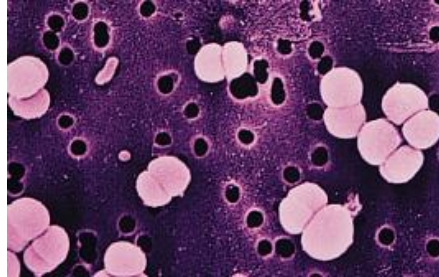


Chapter 7



The Control of Microbial Growth

1

Ch. 7 Objectives:

Students should be able to.....

1. Ch. 7: List **5 physical methods of controlling microbial growth**, and give an example of each.
 - *** Why is moist heat much more effective than dry air?
2. Describe how to measure the **effectiveness of a chemical disinfectant**.
3. List **6 different types of chemical disinfectants** and how they damage microbial cells.
 - *** Distinguish between **biocidal and biostatic** treatments.
4. What are the two **most Resistant**, and the two **most Sensitive** microbial structures to antiseptic treatments? Why are these so?

2

The Control of Microbial Growth

- **Sepsis** refers to microbial contamination.
- **Asepsis** is the absence of significant contamination.
 - Aseptic surgery techniques prevent microbial contamination of wounds.

3

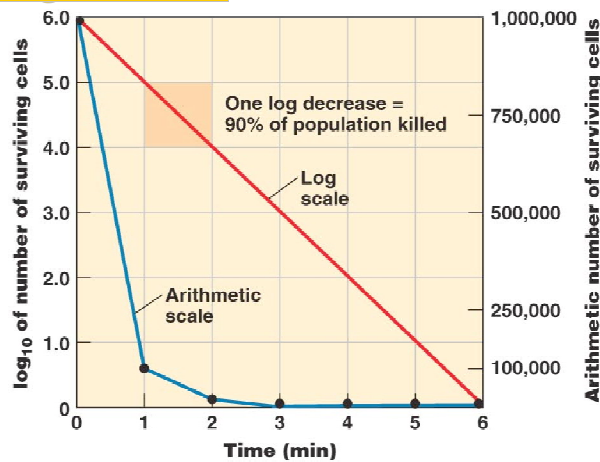
Terminology

1. **Sterilization**: Removal of all microbial life.
2. **Commercial Sterilization**: process kills *C. botulinum* endospores. (= the test organism!)
3. **Disinfection**: Removal of pathogens.
 - ❖ **Fomite** = nonliving surface or inanimate object that may carry infectious microbes.
4. **Antisepsis**: Removal of pathogens from living tissue. (Treatments = less harsh, to preserve tissue.)
5. **Degerming**: Removal of microbes from a limited area.
6. **Sanitization**: Lowering microbial counts on eating utensils.
7. **Biocide/Germicide/Bacteriocide**: Kills microbes.
8. **Germistasis/Bacteriostasis**: Inhibiting, not killing, microbes.

4

Microbial Death

- **Bacterial populations die at a constant, logarithmic rate.**



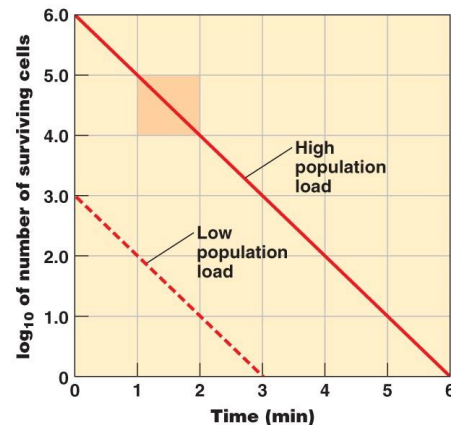
(a) The data are plotted logarithmically (red line) and arithmetically (blue line).

Figure 7.1a

5

Effectiveness of antimicrobial treatment depends on:

1. **Number** of microbes.
2. **Environment**
 - (organic matter, pH, temperature, biofilms)
3. **Time** of exposure
4. **Microbial characteristics**



(b) The effect of high or low initial load of microbes. If the rate of killing is the same, it will take longer to kill all members of a larger population than a smaller one. This is true for both heat and chemical treatments.

Figure 7.1b₆

** Actions of Microbial Control Agents **

1. Alteration of Membrane permeability.

- a) Leakage
- b) Dissolution of PL bilayer

2. Damage to Proteins.

- a) Destruction
- b) Denaturation

3. Damage to Nucleic Acids.

- a) Chemical alterations
- b) Destruction or mutations

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7.1) Physical Methods of Microbial Control

A. Heat:

- Thermal death point (TDP): Lowest temperature at which ALL cells in a culture are killed in 10 min.
- Thermal death time (TDT): Time to kill ALL cells in a culture (at a given temperature!).
- Decimal reduction time (DRT): Minutes to kill 90% of a population at a given temperature.
 - Drop population by an order of magnitude!!

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A. Moist Heat

- denatures proteins

1. Autoclave:

Steam under pressure

- Much higher temps reached at > 1 atm

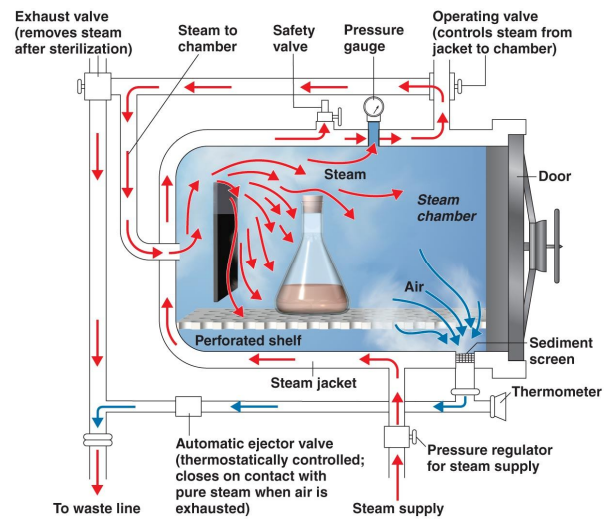


Figure 7.2

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Moist Heat: (#2)

2. Pasteurization reduces 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.
 - Thermophilic** organisms survive (& endospores).

10

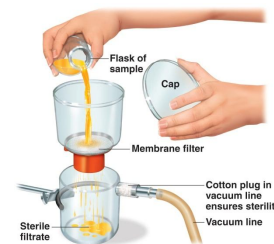
B. Dry Heat

- **Dry Heat** sterilization kills by **oxidation**.
 - Flaming
 - Incineration
 - Hot-air sterilization
 - Not as effective as Autoclave (moist heat, high press.)

	Hot-air	Autoclave
Equivalent treatments	170°C, 2 hr	121°C, 15 min

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Other Physical Methods of Microbial Control



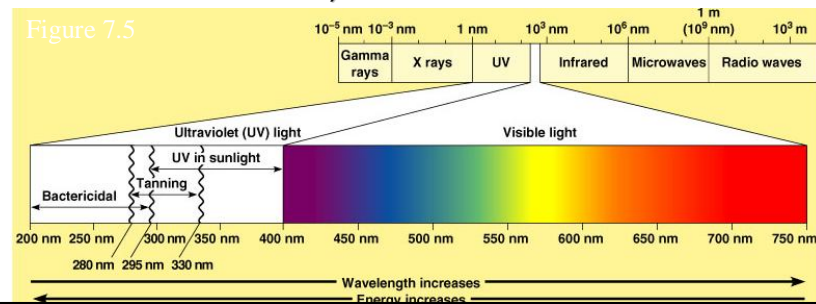
- C. Filtration** removes microbes.
- D. Low temperature** inhibits microbial growth.
 - 1) Refrigeration
 - 2) Deep freezing
 - 3) Lyophilization
- E. High pressure** denatures proteins.
- F. Desiccation** prevents metabolism.
- G. Osmotic pressure** causes plasmolysis.

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Physical Methods of Microbial Control

H. Radiation damages DNA

- Ionizing radiation (X rays, gamma rays, electron beams) → •OH
- Nonionizing radiation (UV)
- (Microwaves kill by heat; not especially antimicrobial)



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7.2) Chemical Methods of Microbial Control

- Principles of effective disinfection
 - Factors for consideration:
 1. Concentration of disinfectant
 2. Organic matter
 3. pH
 4. Time

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A. Chemical Methods of Microbial Control: Evaluation

• Evaluating a Disinfectant:



1. Use-dilution test:

- Metal rings dipped in test bacteria are dried.
- Dried cultures placed in disinfectant for 10 min at 20°C.
- Rings transferred to culture media to determine whether bacteria survived treatment.

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Evaluating a Disinfectant:

2. Disk-diffusion method:

- **Zone of Inhibition/ZOI** = diameter of clearing / sensitivity in mm.

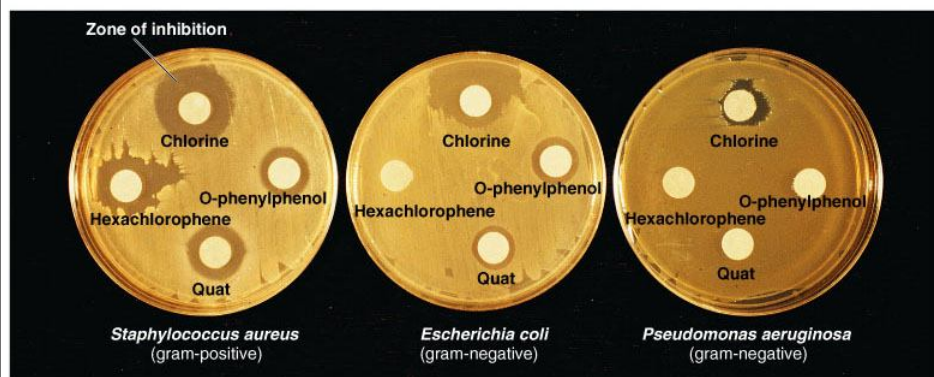


Figure 7.6 16

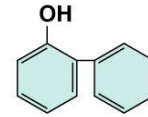
B. Types of Disinfectants

1. Phenol:

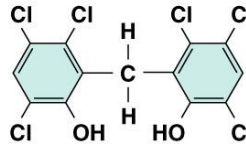
- *Denature proteins.*
❖ “*chaotropic agents*”
- *Disrupt plasma membranes.*



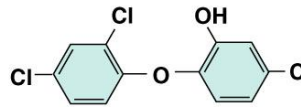
(a) Phenol



(b) O-phenylphenol



(c) Hexachlorophene (a bisphenol)



(d) Triclosan (a bisphenol)

Figure 7.7₁₇

a) **Phenolics:** Lysol

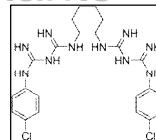
b) **Bisphenols:**
Hexachlorophene,
Triclosan

Types of Disinfectants

2. Biguanides:

Chlorhexidine.

- Disrupt plasma membranes.



3. Halogens: Iodine,

Chlorine.

- Oxidizing agents.
- **Bleach** is hypochlorous acid (HOCl).
- **Iodophores** = betadine (organic combo w/ I).

4. Alcohols: Ethanol,

Isopropanol.

- Denature proteins, dissolve lipids.

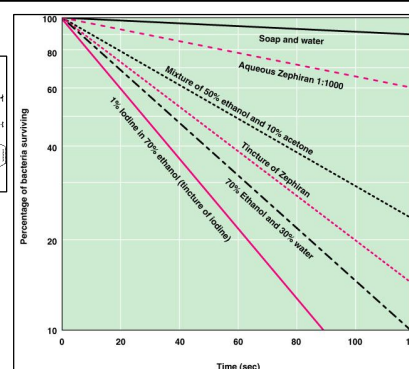


TABLE 7.6 Biocidal Action of Various Concentrations of Ethanol in Aqueous Solution Against *Streptococcus pyogenes*

Concentration of Ethanol (%)	Time (sec)				
	10	20	30	40	50
100	–	–	–	–	–
95	+	+	+	+	+
90	+	+	+	+	+
80	+	+	+	+	+
70	+	+	+	+	+
60	+	+	+	+	+
50	–	–	+	+	+
40	–	–	–	–	–

NOTE: A minus sign indicates no biocidal action (bacterial growth); a plus sign indicates biocidal action (no bacterial growth). The highlighted area represents bacteria killed by biocidal action.

Types of Disinfectants

5. Heavy Metals: Ag, Hg, Cu, Zn

– Oligodynamic action:

- *Very small amounts exert antimicrobial activity!*
- *Denature proteins.*
 - Highly cationic.
 - Large, attractive surfaces.



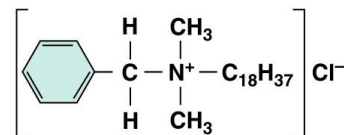
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Types of Disinfectants

6. Surface-Active Agents or "Surfactants":

- Detergents
- Emulsifiers



Benzalkonium chloride

COMPOUND

ACTIVITY

Soap	Degerming
Acid-anionic detergents	Sanitizing
Quarternary ammonium compounds (" <u>Quats</u> ") -- Cationic detergents	Bactericidal, Denature proteins, disrupt plasma membrane

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Types of Disinfectants

7. Chemical Food Preservatives:

a) Organic Acids

- Inhibit metabolism.
- Sorbic acid, benzoic acid, calcium propionate
- Control molds and bacteria in foods and cosmetics

b) Nitrite prevents endospore germination

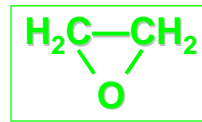
c) Antibiotics: Nisin and natamycin prevent spoilage of cheese

8. Aldehydes:

- Inactivate proteins by covalently cross-linking with reactive functional groups ($-\text{NH}_2$, $-\text{OH}$, $-\text{COOH}$, $-\text{SH}$).
- **Glutaraldehyde, formaldehyde**

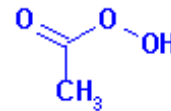
9. Gaseous Sterilants:

- Denature proteins.
- **Ethylene oxide**



10. Peroxygens:

- Oxidizing agents.
- O_3 , H_2O_2 , **peracetic acid**



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Microbial Characteristics and Microbial Control

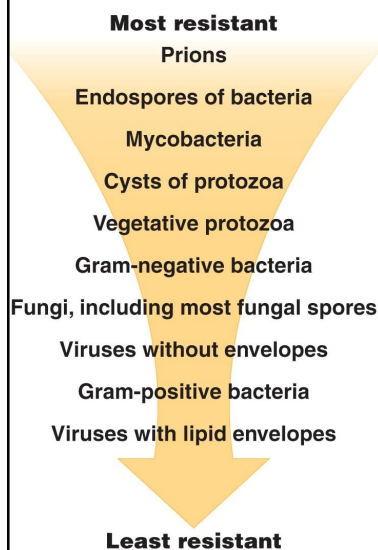


Figure 7.11

Acellular & Cellular Microbes:

1. Prion 3D prot. Strx (acellular)

- Refolds easily
- Must be incinerated!

2. Endospore resistance

- & Protistan cysts

3. Gram- : LPS/OM porins

4. Unenveloped viruses (acell.)

❖ **All above have relatively enhanced resistance to chemical biocides.**

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Microbial Characteristics and Microbial Control

<u>Chemical agent</u>	<u>Effectiveness against:</u>	
	<u>Endospores</u>	<u>Mycobacteria</u>
Phenolics	Poor	Good
Quats	None	None
Chlorines	Fair	Fair
Alcohols & Iodine	Poor	Good
Glutaraldehyde	Fair	Good

***** Microbial control methods, especially biocides, are not equally effective against all microbes. *****