The Control of Microbial Growth

Learning outcomes

1. Distinguish the sterilization and disinfection concepts.
2. List and explain the factors that affect the effectiveness of the process of killing bacteria.
3. Explain the mechanism of action of physical and chemical agents used to kill bacteria.
4. Explain the methods of short-term and long-term preservation of bacterial cells.

STERILIZATION & DISINFECTION

- **Sterilization**
  - Kills all bacteria (vegetative cells + spores).
  - The usual method is high temperature treatment.

STERILIZATION & DISINFECTION

❑ Disinfection

- ✓ Kills pathogenic bacteria (kills non-spore-forming vegetative cells of pathogenic bacteria).
- ✓ Uses chemicals, UV rays, boiling water, steam, etc.
- ✓ When applied directly to living tissue, disinfection is called antisepsis and the chemical used is called an antiseptic.

Factors affecting the effectiveness of the bactericidal process

- **The Rate of Microbial Death:**
  - ✓ When subjected to heat treatment, bacteria are generally killed at a constant rate.

TABLE 7.2 Microbial Exponential Death Rate: An Example

Time (min)	Deaths per Minute	Number of Survivors
0	0	1,000,000
1	900,000	100,000
2	90,000	10,000
3	9000	1000
4	900	100
5	90	10
6	9	1

Factors affecting the effectiveness of the bactericidal process

- ✓ **The number of microbes:** The more microbes there are to begin with, the longer it takes to eliminate the entire population.

- ✓ **Microbial characteristics:**

  - o Spores are more difficult to destroy than vegetative cells;

  - o Different vegetative cells have different sensitivities to a certain physical or chemical agent.

Factors influencing the effectiveness of antimicrobial treatments

- ✓ **Environmental influences**
  - Fats and proteins the solution tend to protect bacteria.
  - The lower the pH, the easier it is to kill bacteria.

- ✓ **Time of exposure**
  - The lower the temperature, the longer the time of exposure.
  - It takes longer to kill spores than vegetative cells.

Actions of Microbial Control Agents

- ✓ Alteration of membrane permeability: alters the lipid and protein structures of the membrane.

- ✓ Damage to proteins and nucleic acids: breaks chemical bonds.

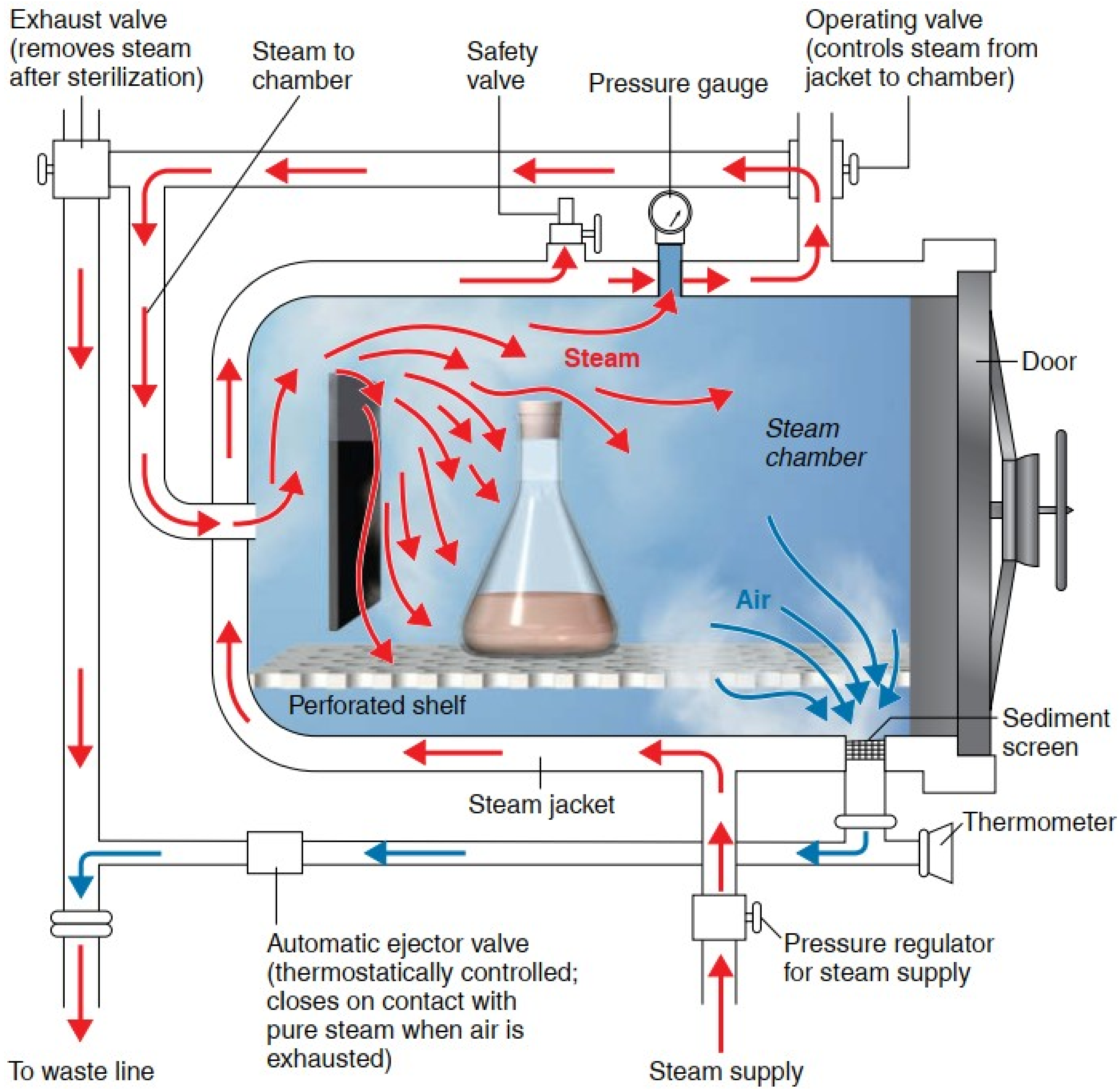
Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

1. Heat

1.1. Moist Heat Sterilization: breaks down hydrogen bonds between protein chains -> coagulate bacterial proteins.

- ✓ Boiling (100 ^{o}C, 1 atm)
- ✓ Steam (uncompressed, 100 ^{o}C)
- ✓ Autoclave (121 ^{o}C, 1 atm):
  - o destroy vegetative cells and spores.
  - o steam should be allowed to come into contact with the surface of the instrument.



Exhaust valve (removes steam after sterilization)
Steam to chamber
Safety valve
Pressure gauge
Operating valve (controls steam from jacket to chamber)
Steam
Steam chamber
Door
Air
Perforated shelf
Sediment screen
Steam jacket
Thermometer
Automatic ejector valve (thermostatically controlled; closes on contact with pure steam when air is exhausted)
Pressure regulator for steam supply
To waste line
Steam supply

TABLE 7.4 The Effect of Container Size on Autoclave Sterilization Times for Liquid Solutions*

Container Size	Liquid Volume	Sterilization Time (min)
Test tube: 18 × 150 mm	10 ml	15
Erlenmeyer flask: 125 ml	95 ml	15
Erlenmeyer flask: 2000 ml	1500 ml	30
Fermentation bottle: 9000 ml	6750 ml	70

*Sterilization times in the autoclave include the time for the contents of the containers to reach sterilization temperatures. For smaller containers, this is only 5 min or less, but for a 9000-ml bottle it might be as much as 70 min. Liquids in an autoclave boil vigorously, so their containers usually are filled only up to 75% of capacity.

Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

1. Heat

1.1. Moist Heat Sterilization: breaks down hydrogen bonds between protein chains -> coagulate bacterial proteins.

- ✓ Pasteurization: mild heating, which was sufficient to kill the organisms that caused the particular spoilage problem without seriously damaging the taste of the product.

  - ❖ *Pasteurization of milk:*
    - o Traditional: 63^{o}C , 30 minutes
    - o Modern:
      - HTST: 72 ^{o}C, 15 seconds.
      - UHT: 140 ^{o}C, < 1 second.

Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

1. Heat

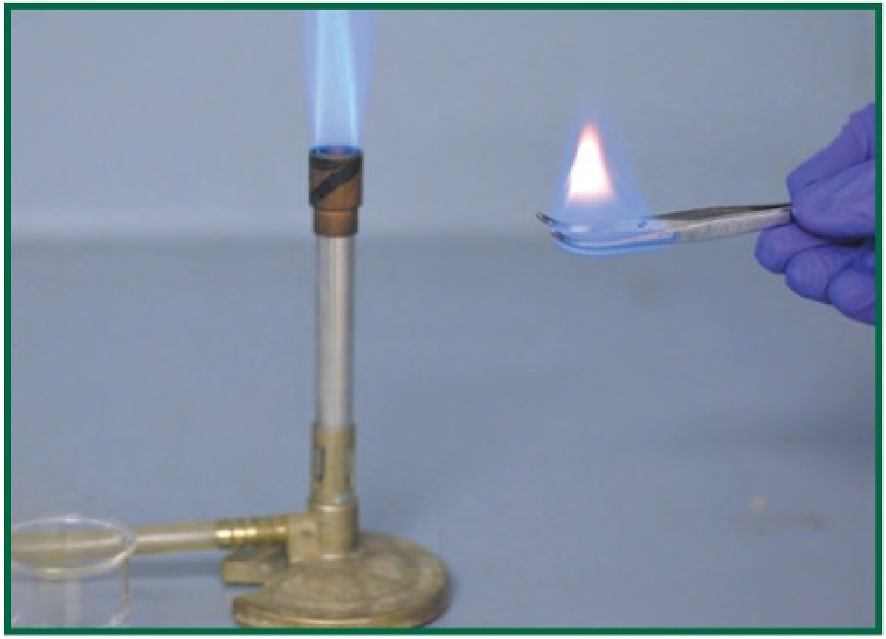
1.2. Dry Heat Sterilization: Dry heat kills microbes by oxidation effects.

- ✓ Direct flaming: sterilizes inoculating loops.
- ✓ Incineration: sterilize and dispose of contaminated paper cups, bags, and dressings, etc.
- ✓ Hot-air sterilization: oven, 170 ^{o}C, 2 hours.

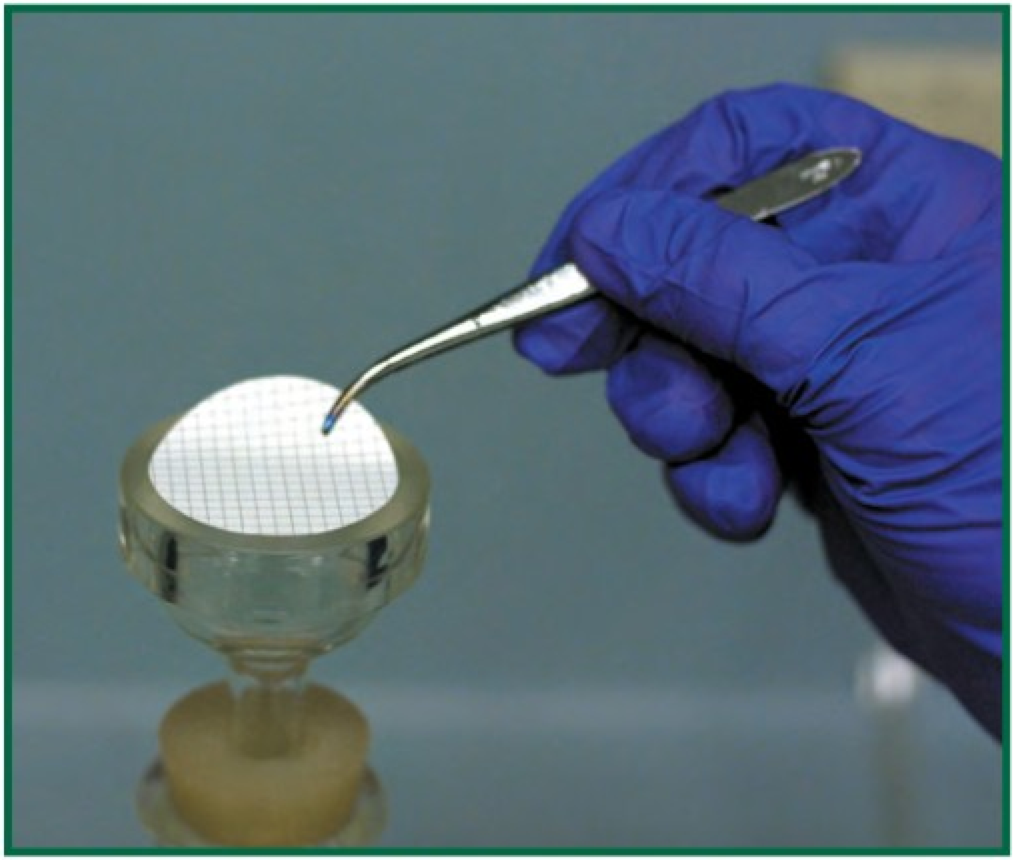
Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

2. Filtration:



8-38 ALCOHOL-FLAMING THE FORCEPS ✦ Dip the forceps into the beaker of alcohol. Remove the forceps and quickly pass them through the Bunsen burner flame—just long enough to ignite the alcohol. **Be sure to keep the flame away from the beaker of alcohol.**



8-39 PLACING THE FILTER ON THE FILTER HOUSING ✦ Carefully place the filter on the bottom half of the filter housing. Clamp the filter funnel over the filter.

Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

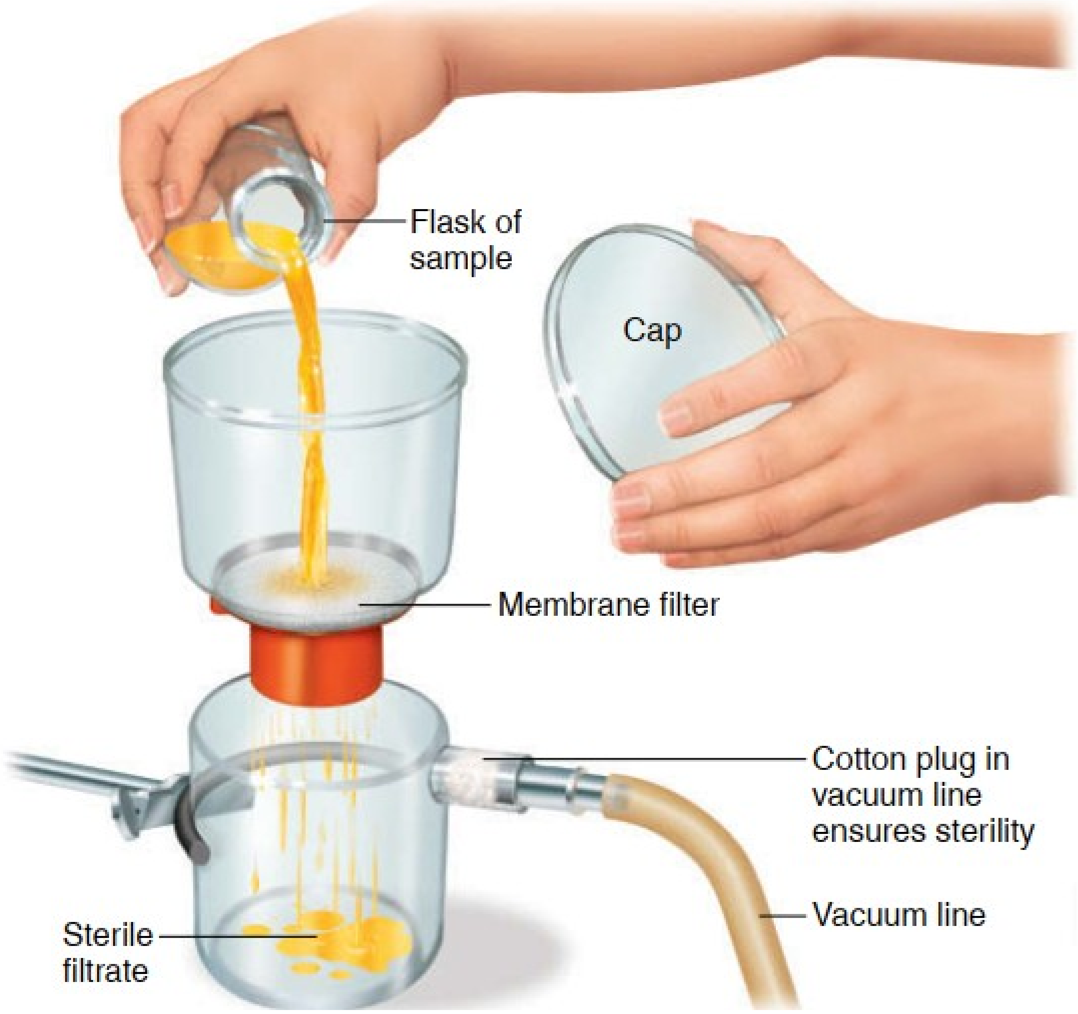
2. Filtration:

8-40 MEMBRANE FILTER ASSEMBLY ✦ The membrane filter assembly is made up of a two-piece funnel and clamp. The membrane filter is inserted between the two funnel halves, and the whole assembly is clamped together.

Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

2. Filtration:



Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

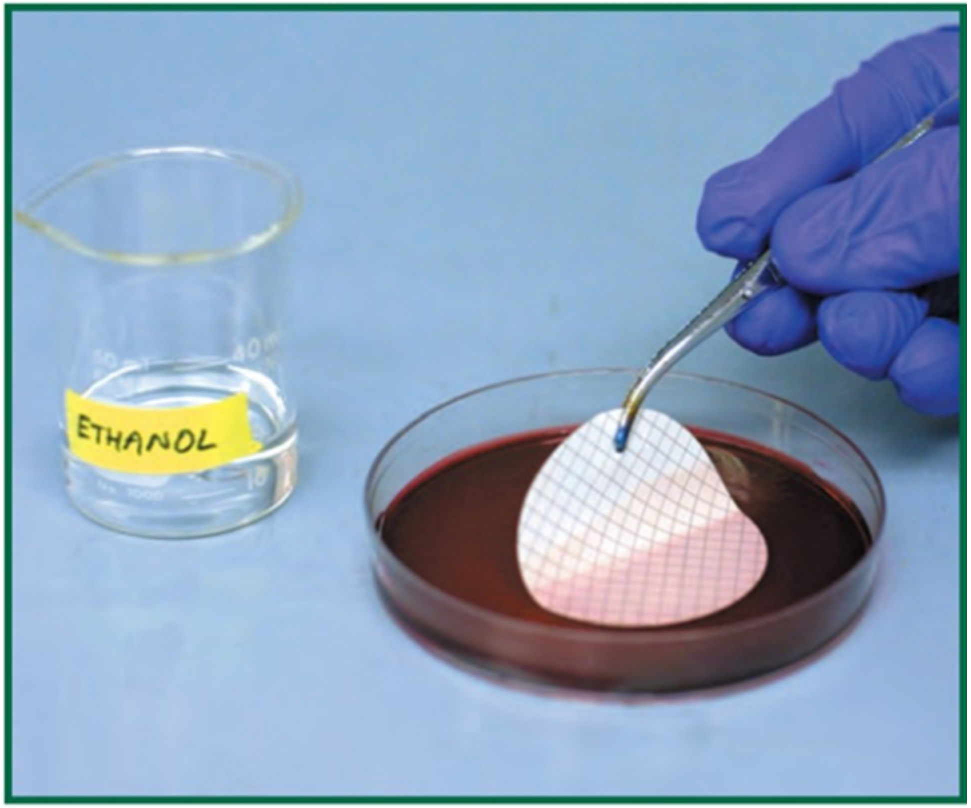
2. Filtration:

8-37 MEMBRANE FILTER APPARATUS ✦ Assemble the membrane filter apparatus as shown in this photograph. Use two suction flasks (as shown) to avoid getting water into the vacuum source. Secure the flasks on the table, as the tubing may make them top-heavy.

Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

2. Filtration:

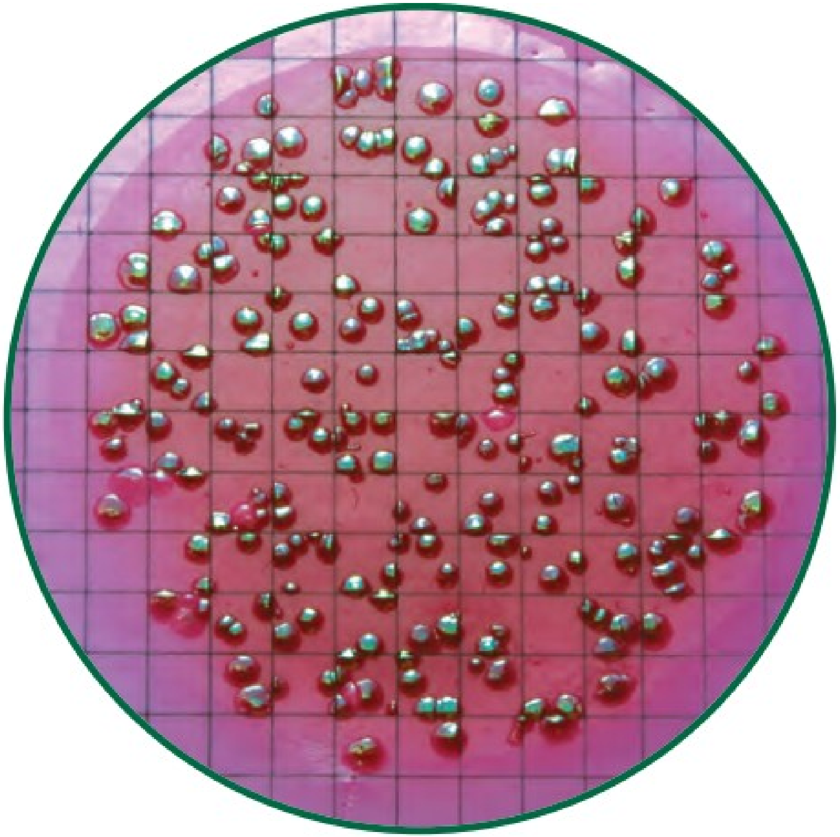


8-41 PLACING THE FILTER ON THE AGAR PLATE ✦ Using sterile forceps, carefully place the filter onto the agar surface with the grid facing up. Try not to allow any air pockets under the filter, because contact with the agar surface is essential for growth of bacteria. Allow a few minutes for the filter to adhere to the agar before inverting the plate.

Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

2. Filtration:



8-36 COLIFORM COLONIES ON A MEMBRANE FILTER ✦ Note the characteristic dark colonies with gold metallic sheen, indicating that this water sample is contaminated with fecal coliforms. Potable water has less than one coliform per 100 mL of sample tested.

Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

2. Filtration: the passage of a liquid or gas through a screenlike material with pores small enough to retain microorganisms.

- ✓ Is used to sterilize heat-sensitive materials, such as some culture media, enzymes, vaccines, and antibiotic solutions.
- ✓ There are different types of membranes with different pore sizes for different filtration purposes.

Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

3. Desiccation:

- ✓ Dehydration -> bacteria cannot grow or reproduce but can remain viable for years. Then, when water is made available to them, they can resume their growth and division. This is the principle that underlies lyophilization, or freeze-drying.

Actions of Microbial Control Agents

- **Physical Methods of Microbial Control**

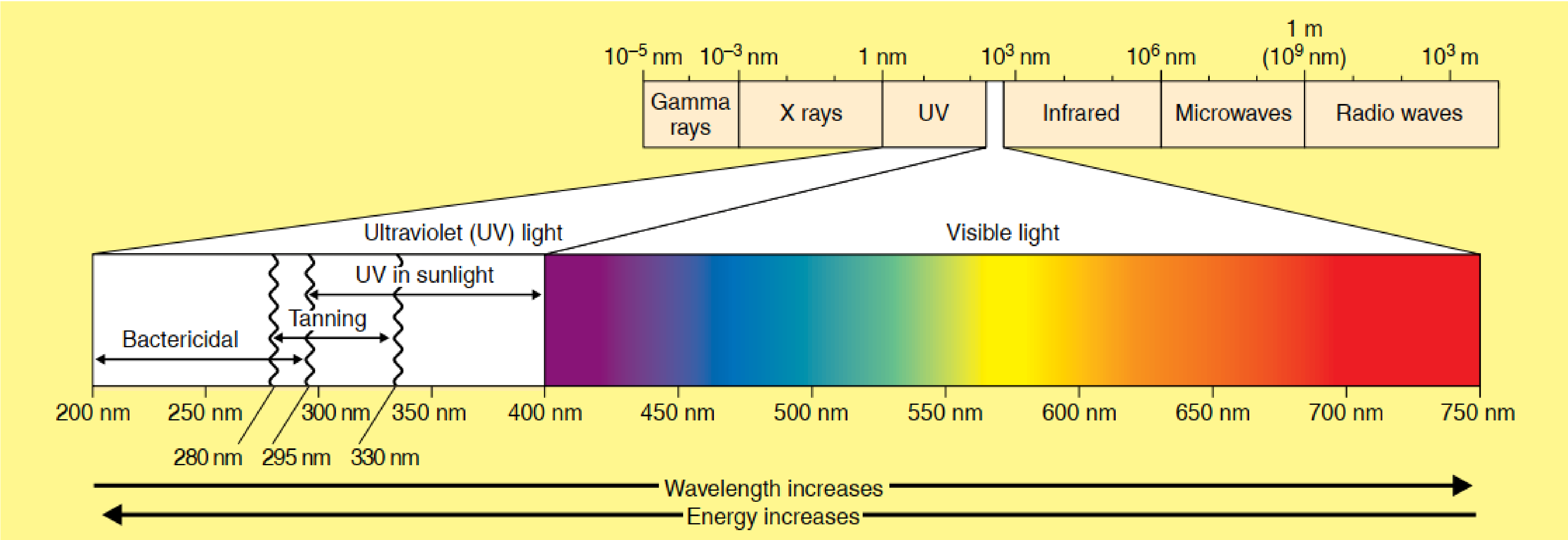
4. Osmotic pressure:

- ✓ Create a hypertonic environment

Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

5. Radiation:



Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

5. Radiation:

- ✓ The effect of radiation on cells depends on wavelength, intensity, and duration of radiation exposure.

Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

5. Radiation:

5.1. Ionizing radiation: gamma rays, X rays, and high-energy electron beams—has a wavelength shorter than that of nonionizing radiation, less than about 1 nm. Therefore, it carries much more energy.

- γ rays, X-rays or high-energy electron beams have wavelengths < 1 nm -> carry a lot of energy -> have the ability to penetrate deeply.

- Ionizes water molecules into active free radicals -> reacts with organic components of cells, especially DNA.

- electron beams are widely used to sterilize pharmaceuticals and disposable medical instruments.

Actions of Microbial Control Agents

❑ Physical Methods of Microbial Control

5. Radiation:

5.2. Nonionizing radiation:

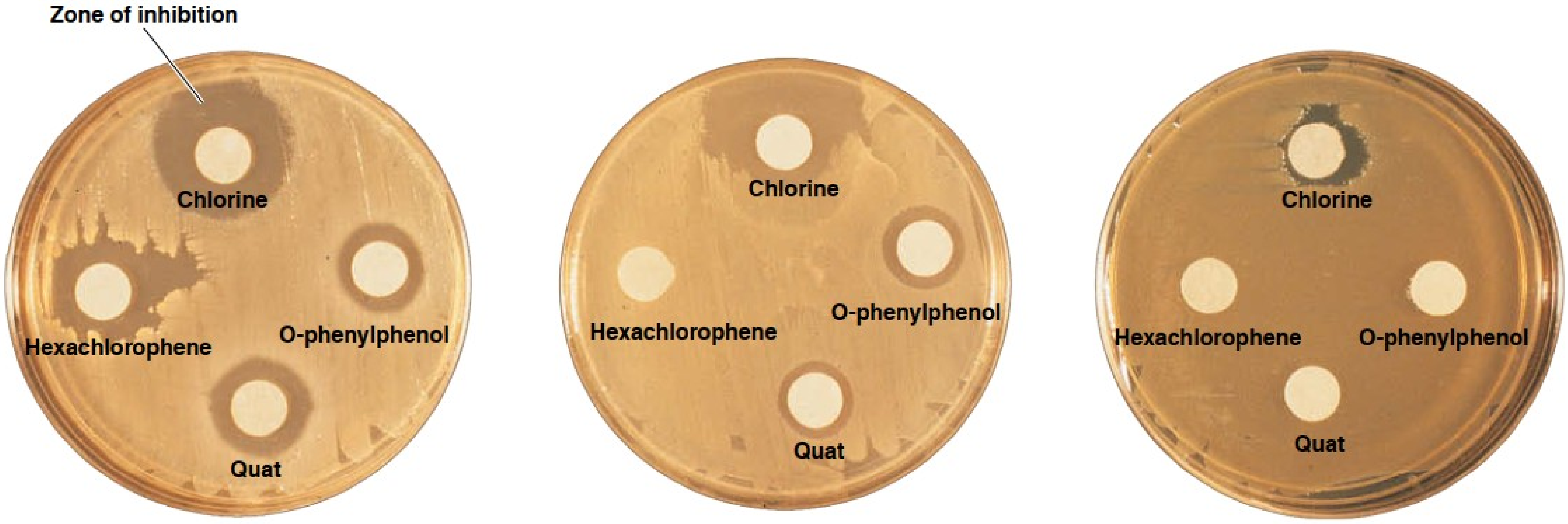
- Wavelengths > 1 nm; cannot penetrate deeply -> need direct irradiation.

- UV rays are strongly absorbed by DNA at 260nm; create bonds between adjacent thymines -> DNA can be copied incorrectly; is also used to disinfect vaccines and other medical products.

- Microwaves: kill pathogenic bacterial cells.

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

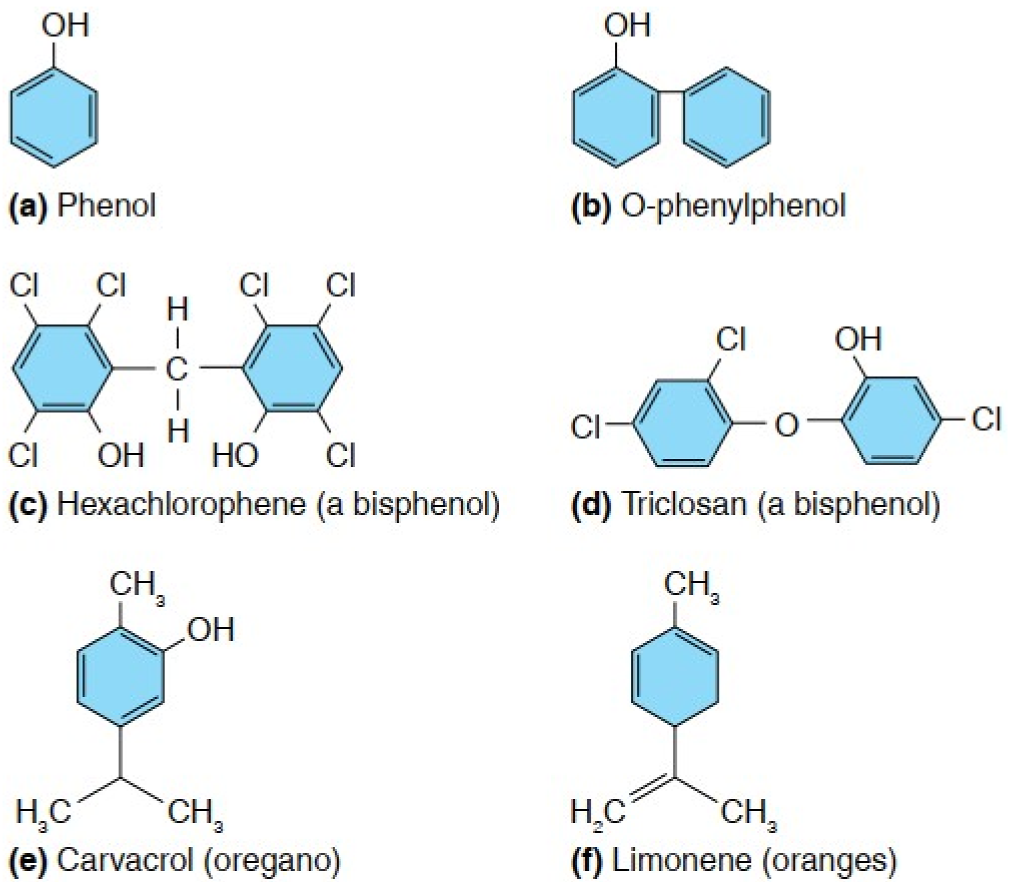


Staphylococcus aureus (gram-positive) | *Escherichia coli* (gram-negative) | *Pseudomonas aeruginosa* (gram-negative)

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

1. Phenol and phenolics



(a) Phenol

(b) O-phenylphenol

(c) Hexachlorophene (a bisphenol)

(d) Triclosan (a bisphenol)

(e) Carvacrol (oregano)

(f) Limonene (oranges)

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

1. Phenol and phenolics

- ✓ Phenol and O-phenylphenol damage the lipid-containing plasma membrane -> loss of cytoplasmic components.

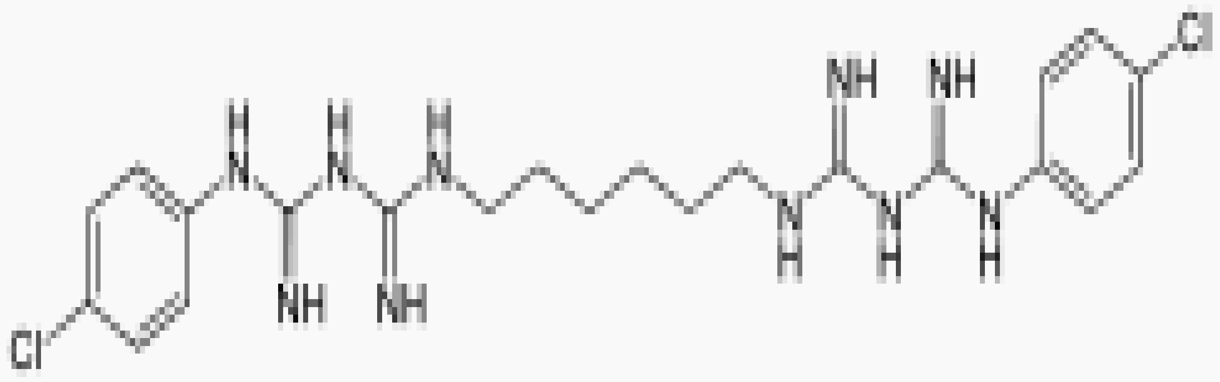
- ✓ Triclosan inhibits the lipid biosynthesis enzyme of the plasma membrane.

! *Pseudomonas aeruginosa* is resistant to triclosan.

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

2. Chlorhexidine: membrane structure intervention.



N′,N′′′′′-hexane-1,6-diylbis[N-(4-chlorophenyl)(imidodicarbonimidic diamide)]

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

3. Essential Oils

- ✓ are a mixture of hydrocarbons extracted from plants: peppermint oil, pine oil, orange oil, cajeput oil, etc.
- ✓ their antimicrobial action is primarily due to phenolics (carvacrol) and terpenes (limonene).

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

4. Iodine (I_2)

- ✓ Iodine impairs protein synthesis and alters cell membranes, apparently by forming complexes with amino acids and unsaturated fatty acids.

- ✓ Common commercial preparation is Betadine®, which is a povidone-iodine. Povidone is a surface-active iodophor (a combination of iodine and an organic molecule (N-vinylpyrrolidone polymer), from which the iodine is released slowly that improves the wetting action and serves as a reservoir of free iodine.

- ✓ Iodines are used mainly for skin disinfection and wound treatment.

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

5. Chlorine (Cl_2):

✓ HOCl is a strong oxidizing agent that damages the function of the cell's enzyme system.

(1)

$$\underset{\text{Chlorine}}{Cl_2} + \underset{\text{Water}}{H_2O} \rightleftharpoons \underset{\text{Hydrogen ion}}{H^+} + \underset{\text{Chloride ion}}{Cl^-} + \underset{\text{Hypochlorous acid}}{HOCl}$$

(2)

$$\underset{\text{Hypochlorous acid}}{HOCl} \rightleftharpoons \underset{\text{Hydrogen ion}}{H^+} + \underset{\text{Hypochlorite ion}}{OCl^-}$$

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

6. Alcohols: ethanol, isopropanol

- ✓ kills bacteria (and fungi) but not endospores (and non-membrane viruses).

- ✓ alcohol denatures proteins (requires water), perforates the plasma membrane and dissolves membrane lipids.

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

7. Heavy Metals (Ag, Hg, Cu) and Their Compounds

- ✓Heavy metal ions interact with the sulfhydryl group of the proteins
  -> protein denaturation.

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

8. Surface-Active Agents

Surface-active agents, or surfactants, can decrease surface tension among molecules of a liquid.

- ✓ **Soaps and detergents:** have an important function in the mechanical removal of microbes through scrubbing.

✓ **Quaternary Ammonium Compounds (Quats):**

- o Their cleansing ability is related to the positively charged portion—the cation—of the molecule: strongly bactericidal against gram-positive bacteria and less active against gram-negative bacteria.
- o They change the cell’s permeability and cause the loss of essential cytoplasmic constituents.

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

9. Chemical Food Preservatives

- ✓ **Organic acids** (sorbic acid and benzoic acid at low pH): can inhibit bacterial cell metabolism (organic acids often inhibit the metabolism of filamentous fungi).
- ✓ **Nitrates/nitrites:** inhibit iron-containing enzymes of anaerobic bacteria.

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

10. Antibiotics

- ✓ **Nisin** is often added to cheese to inhibit the growth of certain endospore-forming spoilage bacteria. It is an example of a bacteriocin, a protein that is produced by one bacterium and inhibits another. Nisin is present naturally in small amounts in many dairy products. It is tasteless, readily digested, and nontoxic.
- ✓ **Natamycin** (pimaricin) is an antifungal antibiotic approved for use in foods, mostly cheese.

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

11. Aldehyde

formaldehyde and **glutaraldehyde:** inactivate proteins by forming covalent cross-links with several organic functional groups on proteins (—NH_2 —OH, —COOH, and —SH).

- ✓ **Formaldehyde** gas is an excellent disinfectant.
- ✓ **Formalin** (a 37% aqueous solution of formaldehyde gas): preserves biological specimens and inactivate bacteria and viruses in vaccines.

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

11. Aldehydes

- ✓ **Glutaraldehyde**:
  - o less irritating and more effective than formaldehyde.
  - o used to disinfect hospital instruments, including endoscopes and respiratory therapy equipment: when used in a 2% solution (Cidex®), glutaraldehyde is bactericidal, tuberculocidal, and virucidal in 10 minutes and sporicidal in 3 to 10 hours.

Actions of Microbial Control Agents

❑ Chemical Methods of Microbial Control

12. H_2O_2

- ✓ oxidation

Actions of Microbial Control Agents

- Note: The above physical and chemical agents can act on the cells of other microorganisms by similar mechanisms.

Bacterial cell preservation

- **Short-term:** 4^{o}C in short time

Bacterial cell preservation

- **Long-term:**
  - ***Deep freezing method:*** bacterial suspension solution (glycerol 10-30%) -> quick freezing at -70 ^{o}C -> -95 ^{o}C (subculture after several years).

  - ***Freeze-drying method:*** Bacteria-containing medium (emulsion, serum, or sodium glutamate) → capsule → rapid cooling (-54 ^{o}C → -72 ^{o}C) → water separation by vacuum machine → sealed → stored in refrigerator (>= 10 years).