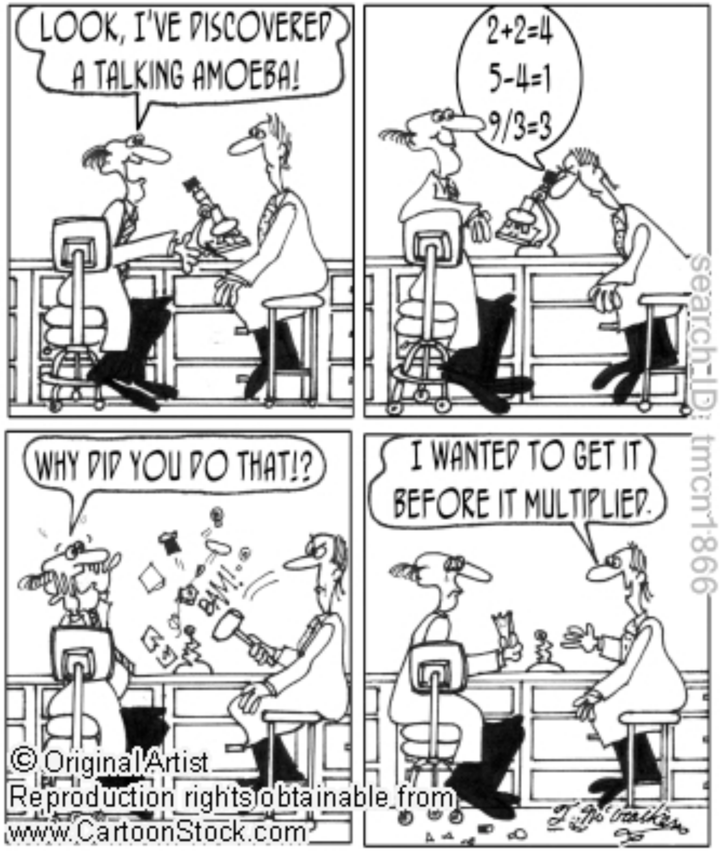
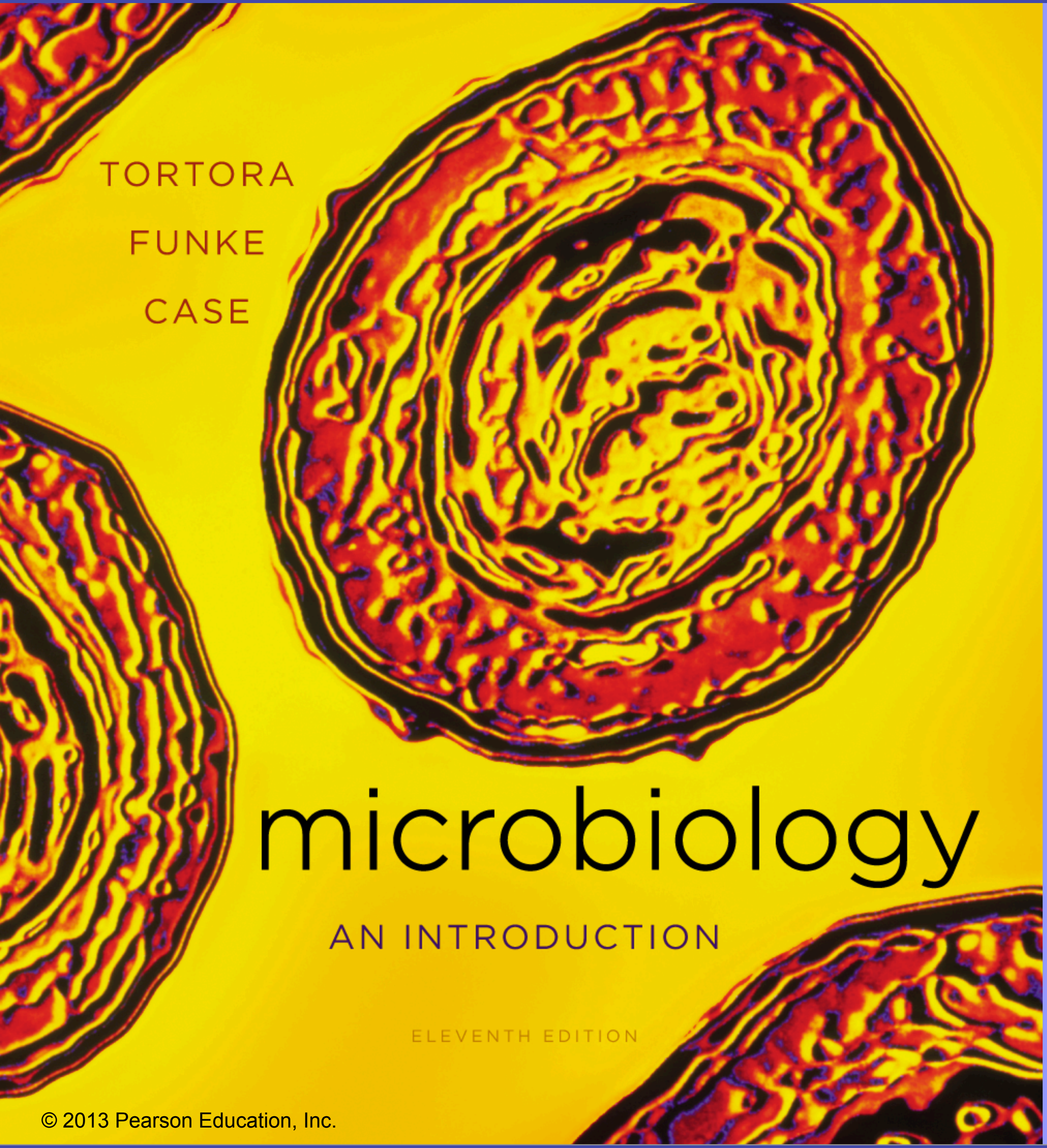


Module 5

The Control of Microbial Growth



Chapter 7

The Control of Microbial Growth

Lectures prepared by Helmut Kae

The Terminology of Microbial Control

- **Sepsis** refers to microbial contamination
- **Asepsis** is the absence of significant contamination
- Aseptic surgery techniques prevent microbial contamination of wounds
- **Biocide/germicide**: killing microbes
- **Bacteriostasis**: inhibiting, not killing, microbes

The Terminology of Microbial Control

- **Sterilization**: removing all microbial life
  - Most commonly done by heating
  - **Sterilant** is sterilizing agent
- **Commercial sterilization**: killing *C. botulinum* endospores
  - Limited heat treatment
  - Attempts to retain quality of food product

The Terminology of Microbial Control

- **Disinfection**: removing pathogens
- **Antisepsis**: removing pathogens from *living tissue*
  - A chemical can be either a disinfectant or an antiseptic, or both

The Terminology of Microbial Control

- **Degerming**: removing microbes from a limited area
- **Sanitization**: lowering total microbial counts
  - Commonly used when cleaning eating utensils

Rate of Microbial Death

- Bactericidal treatments kill bacteria
- Bacterial populations die at a constant logarithmic rate

Table 7.2 Microbial Exponential Death Rate: An Example

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

Figure 7.1a Understanding the Microbial Death Curve.

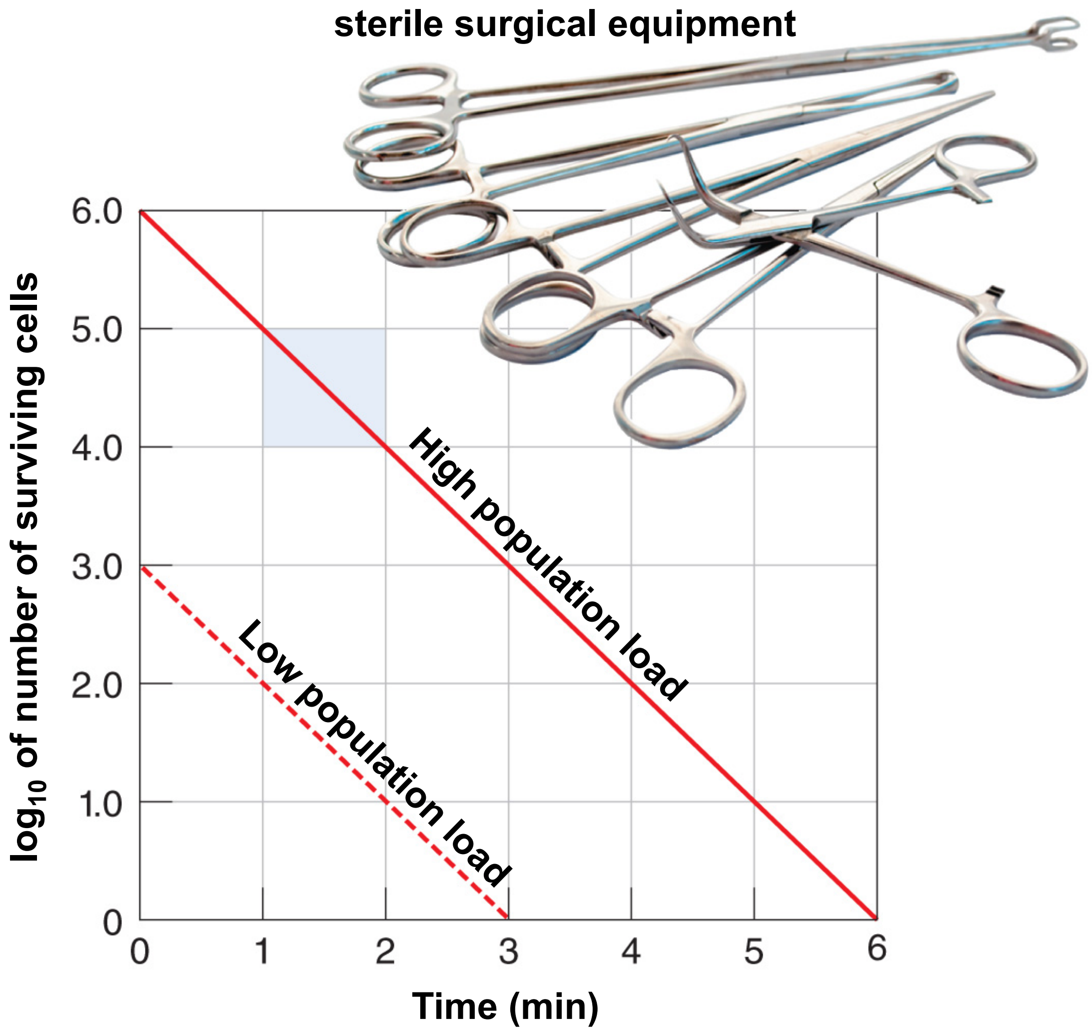
Plotting the typical microbial death curve logarithmically (red line) results in a straight line.

(a) Plotting the typical microbial death curve arithmetically (blue line) is impractical: at 3 minutes the population of 1000 cells would only be a hundredth of the graphed distance between 100,000 and the baseline.

Effectiveness of Treatment

- Depends on:
  - Number of microbes
    - More microbes, harder to kill
  - Environment (organic matter, temperature, biofilms)
    - Presence of some organics can inhibit chemicals
  - Time of exposure
    - Longer exposure, more effective
  - Microbial characteristics

Figure 7.1b Understanding the Microbial Death Curve.



(b) Logarithmic plotting (red) reveals that if the rate of killing is the same, it will take longer to kill all members of a larger population than a smaller one, whether using heat or chemical treatments.

Actions of Microbial Control Agents

- Alteration of membrane permeability
  - Results in leakage
- Damage to proteins
  - Alters or inhibits enzyme function
- Damage to nucleic acids
  - Prevents replication and/or protein synthesis

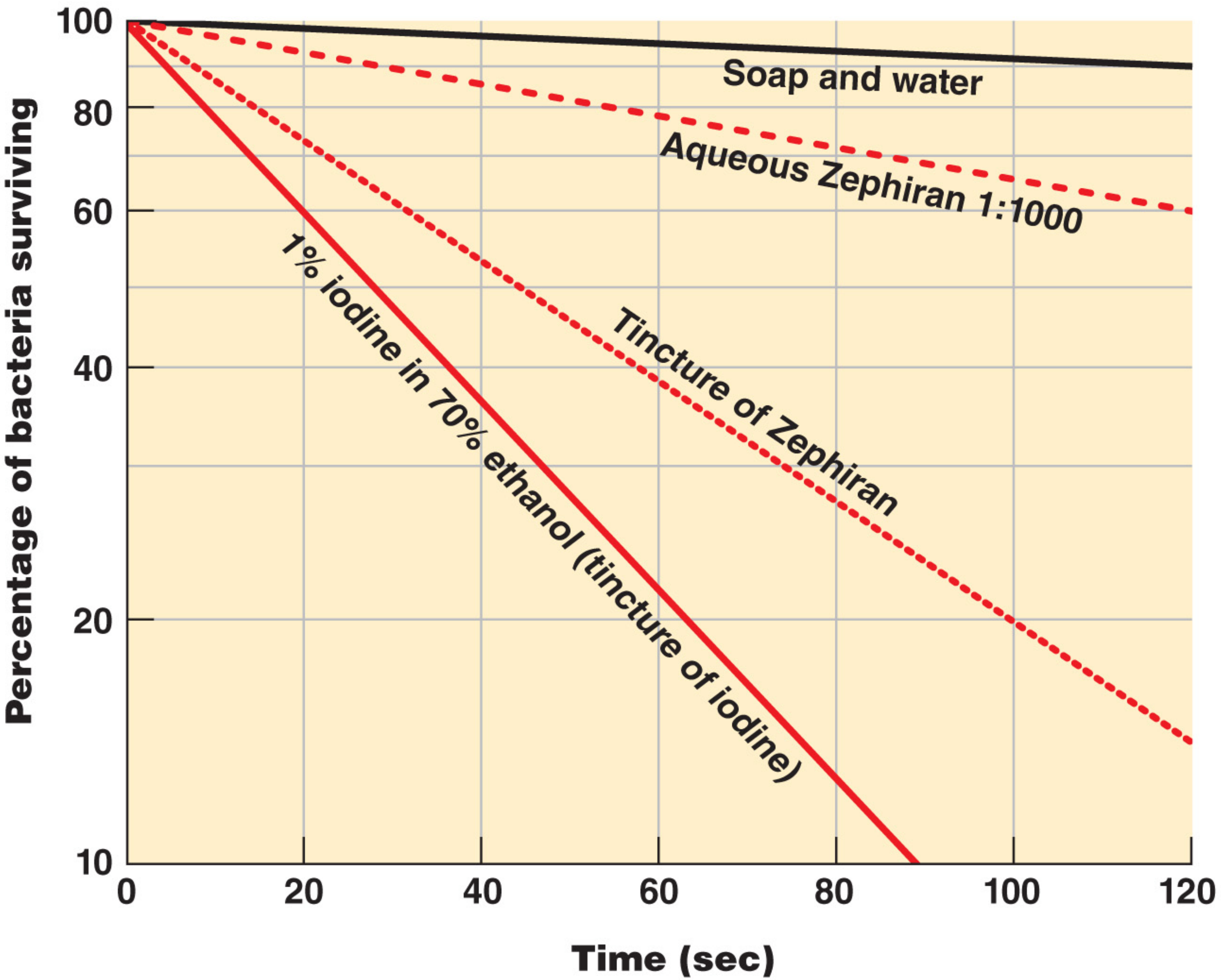
Physical Methods of Microbial Control

- Heat
- Filtration
- Osmotic Pressure
- Radiation

Heat

- Heat denatures proteins – kills microbes
- **Thermal death point (TDP)**: lowest temperature at which all cells in a culture are killed
- **Thermal death time (TDT)**: time during which all cells in a culture are killed
- **Decimal Reduction time (DRT)**: time to kill 90% of a population
- All three are indications of severity of treatment required to kill a population of bacteria

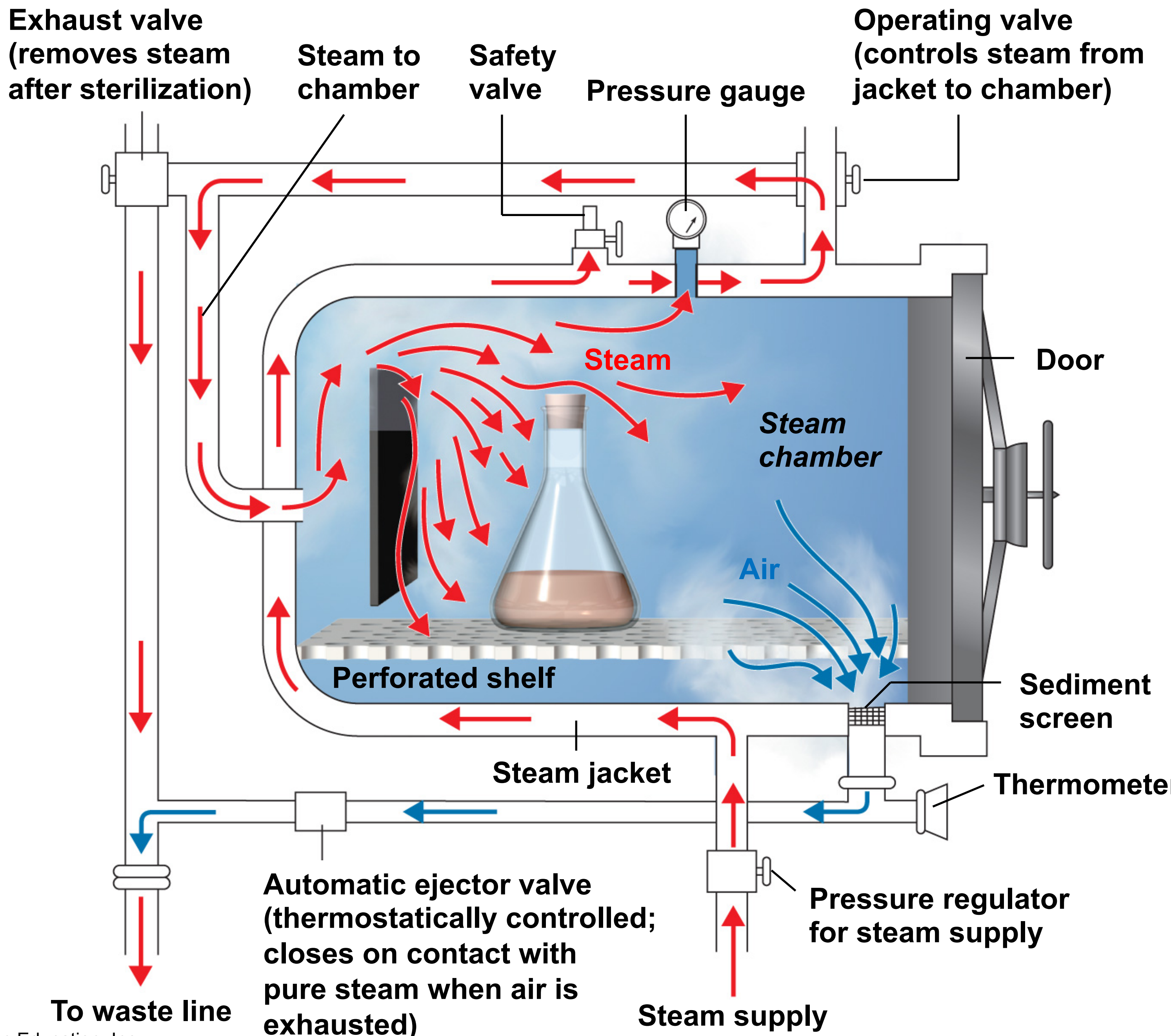
Figure 7.10 A comparison of the effectiveness of various antiseptics.



Moist Heat Sterilization

- **Moist heat** more effective than dry heat
- Kills by coagulating (clumping) proteins
  - Breaks hydrogen bonds that hold structure
- Boiling kills much faster than dry heat
- **Autoclave**: steam under pressure

Figure 7.2 An autoclave.



Steam Sterilization

- Steam effective sterilant
  - Steam must contact item's surface

Figure 7.3 Examples of sterilization indicators.

Pasteurization

- Mild heating to reduce spoilage organisms and pathogens
- Equivalent treatments
  - 63°C for 30 min
  - **High-temperature short-time**: 72°C for 15 sec
  - **Ultra-high-temperature**: 140°C for <1 sec
- Does not significantly damage taste of food

Dry Heat Sterilization

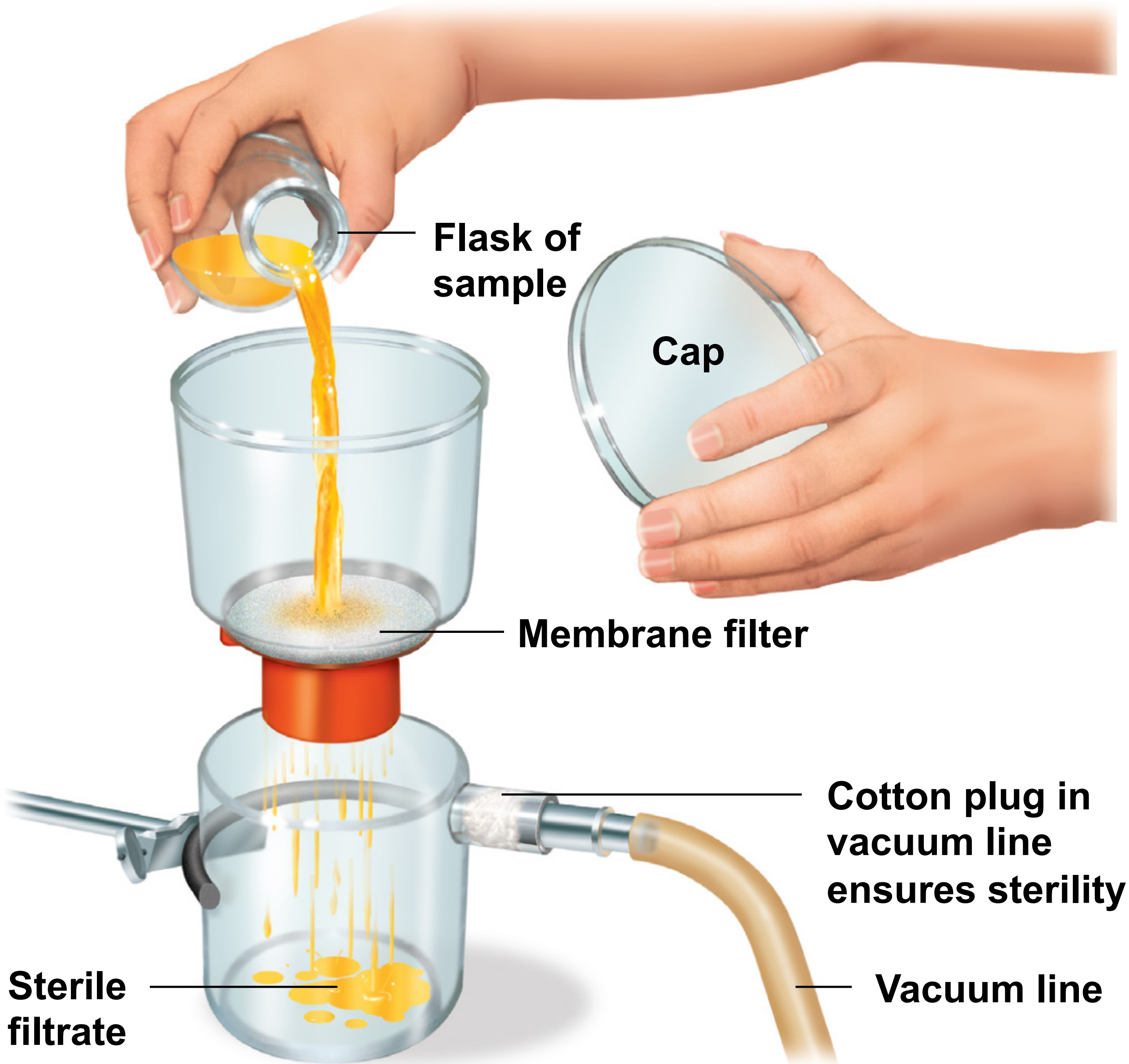
- Kills by oxidation
  - Dry heat
  - Flaming
  - Incineration
  - Hot-air sterilization

	Hot-Air	**Autoclave**
Equivalent Treatments	170˚C, 2 hr	121˚C, 15 min

Filtration

- Liquids can be sterilized by removing microbes
- Used on heat sensitive materials
  - Antibiotics, enzymes, vaccines
- **HEPA** (high-efficiency particulate air) filters remove microbes larger than 0.3 μm
- Membrane filters made of cellulose, plastic polymers
  - Common pore size **0.22 μm**
  - Filter out almost all microbes

Figure 7.4 Filter sterilization with a disposable, presterilized plastic unit.



Physical Methods of Microbial Control

- **Low temperature** inhibits microbial growth
  - Refrigeration
  - Deep-freezing
  - Lyophilization
- **High pressure** denatures proteins
- **Desiccation** prevents metabolism
- **Osmotic pressure** causes plasmolysis

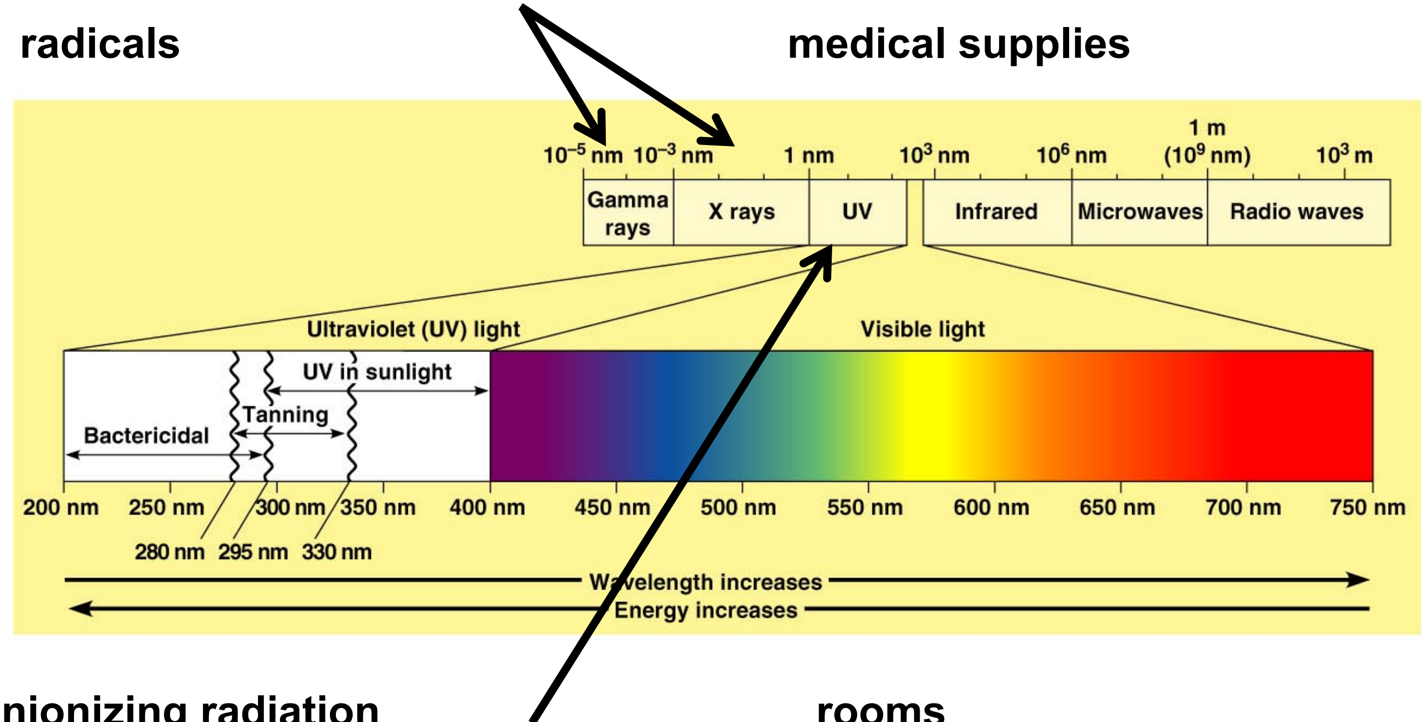
Radiation

- Wavelengths of light smaller than visible light
- **Ionizing radiation** (X rays, gamma rays)
  - Ionizes water to release hydroxyl radicals
  - Damages DNA
- **Nonionizing radiation** (UV light)
  - Damages DNA
- **Microwaves** kill by heat; not especially antimicrobial

Figure 7.5 The radiant energy spectrum.

<u>Ionizing radiation</u>

- Ionizes water, creates hydroxyl radicals
- Radicals damage DNA
- Used to sterilize foods, dental and medical supplies



<u>Nonionizing radiation</u>

- Damages DNA by breaking bonds between thymines
- UV light used to disinfect hospital rooms
- Not very penetrating, microbes must be on surface

Chemical Methods of Microbial Control

- Chemical agents are used on living tissue (as antiseptics) and on inanimate objects (as disinfectants)
- Few chemical agents achieve sterility, but can disinfect
- No single disinfectant is appropriate for all circumstances

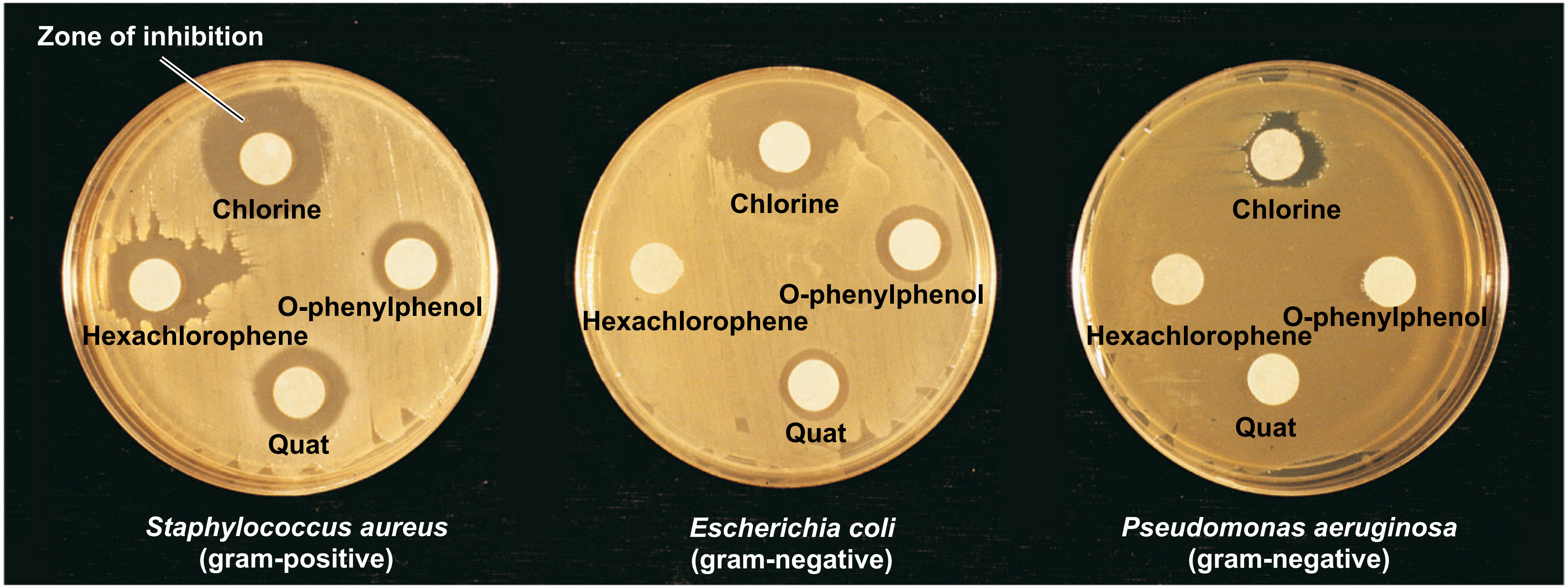
Principles of Effective Disinfection

- Concentration of disinfectant
  - Higher concentration, more effective
- Organic matter
  - Can inhibit some chemicals
- pH
- Time
  - Longer exposure, more effective
  - Longer exposure times can compensate for less ideal conditions

Disk-diffusion Method

- **Disk-diffusion method** - disk of filter paper is soaked with chemical and placed on an inoculated agar plate
  - A zone of inhibition indicates effectiveness
- Can perform test on any desired test bacteria
  - Determine best chemical for inhibiting specific microbe

Figure 7.6 Evaluation of disinfectants by the disk-diffusion method.

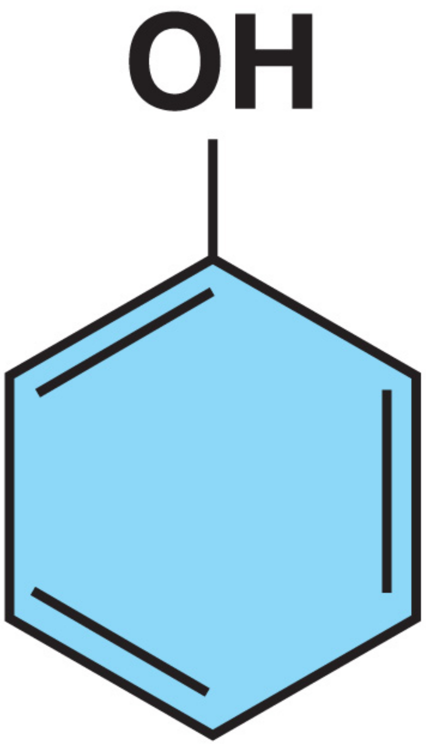


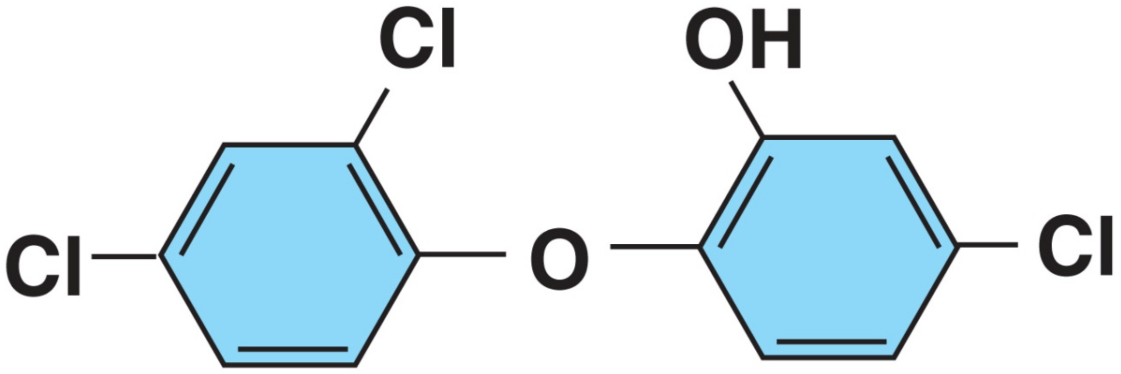
Chemical Methods of Microbial Control

- Chemical disinfectants and antiseptics do not show selective toxicity.
  - Cannot be used internally

Chemical Methods of Microbial Control

- Disrupt plasma membranes
  - Phenol and phenolics
  - Triclosan (a bisphenol)





Chemical Methods of Microbial Control

- Iodine
  - **Tinctures**: mixtures in aqueous alcohol
  - **Iodophors**: iodine in organic molecules
  - Alter protein synthesis and membranes
- Chlorine
  - Bleach: hypochlorous acid (HOCl)
  - Chloramine: chlorine + ammonia
  - Oxidizing agents

Chemical Methods of Microbial Control

- Ethanol, isopropanol
  - Denature proteins, dissolve lipids
  - Require water

Table 7.6 Biocidal Action of Various Concentrations of Ethanol in Aqueous Solution against *Streptococcus pyogenes*

TABLE 7.6 Biocidal Action of Various Concentrations of Ethanol in Aqueous Solution against *Streptococcus pyogenes*

Concentration of Ethanol (%)	Time of Exposure (sec)				
	10	20	30	40	50
100	G	G	G	G	G
95	NG	NG	NG	NG	NG
90	NG	NG	NG	NG	NG
80	NG	NG	NG	NG	NG
70	NG	NG	NG	NG	NG
60	NG	NG	NG	NG	NG
50	G	G	NG	NG	NG
40	G	G	G	G	G

Note:
G = growth
NG = no growth

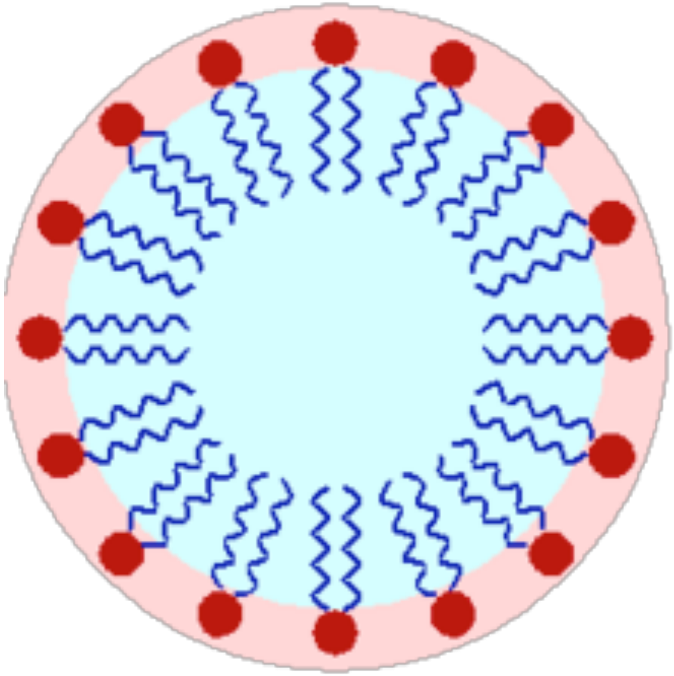
Chemical Methods of Microbial Control

- Heavy Metals
- **Ag, Hg, and Cu**
  - Silver sulfadiazine used as a topical cream on burns
  - Copper sulfate is an algicide
- Work via oligodynamic action
  - Mechanism not clear, but bind to and react with proteins
  - Causes denaturation

Figure 7.8 Oligodynamic action of heavy metals.

Surface-Active Agents, or Surfactants

- Amphipathic molecules
  - Both hydrophobic and hydrophilic
  - Surround particles, rinsed away with water
- Soap works via degerming



Micelle formed by surfactant

Chemical Food Preservatives

- Organic acids
  - Inhibit metabolism
  - Sorbic acid, benzoic acid, and calcium propionate
  - Control molds and bacteria in foods and cosmetics
- Nitrite prevents endospore germination
- Antibiotics
  - Nisin and natamycin prevent spoilage of cheese

Microbial Characteristics and Microbial Control

- Different microbes have varying resistance to types of disinfectants and antiseptics
- Biocides tend to be more effective against gram-positive than gram-negative
  - Outer membrane (OM) helps provide resistance
- Within gram-negative *Pseudomonas* unusually resistant
  - Contain porins in OM, effective at keeping away chemicals from inner membrane
- Endospores, acid-fast cell wall have major effects on treatments

Thermal Death Point

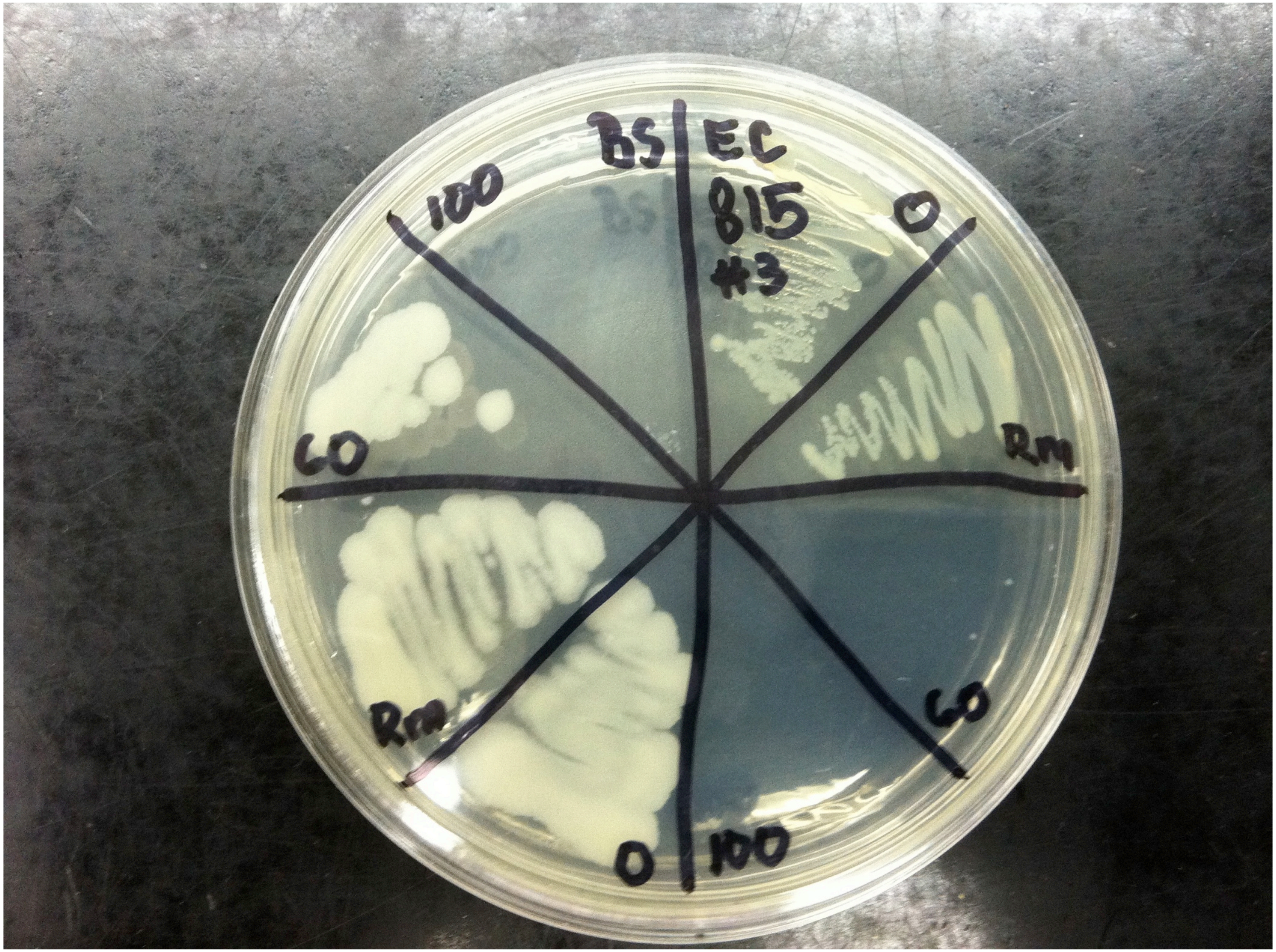


Figure 7.11 Decreasing order of resistance of microorganisms to chemical biocides.

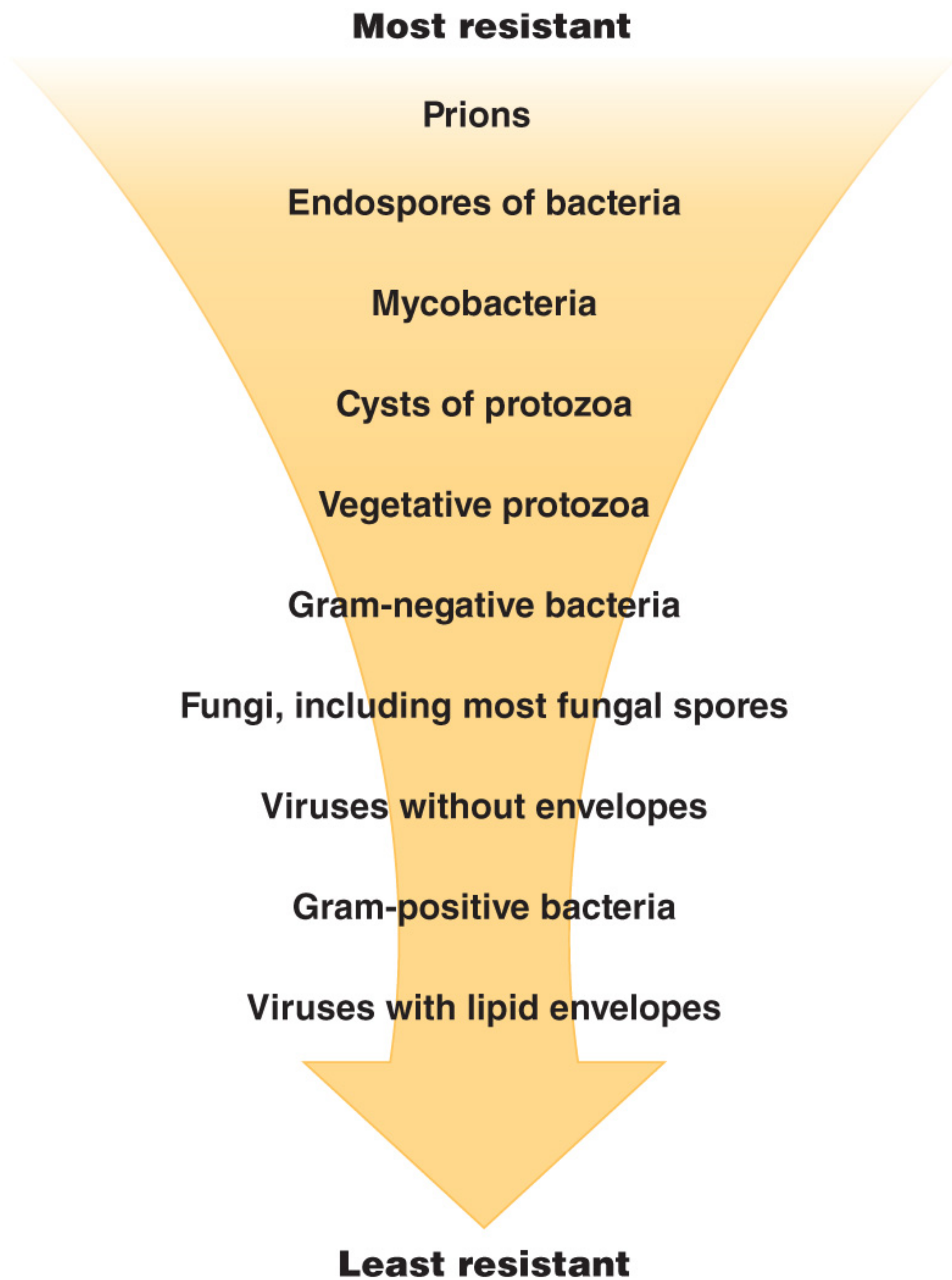


Table 7.7 The Effectiveness of Chemical Antimicrobials against Endospores and Mycobacteria

TABLE 7.7 The Effectiveness of Chemical Antimicrobials against Endospores and Mycobacteria

Chemical Agent	Endospores	Mycobacteria
Mercury	No activity	No activity
Phenolics	Poor	Good
Bisphenols	No activity	No activity
Quats	No activity	No activity
Chlorines	Fair	Fair
Iodine	Poor	Good
Alcohols	Poor	Good
Glutaraldehyde	Fair	Good
Chlorhexidine	No activity	Fair

Microbial Characteristics and Microbial Control

- In general, characteristics of specific bacterial species offer different levels of resistance to disinfectants, antiseptics, antibiotics
  - Biocides are not uniformly effective against all microbes