Microbial Control

Terminology

- **Sterilization:** Removal of all microbial life
- **Commercial Sterilization:** Killing *C. botulinum* endospores
- **Disinfection:** Removal of pathogens
- **Antisepsis**: Removal of pathogens from living tissue
- **Degerming:** Removal of microbes from a limited area
- **Sanitization**: Lower microbial counts to safe level
- **Biocide/Germicide:** Kills microbes
- **Bacteriostasis:** Inhibiting, not killing, microbes
- **Lyophilization:** a good method of Preserving microorganisms for future use

Terminology

- -**cidal** means to kill
  - Bacteriocidal agent kills bacteria
- -**static** means to inhibit or prevent
  - Bacteriostatic agent will only inhibit or prevent bacterial growth
  - Growth will resume after the agent is removed
- **Sepsis, Asepsis, Aseptic Technique, Antisepsis, and Antiseptic Technique**

Disinfection and Antisepsis

Disinfection

- Removal of harmful organisms from surfaces
- NOT sterile
- Generally involves the use of chemicals
- Example - Vespene

Antisepsis

- Removal of harmful organisms from the skin
- NOT sterile
- Use of a less harsh chemical substance
- Antibacterial washes

Physical Methods to Control Microbial Growth

1. Autoclaving
2. pasteurization
3. Heat – moist or dry
4. Filtration
5. Low temperature
6. Desiccation
7. Osmotic pressure
8. Radiation

1. Autoclave

- Steam under pressure
- Achieves temperatures above 100°C
- Very high penetrating power
- Kills bacteria and spores by denaturing proteins
- Typical cycle is 30 min, 18psi, 122°C
- Items to be sterilized must be heat stable
- Examples : media, glassware, lab coats, surgical instruments

2. Pasteurization

- Method for treating milk to eliminate pathogenic microbes
- Low temperature pasteurization: 72°C for 15 sec
- Kills pathogens but the milk is NOT sterile
- Ultra high temperature pasteurization: 74°C to 140°C for 5 sec and back to 74°C does achieve sterility

Pasteurization

63°C for 30 minutes
72°C for 15 seconds
140°C for 1 second

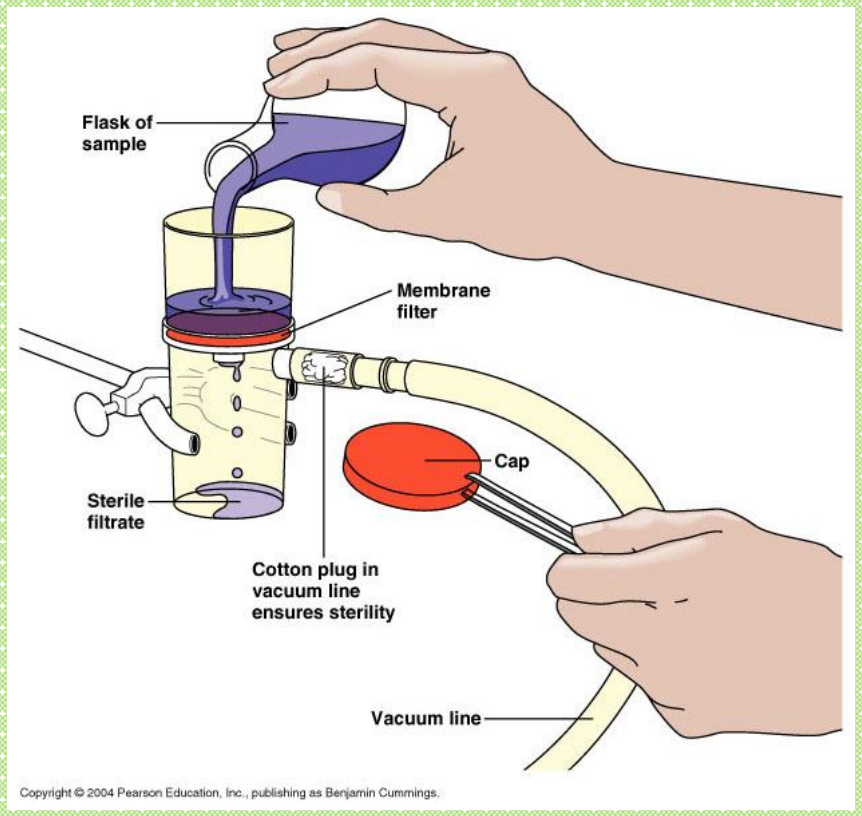
Pasteurization reduces spoilage organisms and pathogens

3. Dry Heat Sterilization

- Kills by oxidation – incineration
- Flaming your inoculating loop is an example
- Hot air sterilization – oven
- Temperature required is 170°C (312°F) for 2 hrs
- Oven sterilization also requires that the items be heat stable

4. Filtration

- Method used to sterilize heat sensitive liquids
- Culture media, antibiotics, vaccines, enzymes
- Solution is passed through a membrane with pours so small that bacteria cannot pass through
- Resultant solution is STERILE!



5. Freezing

- Bacteriostatic effect only
- Slows down bacterial growth but does not kill the bacteria
- Some bacteria may be killed on the freeze-thaw but many survive
- NO STERILITY from freezing

6. Desiccation

- Drying out; removal of water
- Bacteriostatic
- Bacteria will grow once water is added
- Bacterial cultures are often preserved in a freeze dried state;

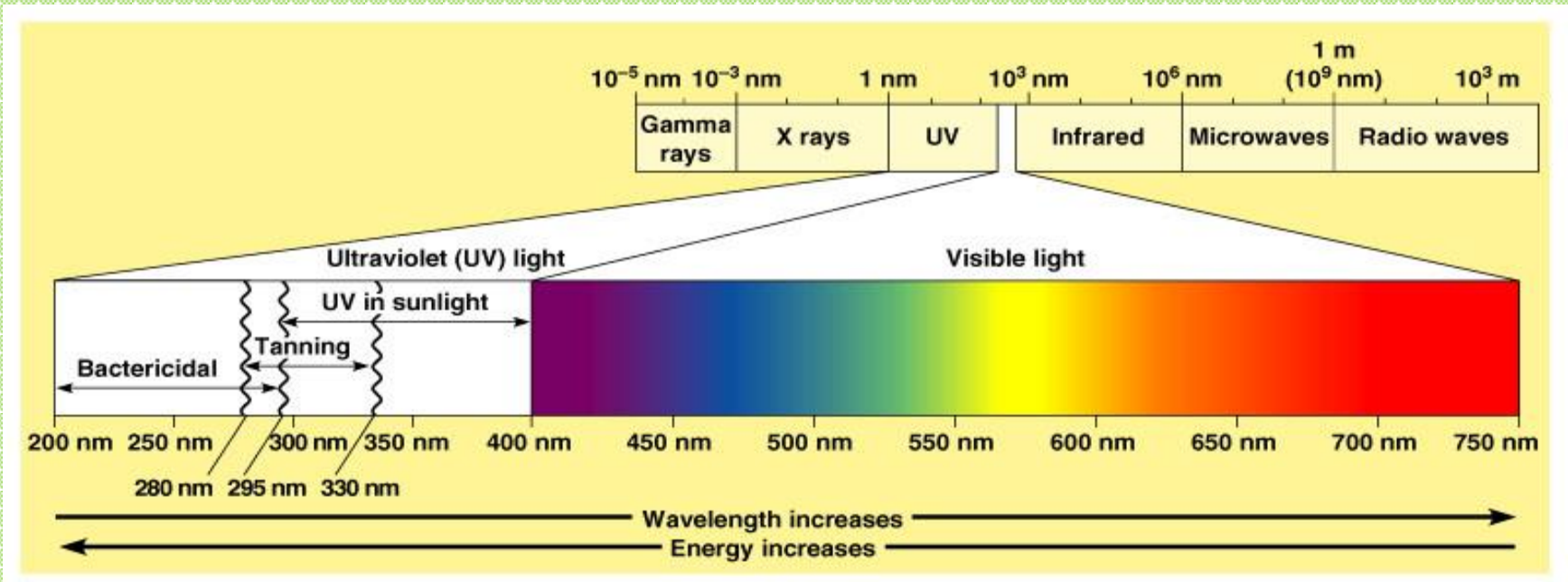
  Add media and presto, live bacteria!
- NO STERILITY from desiccation

7. Osmotic Pressure

- High salt or sugar content can “preserve” foods by preventing bacterial growth
- Bacteriostatic
- Honey, jelly, salted meats resist bacterial growth because of osmotic pressure exerted by the sugar or salt
- Molds and yeast often still grow

8. Radiation

- Radiation damages DNA
  - Ionizing radiation (X rays, gamma rays, electron beams)
  - Nonionizing radiation (UV)- surface sterilization only
  - (Microwaves kill by heat; not especially antimicrobial)



Chemical methods of sterilization

A. Factors which influence effectiveness
   - Dilution, time, pH, organic matter

B. Types of Disinfectants/Antiseptics
   - Phenol and phenolics (e.g.. amphyl)
   - Halogens (Chlorine, iodine, bromine)
   - Alcohols (e.g.. isopropyl alcohol)
   - Heavy metals (Ag, Hg, Cu, Zn)
   - Surface active agents (soaps & detergents)
   - Quaternary Ammonium Cmpds (quats)
   - Aldehydes (e.g.. glutaraldehyde)

Factors which influence effectiveness

- Evaluating a disinfectant
  - Use-dilution test
    1. Metal rings dipped in test bacteria are dried
    2. Dried cultures placed in diluted disinfectant (according to manufacturer's instructions) for min at 20°C
    3. Rings transferred to culture media to determine whether bacteria survived treatment

Factors which influence effectiveness

- Evaluating a disinfectant
  - Disk-diffusion method
  - Particular species are evaluated in each test
  - Zone of inhibition must be at or beyond a certain diameter

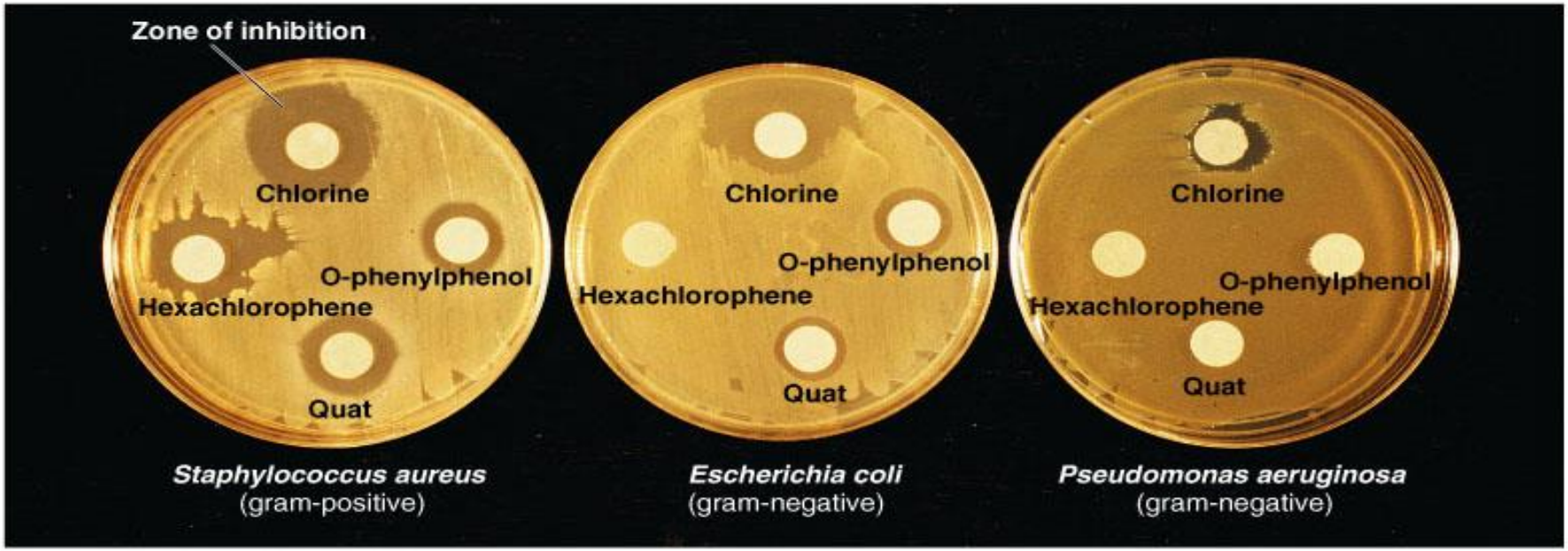


Figure 7.6

Phenols and Phenolics

- Derivatives of carbolic acid
- Found in throat lozenges and soaps
- Primary activity is injury to bacterial plasma membrane
- Well known products that contain these chemicals are Lysol, pHisohex soap, chlorhexidine soaps used in surgical scrubs

Halogens

- Iodine and Chlorine compounds
- Very reactive molecules that kill microbes
- Betadine – iodine compound used to clean surgical sites and wounds
- Chlorine is used to disinfect water
- Household bleach is chlorine and can be used to disinfect home surfaces

Oligodynamic Action

- Action of certain heavy metals to kill bacteria
- Metals such as silver, copper, zinc, and mercury can prevent bacterial growth
- Metal ions combine with proteins to cause denaturation (loss of protein function)

Oligodynamic Action

Alcohols

- Effective agent to kill bacteria
- Denatures proteins
- Ethanol and isopropanol are the most commonly used alcohols
- Need to clean a wound in the wild? Use that Tequila you're carrying, then drink the rest!

Quaternary Ammonium Compounds

- Widely used surface active agents
- Positive charged ammonium group is responsible for anti-microbial action
- Quats are bacteriocidal (G+), fungicidal, amoebicidal, and virucidal
- Two best known quats are
  - Zephiran (benzalkonium chloride)
  - Cepacol (cetylpyridinium chloride)

Aldehydes

- Formaldehyde
- Glutaraldehyde
- Both are strong bacterial killers
- Cause protein inactivation
- Glutaraldehyde is often used to sterilize hospital instruments (Cidex)

Gaseous sterilizers

- Ethylene oxide
- Denatures proteins
- Used in closed areas such as rooms
- High penetrating power, but dangerously explosive in pure form
- Used to sterilize heat sensitive items or large spaces such as rooms