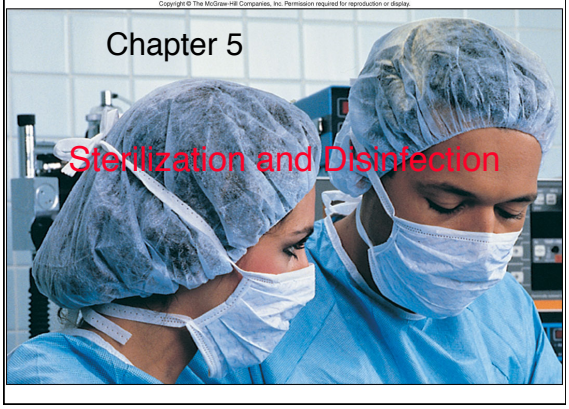


Chapter 5

Sterilization and Disinfection

Remember:

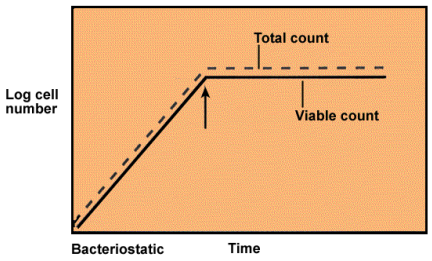
Sterility is an absolute!
Either something is sterile
or it is not!!

To control the growth of microorganisms is to prevent the reproduction of those microorganisms.

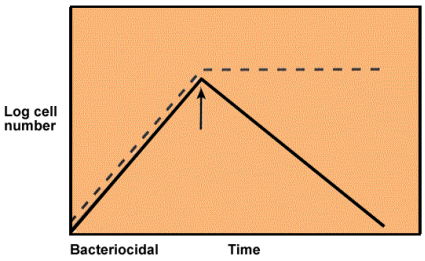
To destroy...use suffix
-cidal
To inhibit growth...use suffix
-static

bactericidal or bacteriostatic?

Bacteriostatic



Bacteriocidal



Different levels of microbial control

Control of Microbial Growth

- ✓ Chemical Methods
- ✓ Physical Methods

Chemical Methods

- ✓ Sterilants
- ✓ Disinfectants
- ✓ Antiseptics
- ✓ Germicides
- ✓ Sanitizers
- ✓ Antibiotics

Sterilants

- ✓ Chemical agents that accomplish sterilization.

Disinfectants

- ✓ Substances that kill vegetative cells, but not necessarily their spores.
- ✓ Typically disinfection is process of killing infectious microorganisms.
- ✓ Used on inanimate objects.

Antiseptics

- ✓ Agents that prevent microorganisms from growing, but may kill them.
- ✓ Typically used on body surfaces, such as on cuts and abrasions.

Germicides

- ✓ Kills vegetative cells, but not necessarily their spores - works rapidly.
- ✓ Very much like disinfectant, but may kill non-infectious microorganisms.

Sanitizers

- ✓ Kills 99.9% microorganisms in a contaminating area.
- ✓ Applied to inanimate objects.
- ✓ Used in hospitals and other public places.

Antibiotics

- ✓ Substances produced by microorganisms that has antimicrobial activity.
- ✓ Active in small quantities.
- ✓ Typically act on the metabolism of cells whose growth they inhibit.

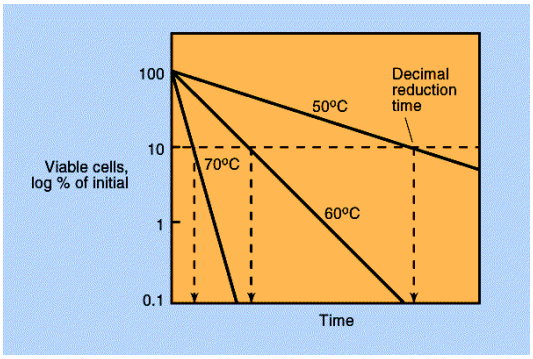
Disinfectants and Antiseptics

- ✓ Phenols
- ✓ Alcohols
- ✓ Halogens
- ✓ Heavy metals
- ✓ Detergents

Conditions Affecting Antimicrobial Activity

- ✓ Size of microbial population.
- ✓ Intensity or concentration of agent.
- ✓ Time exposure to agent.
- ✓ Temperature at which organisms are exposed to agent.
- ✓ Nature of material containing microbes.
- ✓ Characteristics of organisms present.

Exponential death



The Ideal Chemical Agent
(and other wish lists...)

1. Antimicrobial activity.
   preferably broad spectrum
2. Solubility.
   in water or alcohol
3. Stability.
   has some shelf-life

4. Lack of toxicity.
   Must not harm humans or animals.
5. Homogeneity.
   Uniform composition.
6. Minimum inactivation by extraneous materials.
7. Activity at ordinary room temperatures.

8. Ability to penetrate.
   or else activity is limited to application site.
9. Material safety.
   unable to rust or corrode.
10. Deodorizing ability.

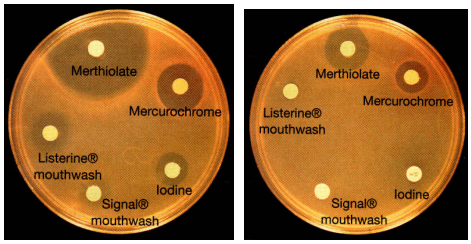
11. Detergent ability.
    Surfactant with mechanical removal of organisms.
12. Availability and low cost.

Measuring Antimicrobial Activity

✓ Tube dilution method
✓ Disk-plate method

Disk-plate method

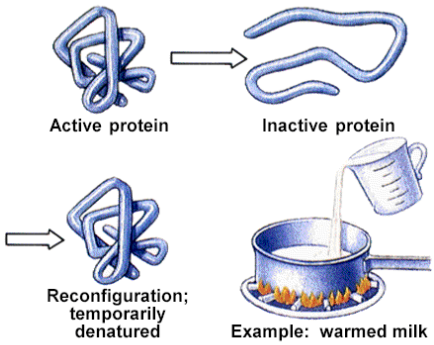
Staphylococcus aureus *Escherichia coli*



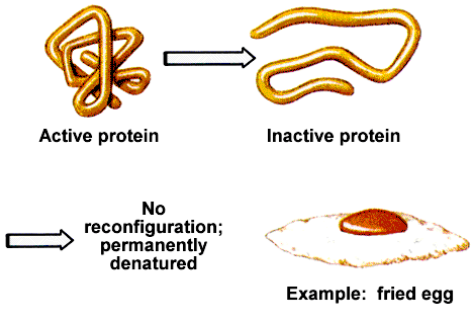
Mechanisms of action of Chemical agents

✓Protein denaturation
- ✒Hydrogen and disulfide bonds are disrupted
- ✒Heat, acids, & alkalis denature
- ✒Permanent denaturation
  - ☞Bacteriocidal
- ✒Temporarily denaturation
  - ☞Bacteriostatic

Temporarily denatured protein



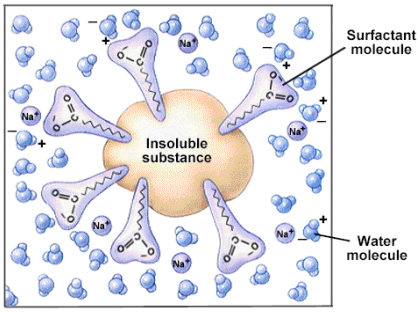
Permanently denatured protein



Mechanisms of action of Chemical agents

✓Reactions on membranes
- ✒Surfactants disrupt lipid membranes
  - ☞Alcohols, detergents, & quarternary ammonium compounds (benalkonium chloride)
  - ☞Dissolve lipids
- ✒Phenols
  - ☞Dissolve lipids
  - ☞Denature proteins

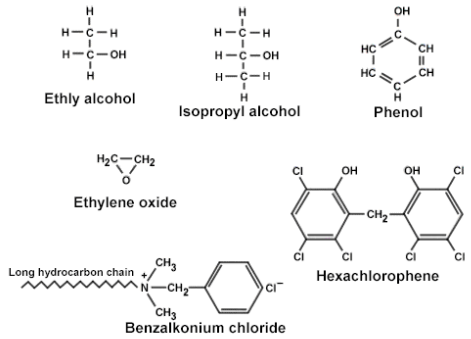
Surfactants reduce surface tension



Mechanisms of action of Chemical agents

✓Alkylating agents
- ✒Ethylene oxide & nitrous acid
  - ☞Alter DNA or RNA
  - ☞Replace chemical groups on the nucleic acids
    - ✒Hydrogen, amino, or alcohol groups

Structures of some important disinfectants (fig. 12.4)



Soaps & Detergents

- ✓Soaps remove dirt and microbes
  - ✒They may kill some
- ✓Quaternary Ammonium Compounds (quats)
  - ✒bacteriocidal for both G+ and G- bacteria
  - ✒low toxicity
  - ✒detergent action
  - ✒high stability
  - ✒ noncorrosive

Mode of Action--Quaternaries

- ✓Denature of cell proteins.
- ✓Interfere with metabolic processes.
- ✓Damage the cytoplasmic membrane.

Acids and Alkalis

- ✓Acids lower pH
  - ✒Denatures proteins
- ✓Alkalis raise pH
  - ✒Denature proteins

Heavy Metals

- ✓Mercury (Hg)
- ✓Lead (Pb)
- ✓Zinc (Zn)
- ✓Silver (Ag)
- ✓Copper (Cu)

Antimicrobial uses of heavy metals

- ✓Mercurochrome, methiolate and metaphen are used to treat minor cuts, wounds and skin infections.
- ✓Silver nitrate - treat eyes of infants at birth to prevent gonococcal infections.
- ✓Copper sulfate is an algicide in water.
- ✓Zinc compounds are fungicidal
- ✓Silver sulfadiazine - burn dressings

Mode of Action--Heavy Metals

✓ Inactivate cellular proteins by combining with them.

Halogens

Halogens include:

✓ iodine

✓ chlorine

✓ bromine

Iodine compounds

- Germicidal against many bacteria
- Inactivated by organic materials
- used typically for disinfection of skin
- can disinfect water and sanitize food utensils.

Mode of Action--Iodine

✓ Strong oxidizing agent.

✓ Inactivates proteins by binding with the amino acid, tyrosine

Chlorine compounds

- chlorine gas (Cl_2) is widely used disinfectant
- inorganic chlorine compounds include calcium and sodium hypochlorite (NaOCl--bleach)
- organic chlorine compounds chloramines--substituted ammonium

✓ Disinfection of drinking water and swimming pool water.

✓ Treatment of waste water from sewage treatment plants.

✓ Used to sanitize eating utensils in restaurants

✓ personal hygiene and household disinfection

Mode of Action--Chlorine

✓Release nascent oxygen when hydrolyzed; damages cell substances

✓Combine with proteins and destroy their biological activity.

Alcohols

Ethyl alcohol (ethanol)

✓Used as a 70% solution

✓Effective against vegetative cells, but does not sterilize

✓Surfactant - removes skin, soil, oil and microbes

✓?Most effective alcohol used

✓Activity is diminished with extraneous proteins

Isopropyl alcohol (rubbing alcohol)

✓ Used as a skin antiseptic

✓ Used as a disinfectant for oral thermometers and some surgical instruments

✓Limited by toxicity

Mode of Action--Alcohols

✓Denature proteins

✓Dissolve lipids

✓Have detergent action

Phenols

Phenol

✓Antiseptic and disinfectant

✓Used as a 5% solution

✓Kills vegetative cells

Phenols

Lysol

✒disinfects inanimate objects

Hexachlorophene

✒bacteriostat against G+ bacteria

✒prolonged applications are toxic

Mode of Action--Phenols

- ✓ Alter selective permeability of cytoplasmic membrane, causing leakage of cell contents.
- ✓ Denature and inactivate proteins and enzymes.
- ✓ Bacteriostatic or bactericidal--depends on concentration used.

Oxidizing agents

- ✓ Disrupt disulfide bonds
  - ✒ Disrupt membranes and proteins
- ✓ H_2O_2
- ✓ O_3
  - ✒ fruits

Alkylating agents

- ✓ Chemical sterilizers
- ✓ Used to sterilize heat-sensitive materials and enclosed sterile areas.
  - ✒ ethylene oxide
  - ✒ β-propiolactone
  - ✒ glutaraldehyde
  - ✒ formaldehyde

Dyes

- ✓ DNA mutations - acridine
- ✓ cell wall synthesis - crystal violet

Physical Control of Microbial Growth

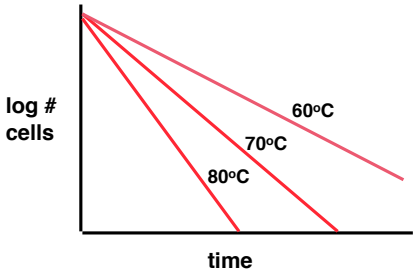
Physical Methods

- ✓ High Temperatures
  - ✒ Moist heat
  - ✒ Dry heat
  - ✒ Incineration
- ✓ Low Temperatures

Physical Methods

- ✓ Radiation
  - ✒ Ionizing radiation
  - ✒ Non-ionizing radiation
- ✓ Filtration
  - ✒ Membrane filters
  - ✒ HEPA filters
- ✓ Desiccation

Thermal Death Time (TDT)



Moist Heat

Kills most likely by protein denaturation

Boiling Water

- ✓ 100°C for 10 minutes
- ✓ Kills vegetative cells on instruments and containers.
- ✓ Not reliable for sterilization; spores are not killed

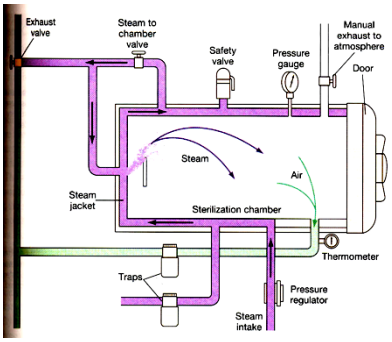
Pasteurization

- ✓ 62.9°C for 30 min/71.7°C for 15 sec.
- ✓ Kills vegetative cells of pathogens and other organisms in beverages.
- ✓ Does not sterilize
  - ✒ UHT 140°C

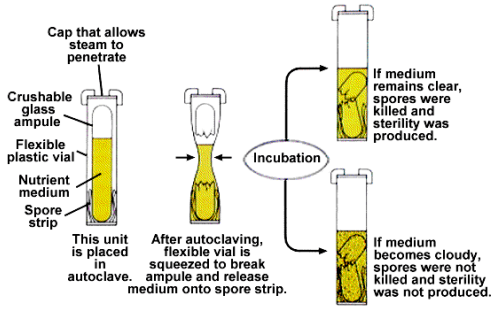
Autoclave

- ✓ 121.6°C, 15 lbs/in², for 15-30 min
- ✓ Sterilizes instruments, linens, utensils, treatment trays, microbiological media and other liquids.
- ✓ Some organisms are not destroyed by steam heat; some materials are destroyed by heat.

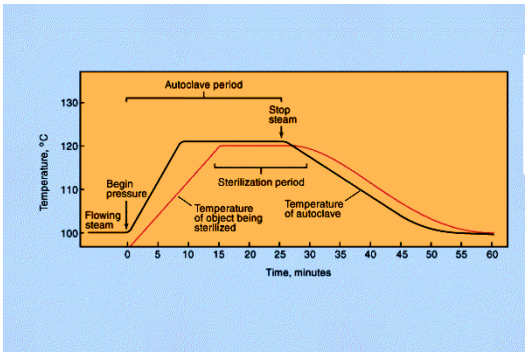
Diagram of an autoclave



Checking for sterility



15 minutes of proper time & temp



Dry Heat

Does most of its damage by oxidizing molecules

Hot-air oven

- ✓ 170-180°C for 1-2 hours.
- ✓ Sterilizing oils, glassware, sharp instruments, metals.
- ✓ Some materials are destroyed by heat.

Incineration

- ✓ Hundreds of degrees.
- ✓ Sterilizes transfer loops, needles.
- ✓ Disposal of animal carcasses and contaminated objects.
- ✓ Some materials are destroyed by high temperatures.

Low Temperatures

Retards growth by slowing down rate of enzymes, but does not kill many microbes

Freezers

- ✓ Less that 0ºC - usually -20°C
- ✓ Preserves foods and other materials.
- ✓ Microbiostatic, rather than microbiocidal.
- ✓ Thaw and refreeze can allow growth of microbes

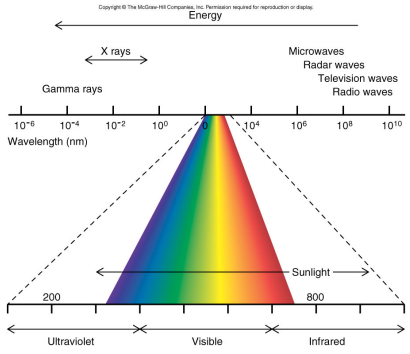
Liquid Nitrogen Refrigerators

- ✓ minus 180ºC.
- ✓ Very effective preservation of microorganisms.
- ✓ Liquid N is expensive.

Drying (Desiccation)

- ✓ Absence of water
  - ✒ Preserves food
  - ✒ Will destroy some pathogens
  - ✒ No effect on most spores
- ✓ Lyophilization
  - ✒ Freeze drying
  - ✒ Long term preservation.
  - ✒ Does not normally kill microbes

Radiation



Non-ionizing radiation

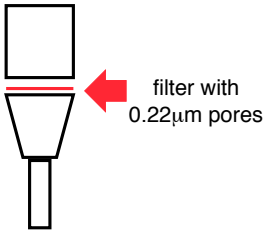
- ✓ Ultraviolet light
- ✓ Only excites most molecules, but damages DNA.
- ✓ Does not penetrate very well.
- ✓ Sterilizes air, surfaces in hospital operating rooms and industrial packing rooms.

Ionizing radiation

- ✓ High energy electron beams, gamma rays and X-rays.
- ✓ Split molecules into radical atoms.
- ✓ Radicals are very reactive and destructive to living cells.
- ✓ Very useful in sterilizing packaged food, medical equipment.

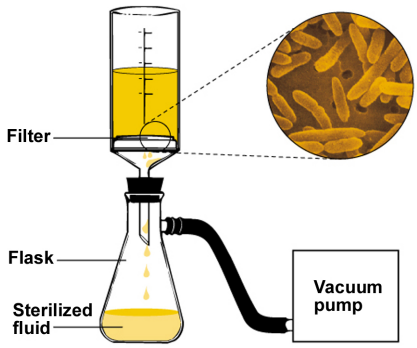
Filtration

Membrane filters



apparatus placed in vacuum flask

Filtration of fluids



Filter sizes vs particles

PORE SIZE (in μm)	PARTICLES THAT PASS THROUGH THEM
10	Erythrocytes, yeast cells, bacteria, viruses, molecules
5	Yeast cells, bacteria, viruses, molecules
3	Some yeast cells, bacteria, viruses, molecules
1.2	Most bacteria, viruses, molecules
0.45	A few bacteria, viruses, molecules
0.22	Viruses, molecules
0.10	Medium-sized to small viruses, molecules
0.05	Small viruses, molecules
0.025	Only the very smalled viruses, molecules
Ultrafilter	Small molecules

Membrane filters

Used for:

- ✓ filtration.
- ✓ separating microorganisms and collecting microbial samples.
- ✓ examination of water samples.

Membrane filters

- ✓ Pores are uniform.
- ✓ Easily manufactured with desired pore size.
- ✓ Absorb very little of fluid filtered.
- ✓ Quite rapid.
- ✓ Disposable

HEPA filters

High-Efficiency Particulate Air filters

- ✓ Traps particulates, such as microorganisms.
- ✓ Very efficient.

Osmotic pressure

- ✓ High concentrations of salts, sugars
- ✓ Removes water from microbes
- ✓ Plasmolysis