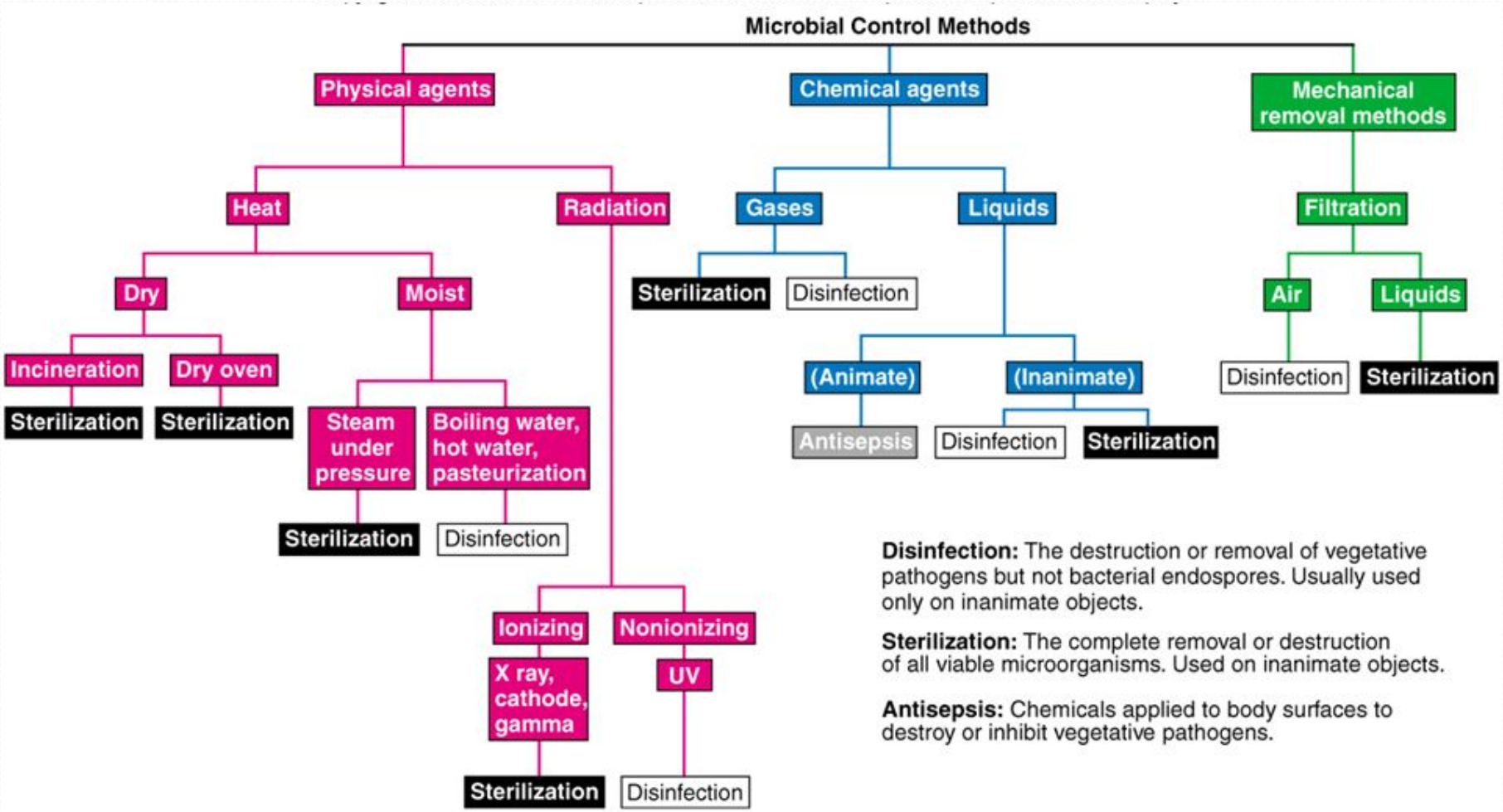
Control of microbes

Physical and chemical methods to destroy or reduce microbes in a given area

Physical and Chemical Agents for the Control of Microbial Growth



Relative resistance of microbes

- Highest resistance
  - Bacterial endospores
- Moderate resistance
  - *Pseudomonas sp.*
  - *Mycobacterium tuberculosis*
  - *Staphylococcus aureus*
  - Protozoan cysts
- Least resistance
  - most vegetative cells
  - Fungal spores
  - enveloped viruses
  - Yeast
  - Protozoan trophozoites

Terms

- **Sterilization**– A physical or chemical process that destroys all viable microbes, including viruses & endospores(15 psi/121°C/15min (Steam under pressure)
- **Disinfection** – a process to destroy vegetative pathogens, not endospores
  - A *disinfectant* is a disinfecting chemical or physical agent , normally applied to non-living material

- **Sanitization**– any cleansing technique that mechanically removes microbes
- **Degermation** – reduces the number of microbes
- **Antiseptic**-antimicrobial agent used on living tissue

Cleaning : the physical removal of organic material or soil from objects, is usually done by using water with or without detergents.

Disinfection :It is killing or removing of harmful microorganisms

Disinfectant: a chemical substance used to kill microbes on surfaces but too toxic to be applied directly to tissue.

Antiseptic :A product that destroys or inhibits the growth of microorganisms in or on living tissue.

Sterilant: an agent or method used to remove or kill all microbes.

Septic: presence of pathogenic microbes in living tissue.

Aseptic: absence of pathogenic microbes.

Sterile: free of life of every kind.

Bacteriostatic: inhibiting bacterial multiplication. Bacteriostatic action is reversible by removal or inactivation of agent.

Bactericidal: killing bacteria

cidal vs. static

- Bactericidal - kills bacteria
- Bacteristatic - inhibits bacterial growth

- Fungicidal
- Fungistatic
- Algacidal
- Algastatic

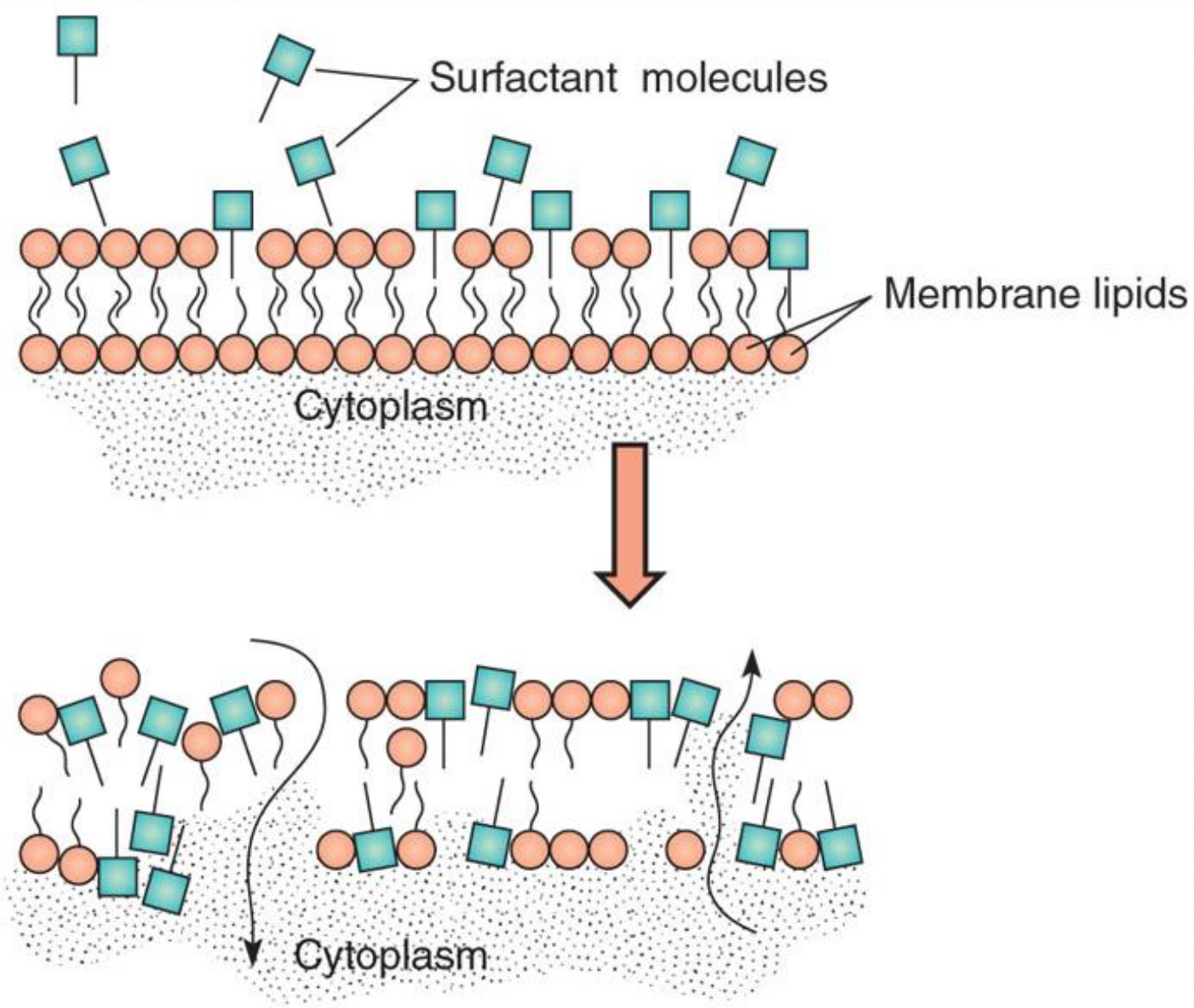
Factors that influence action of antimicrobial agents:

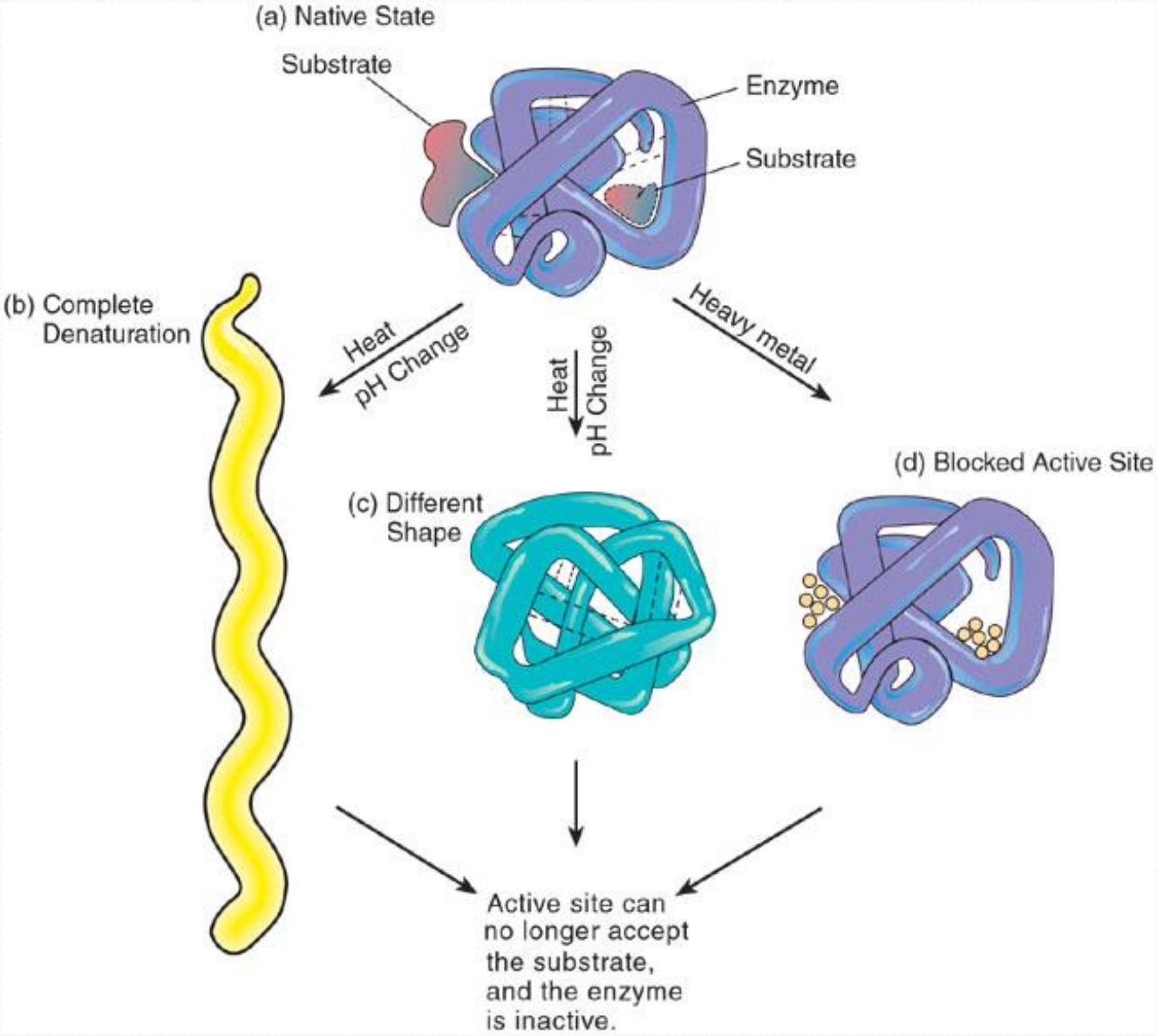
Several critical factors play key roles in determining the effectiveness of an antimicrobial agent, including:

1. Number (population size)
2. Type and activities of microbes
3. Nature of microbes in the population
4. Temperature & pH of environment
5. Time (Duration of exposure)
6. Concentration of antimicrobial agent
7. Presence of organic matter, solvents and inhibitors
8. Biofilm formation(protects communities of microbes)

Cellular targets of control

1. Cell wall
2. Cell membrane(Alterative of cell permeability)
3. Cellular synthetic processes (DNA, RNA)
4. Proteins, Enzymes


Surfactant molecules
Membrane lipids
Cytoplasm
Cytoplasm


(a) Native State
Substrate
Enzyme
Substrate
(b) Complete Denaturation
Heat
pH Change
Heat
pH Change
Heavy metal
(c) Different Shape
(d) Blocked Active Site
Active site can no longer accept the substrate, and the enzyme is inactive.

Methods of Physical Control

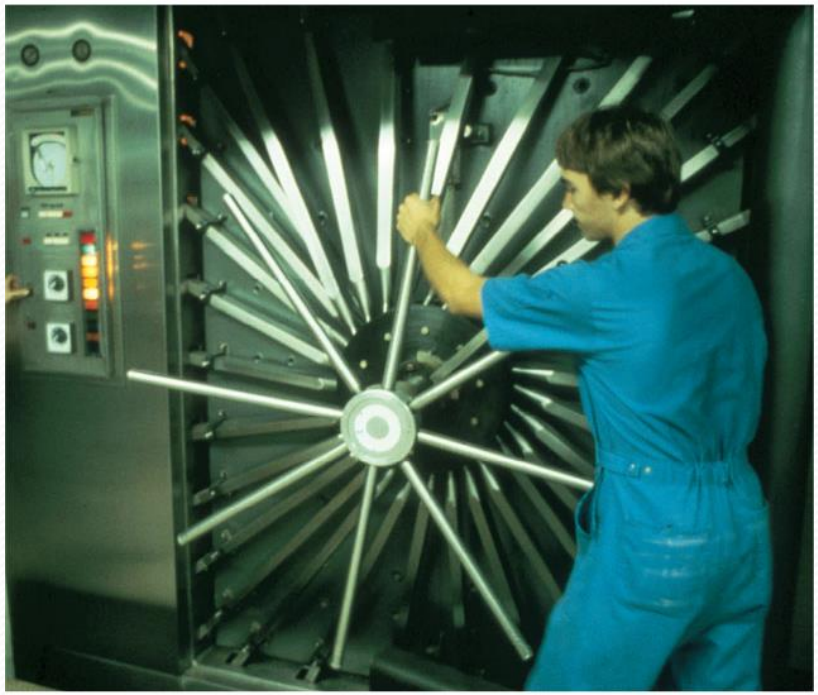
1. Heat
2. Low temperatures
3. Desiccation
4. Radiation
5. Filtration

1. Heat

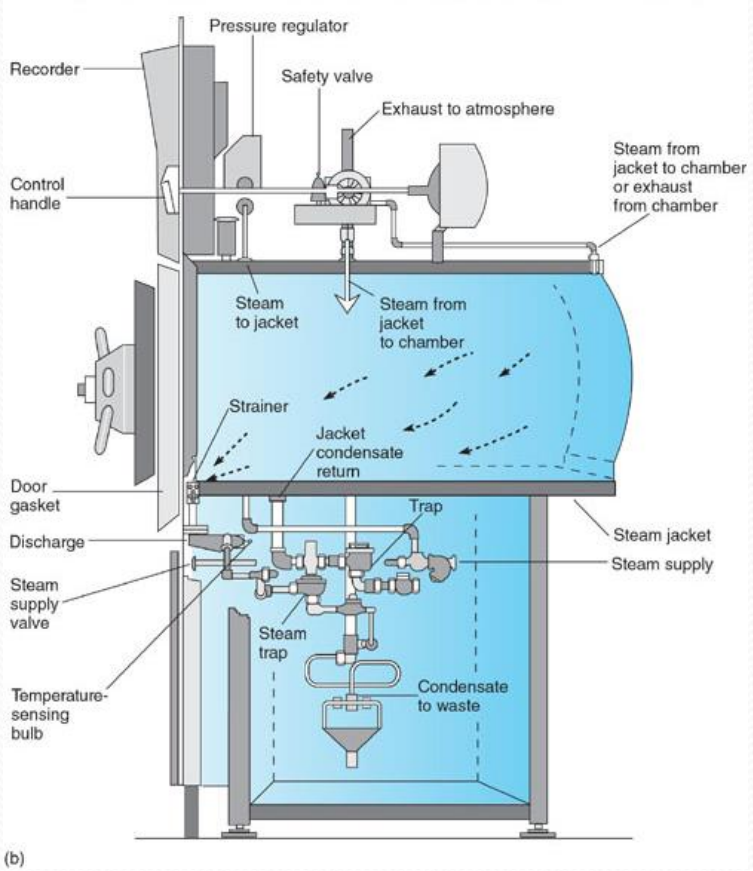
- **Moist heat** – use of hot water or steam .
  - Boiling Water
  - Steam Heat (Autoclave)

- Mode of action – denaturation of proteins, destruction of membranes & DNA
- sterilization
- autoclave 15 psi/121°C/10-40min (Steam under pressure)

- intermittent sterilization – unpressurized steam at 100°C 30-60 min for 3 days
- disinfection
- Pasteurization <100°C for seconds; kills *Salmonella, Listeria, Ricktessia (Q fever): Mycobacterium t(15 sec. 60°C)*
- Boiling at 100°C for 30 minutes to destroy non-spore-forming pathogens
  - kills vegetative bacterial cells, Fungi and many viruses
  - not effective for endospores and some viruses
  - Hepititis (20 min)
  - Some spores may survive boiling water for up to 20 hrs
  - Increasing the pressure raises the Temp.
  - 15 psi **121 C for 15 min.**



(a)


Pressure regulator
Recorder
Safety valve
Exhaust to atmosphere
Steam from jacket to chamber or exhaust from chamber
Control handle
Steam to jacket
Steam from jacket to chamber
Strainer
Jacket condensate return
Door gasket
Trap
Discharge
Steam jacket
Steam supply
Steam supply valve
Steam trap
Condensate to waste
Temperature-sensing bulb
(b)

Dry Heat

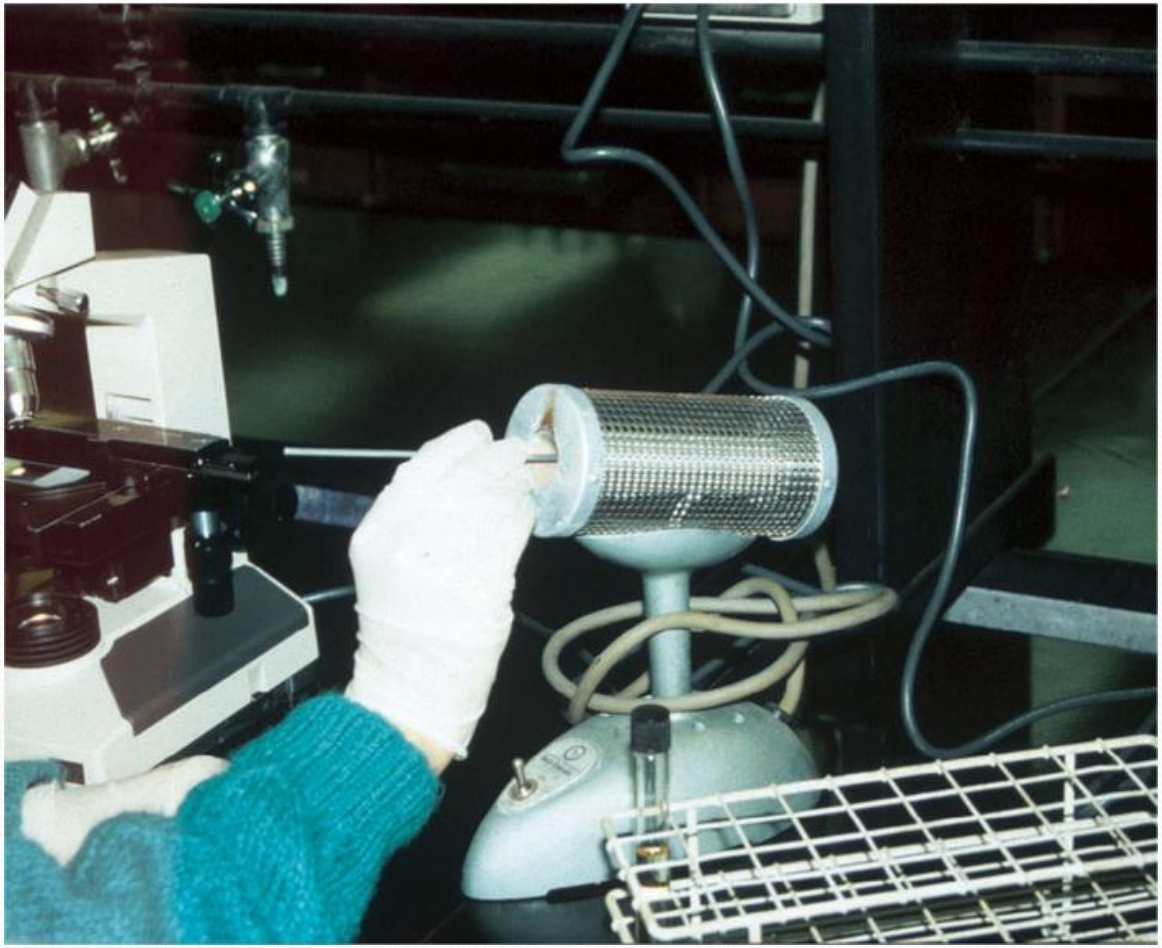
Dry heat using higher temperatures than moist heat, can also sterilize

- Incineration – 600-1200°C combusts & dehydrates cells

disposable wastes (paper cups, bags, dressings)

- dry ovens – 150-180°C- coagulate proteins(170 C for 2 hours) (test tube , pipette , Petri dish .
- Direct Flaming
  - Inoculating Loop and Needle 100% effective
- Hot Air Sterilization
  - used on substances that would be damaged by moist heat sterilization
    - gauzes, dressings or powders

Dry heat



2. Low temperatures

- Microbistatic – slows the growth of microbes
- refrigeration 0-15°C & freezing <0°C
- used to preserve food, media and cultures

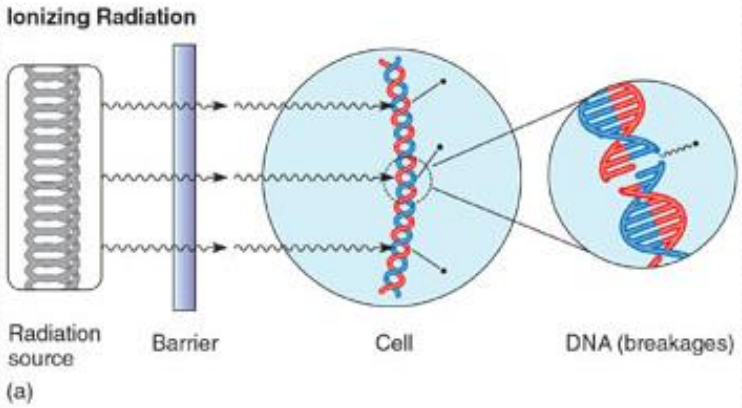
3. Desiccation

- gradual removal of water from cells, leads to metabolic inhibition
- not effective microbial control – many cells retain ability to grow when water is reintroduced

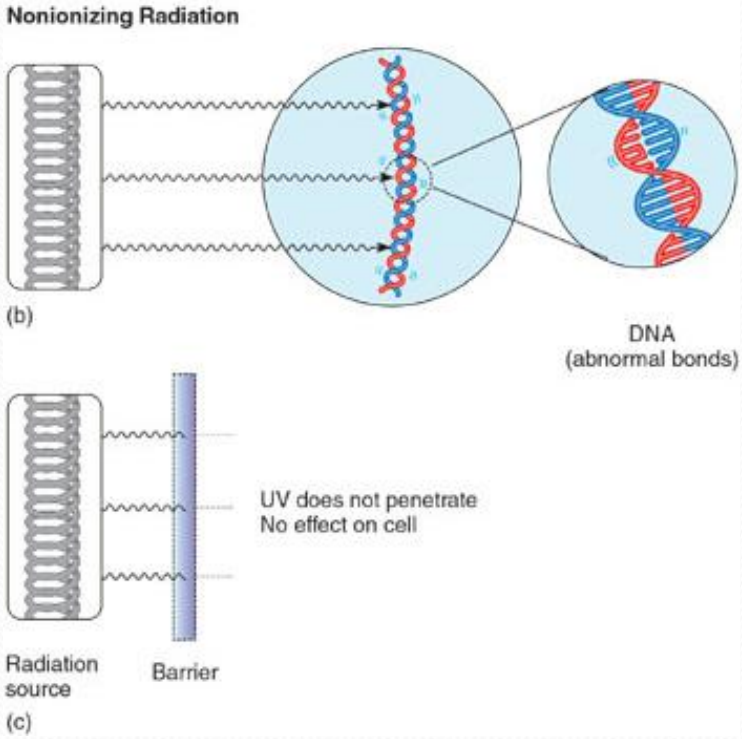
4. Radiation

1. Ionizing radiation – deep penetrating power, breaks DNA,
   - gamma rays, X-rays, cathode rays
   - used to sterilize medical supplies & food products
2. Nonionizing radiation – little penetrating power to sterilize air, water & solid surfaces
   - UV light creates thymine pyrmidines, which interfere with replication

- Used on substances that could be damaged by heat
  - plastic petri dishes
  - plastic syringes
  - surgical gloves



Ionizing radiation breaks DNA

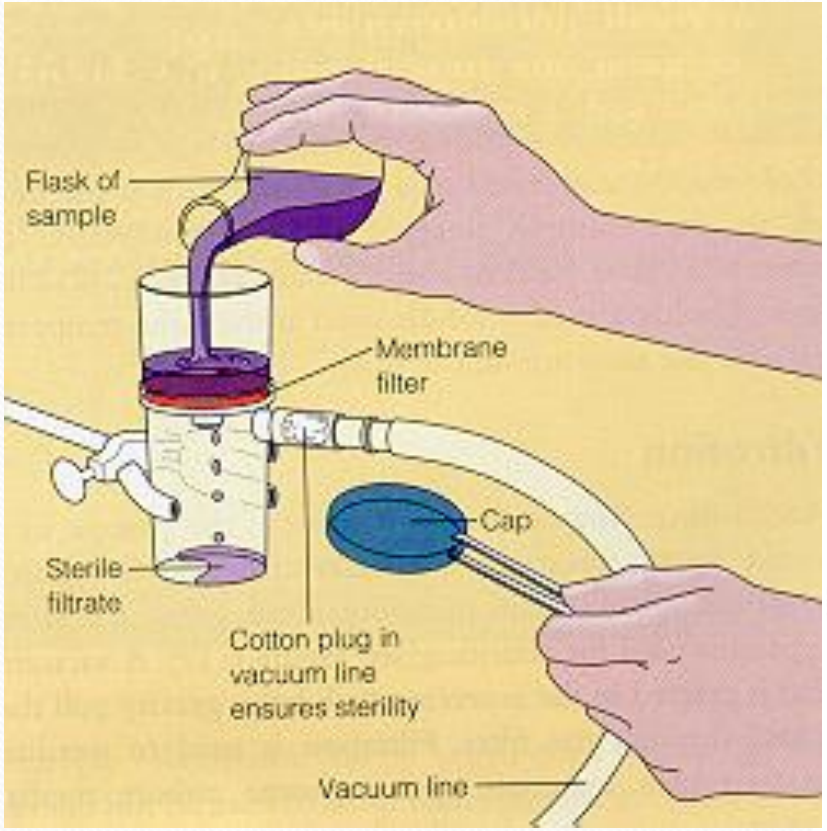


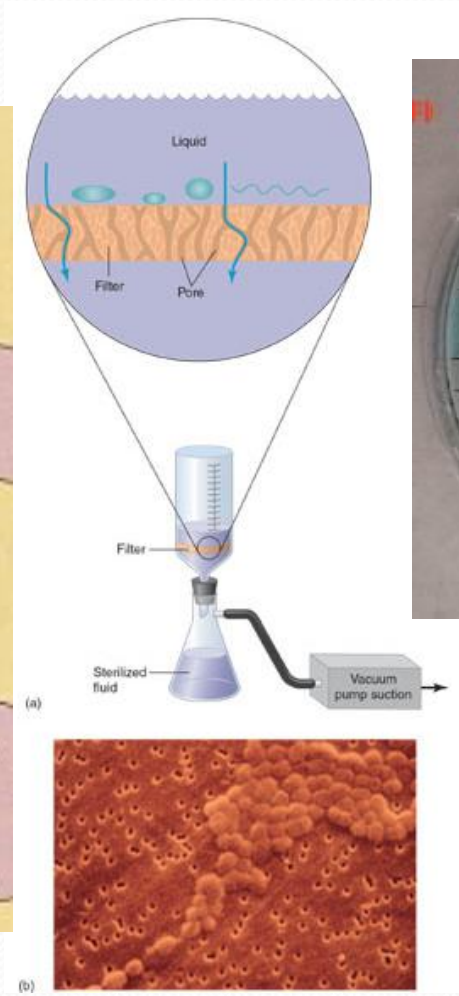
Nonionizing radiation interfere with replication

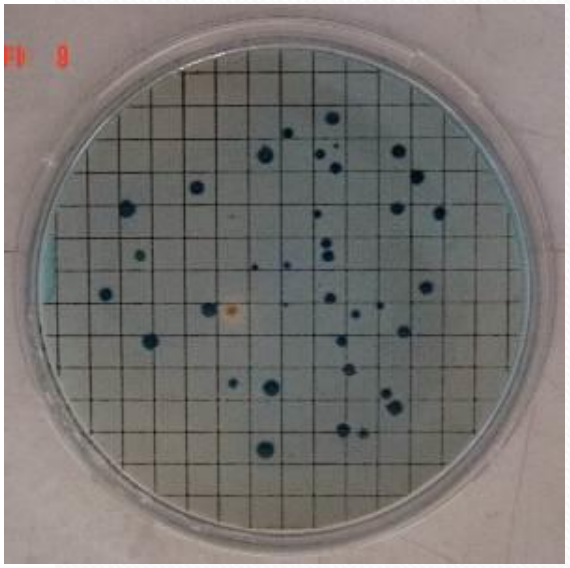
5. Filtration

- physical removal of microorganisms from solutions that might be damaged by heat
- used to sterilize heat sensitive liquids & air in hospital isolation units & industrial clean rooms
  - culture media
  - enzymes
  - vaccines
  - antibiotics

Filtration


Flask of sample
Membrane filter
Cap
Sterile filtrate
Cotton plug in vacuum line ensures sterility
Vacuum line


Liquid
Filter
Pore
Filter
Sterilized fluid
Vacuum pump suction
(a)
(b)



Chemical control

1. Halogens
2. Phenolics
3. Chlorhexidine
4. Alcohols
5. Hydrogen peroxide
6. Detergents & soaps
7. Heavy metals
8. Aldehydes

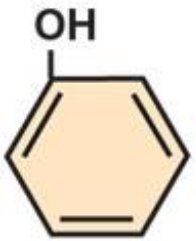
1. Halogens

- Chlorine – Cl_2, hypochlorites (chlorine bleach), chloramines
  - Denaturation of proteins by disrupting disulfide bonds
  - Can be sporicidal

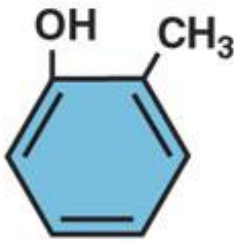
- Iodine - I_2
  - Denature proteins
  - Can be sporicidal
  - Milder medical & dental degerming agents, disinfectants, ointments

2. Phenolics

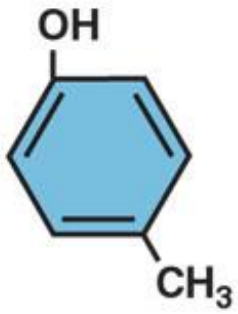
- Disrupt cell membranes & precipitating proteins ; bactericidal, fungicidal, virucidal, not sporicidal
  - Lysol
  - triclosan- antibacterial additive to soaps



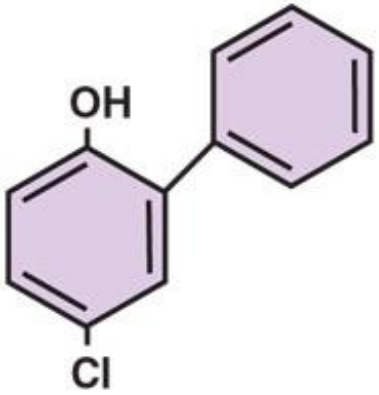
Phenol
(basic aromatic
ring structure)



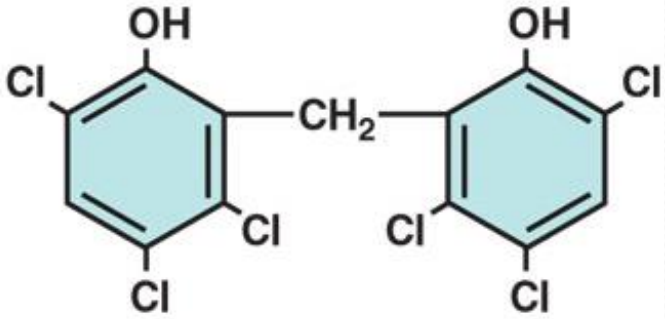
o-cresol



p-cresol



Chlorophene
(a chlorinated phenol)



Hexachlorophene
(a bisphenol)

3. Chlorhexidine:

is a chemical antiseptic. It is effective on both Gram-positive and Gram-negative bacteria

- A surfactant & protein denaturant with broad microbicidal properties
- Not sporicidal
- Used as skin degerming agents for preoperative scrubs, skin cleaning & burns
- Chlorhexidine is harmful in high concentrations, but is used safely in low concentrations in many products, such as mouthwash

4. Alcohols

(is an organic compound in which the hydroxyl functional group(-OH)

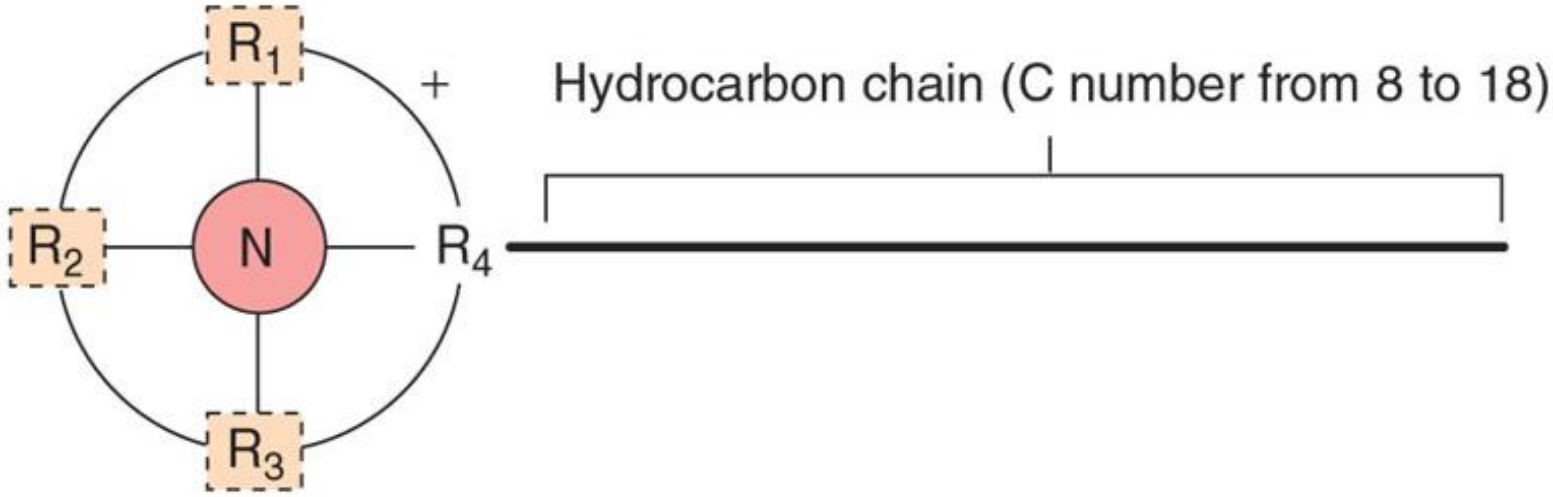
- Ethyl, isopropyl in solutions of 50-90%
- Act as surfactants dissolving membrane lipids and coagulating proteins of vegetative bacterial cells and fungi
- Not sporicidal

5. Hydrogen peroxide (H_2O_2)

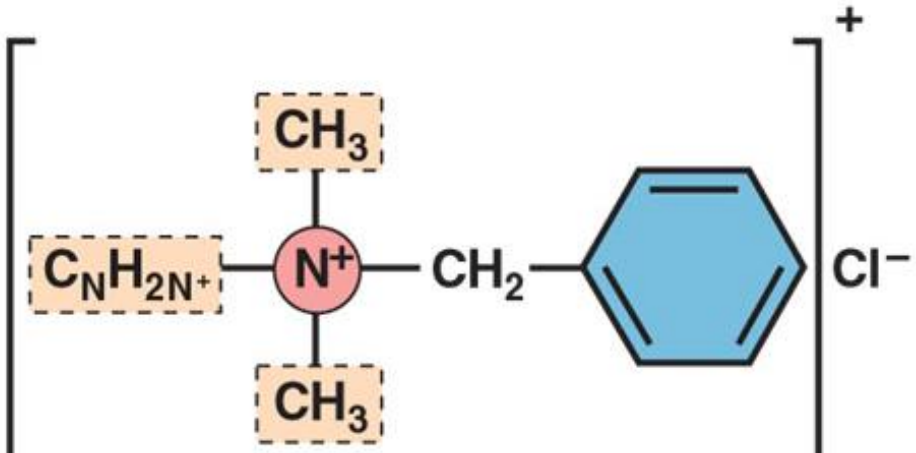
- Weak (3%) to strong (25%)
- Produce highly reactive hydroxyl-free radicals that damage protein & DNA while also decomposing to O_2 gas – toxic to anaerobes
- Strong solutions are sporicidal

6. Detergents & soaps

- Quaternary ammonia act as surfactants that alter membrane permeability of some bacteria & fungi
  - Not sporicidal
- Soaps- mechanically remove soil and grease containing microbes

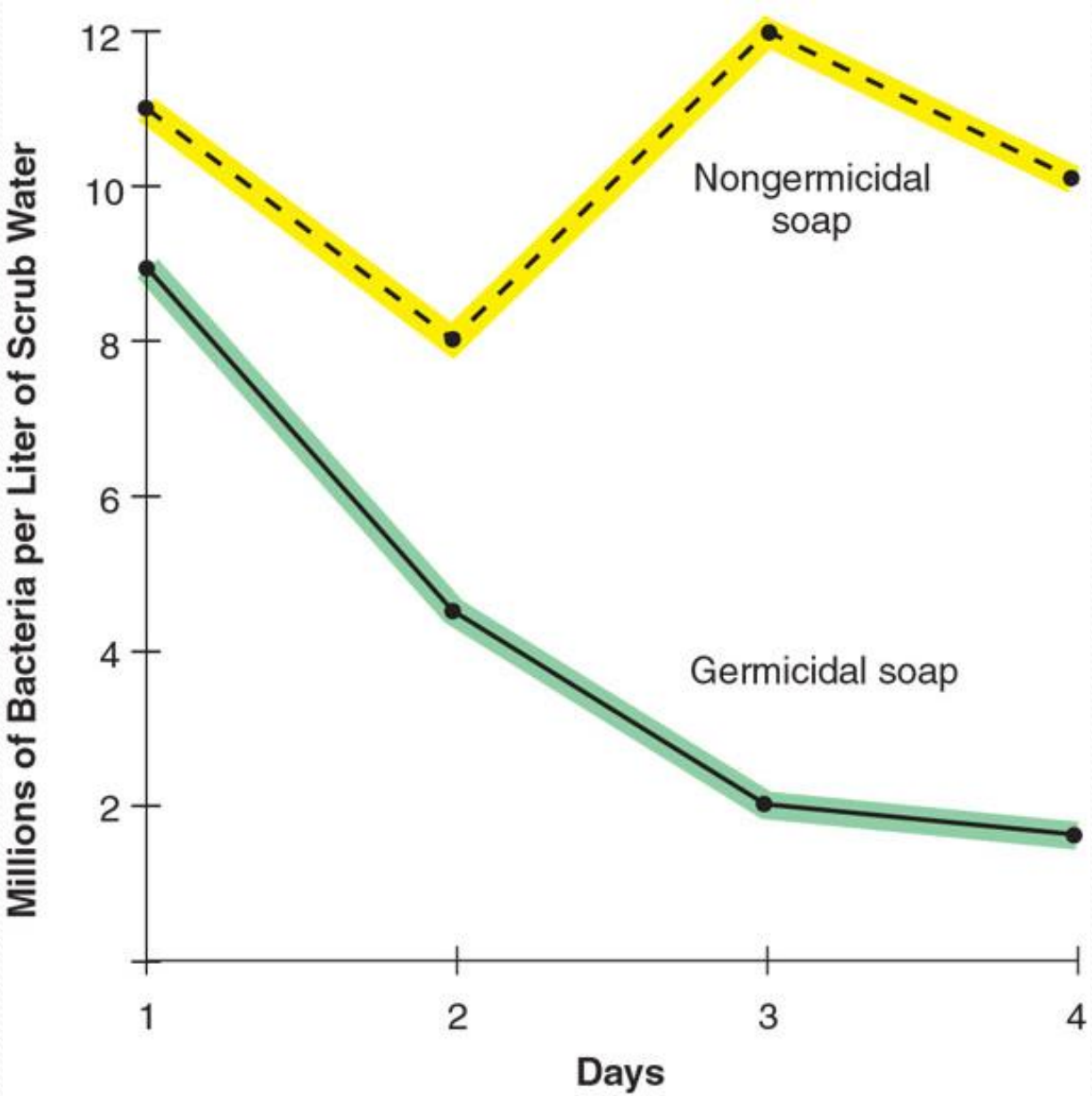


(a)



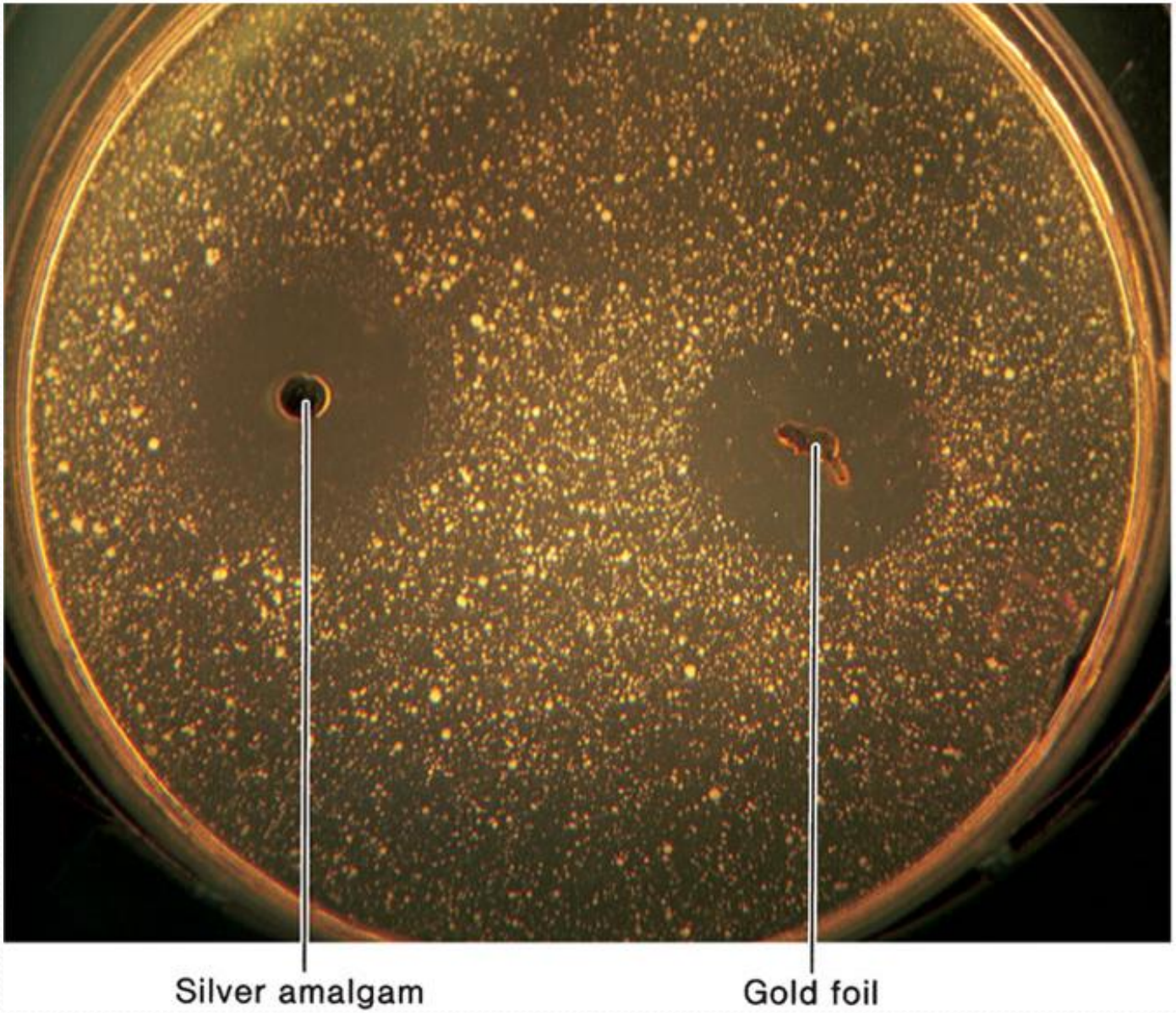
Benzalkonium chloride

(b)


Millions of Bacteria per Liter of Scrub Water
12
10
8
6
4
2
1
2
3
4
Days
Nongermicidal soap
Germicidal soap

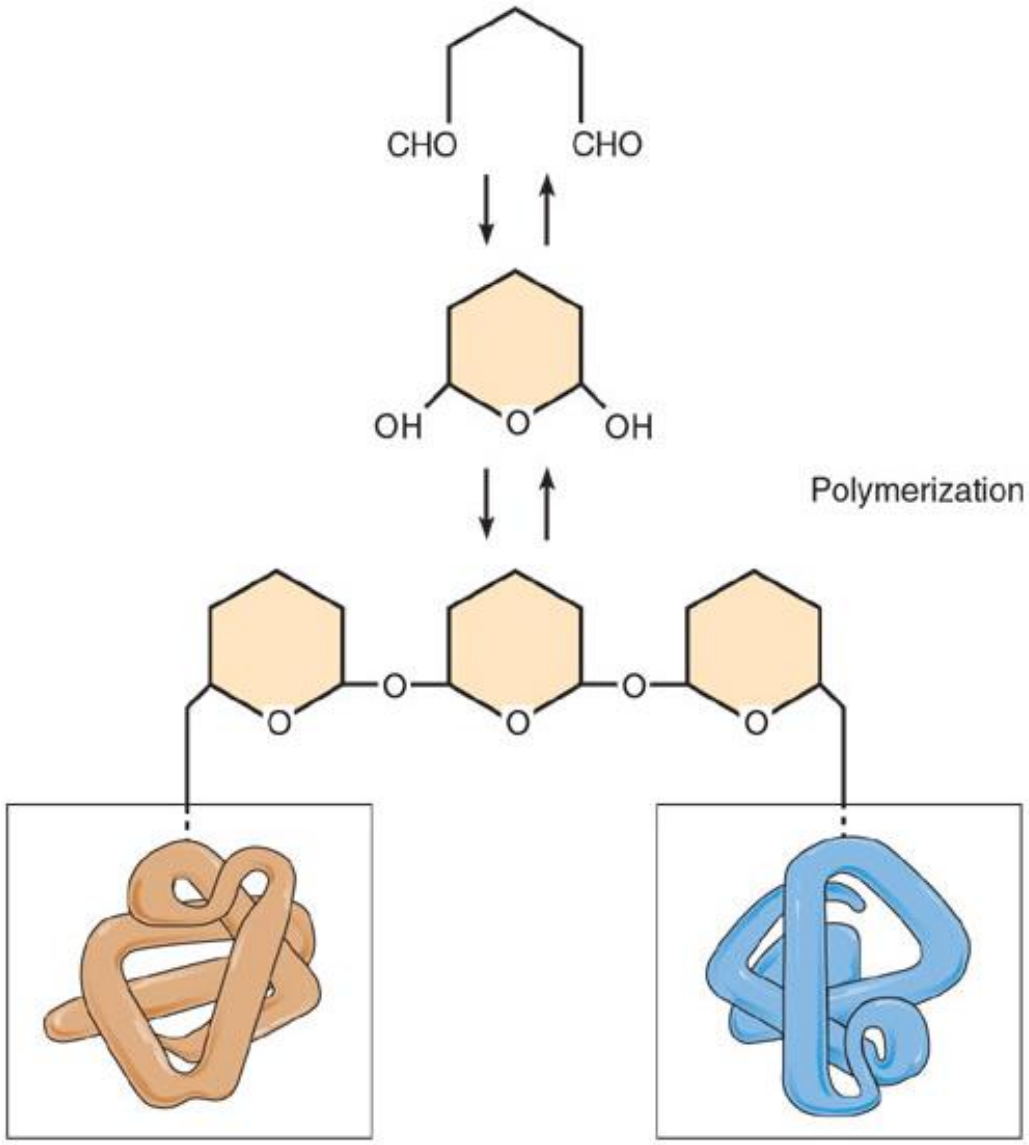
7. Heavy metals

- Solutions of silver & mercury kill vegetative cells in low concentrations by inactivating proteins
- Not sporicidal


Silver amalgam
Gold foil

8. Aldehydes

- Glutaraldehyde & formaldehyde kill by alkylating protein & DNA.
- glutaraldehyde in 2% solution used as sterilant for heat sensitive instruments
- formaldehyde - disinfectant, preservative, toxicity limits use


CHO
CHO
OH
O
OH
Polymerization
O
O
O
O
O
Microbe's surface protein
Microbe's surface protein

Gases

- Ethylene oxide, propylene oxide & chlorine dioxide
- Strong alkylating agents, sporicidal


(a)
(b)
Air intake
Vacuum pump
Air filter
Chamber
Door
Gas mixer
ETO cylinder
CO_2 cylinder

How can sterilize each:

Skin

Class Petri dish

Gauzes

plastic syringes

solid surfaces

Q/ Write type of method(**Sterilization–Disinfection) for each:**

Soap

Hydrogen peroxide

Alcohols

Autoclave

Direct Flaming