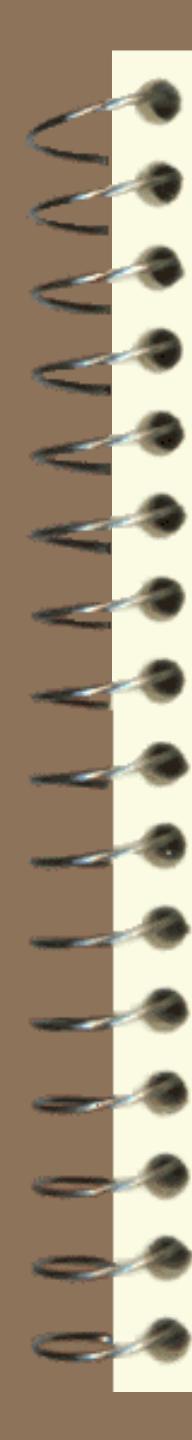


Chapter 9: Controlling Microbial Growth in the Environment

Control of Microbial Growth:

Introduction

- Early civilizations practiced salting, smoking, pickling, drying, and exposure of food and clothing to sunlight to control microbial growth.
- Use of spices in cooking was to mask taste of spoiled food. Some spices prevented spoilage.
- In mid 1800s Semmelweiss and Lister helped developed **aseptic techniques** to prevent contamination of surgical wounds. Before then:
 - Nosocomial infections caused death in 10% of surgeries.
 - Up to 25% mothers delivering in hospitals died due to infection



Control of Microbial Growth:

Definitions

Sterilization: Killing or removing *all forms of microbial life* (including endospores) in a material or an object.

Heating is the most commonly used method of sterilization.

Commercial Sterilization: Heat treatment that kills endospores of *Clostridium botulinum* the causative agent of botulism, in canned food.

Does not kill endospores of thermophiles, which are not pathogens and may grow at temperatures above 45°C.

Control of Microbial Growth: Definitions

Disinfection: Reducing the number of pathogenic microorganisms to the point where they no longer cause diseases. Usually involves the removal of vegetative or non-endospore forming pathogens.

May use physical or chemical methods.

- ◆ **Disinfectant:** Applied to inanimate objects.
- ◆ **Antiseptic:** Applied to living tissue (*antisepsis*).
- ◆ **Degerming:** Mechanical removal of most microbes in a limited area. Example: Alcohol swab on skin.
- ◆ **Sanitization:** Use of chemical agent on food-handling equipment to meet public health standards and minimize chances of disease transmission. E.g: Hot soap & water.

Control of Microbial Growth: Definitions

Sepsis: Comes from Greek for decay or putrid.
Indicates bacterial contamination.

Asepsis: Absence of significant contamination.

Aseptic techniques are used to prevent
contamination of surgical instruments, medical
personnel, and the patient during surgery.

Aseptic techniques are also used to prevent
bacterial contamination in food industry.

Control of Microbial Growth: Definitions

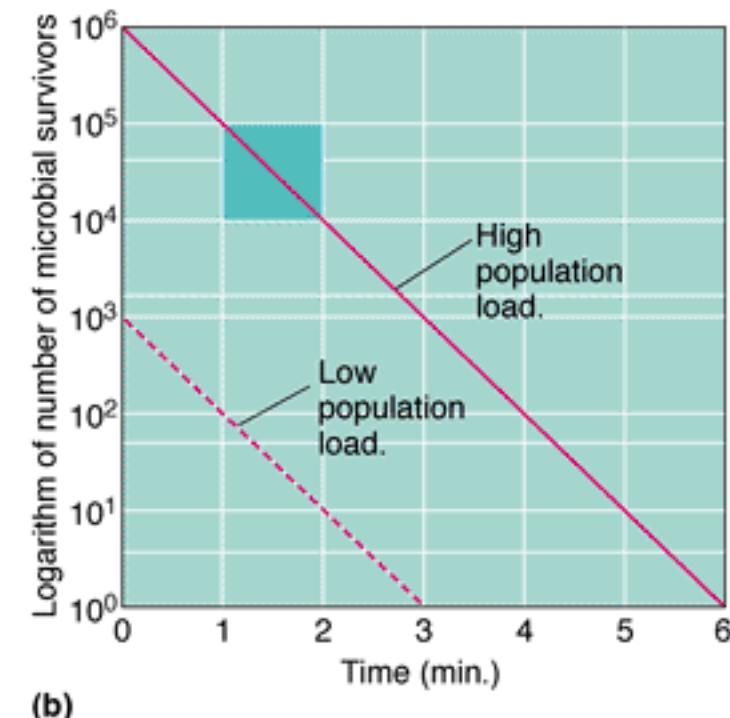
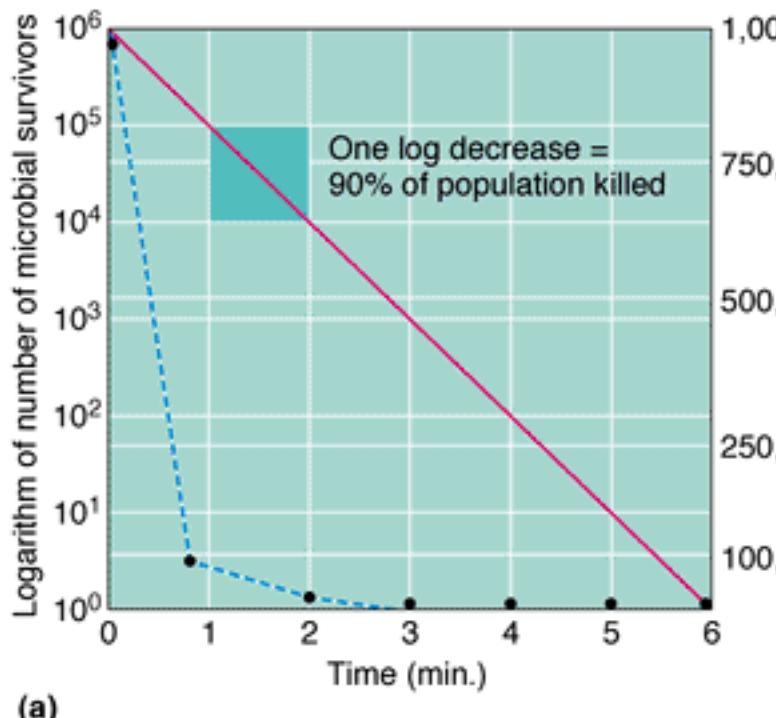
Bacteriostatic Agent: An agent that *inhibits* the growth of bacteria, but does not necessarily kill them. Suffix stasis: To stop or steady.

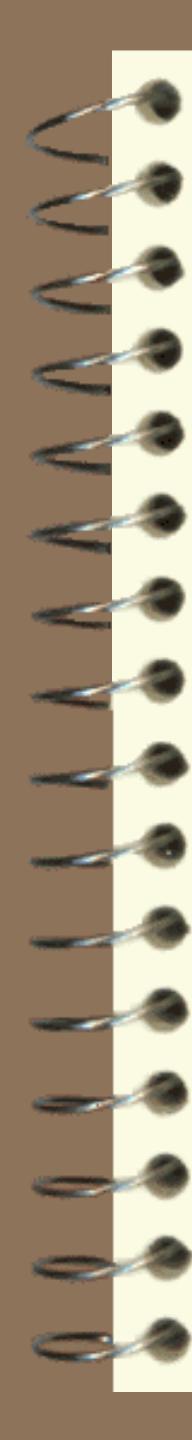
Germicide: An agent that kills certain microorganisms.

- ◆ **Bactericide:** An agent that kills bacteria. Most do not kill endospores.
- ◆ **Viricide:** An agent that inactivates viruses.
- ◆ **Fungicide:** An agent that kills fungi.
- ◆ **Sporocide:** An agent that kills bacterial endospores or fungal spores.

Control of Microbial Growth: Rate of Microbial Death

When bacterial populations are heated or treated antimicrobial chemicals, they usually die at a constant rate.





Factors Affecting the Efficacy of Antimicrobial Methods:

- 1. Site to be Treated:** Will determine the choice of antimicrobials that can be used.
- 2. Number of Microbes:** The more microbes present, the more time it takes to eliminate population.
- 3. Type of Microbes:** Endospores are very difficult to destroy. Vegetative pathogens vary widely in susceptibility to different methods of microbial control.
- 4. Environmental influences:** Presence of organic material (blood, feces, saliva) tends to inhibit antimicrobials, pH etc.
- 5. Temperature and Time of Exposure:** Chemical antimicrobials and radiation treatments are more effective at longer times. In heat treatments, longer exposure compensates for lower temperatures.

Most resistant

Prions

Bacterial endospores

Mycobacteria

Cysts of protozoa

Active-stage protozoa (trophozoites)

Gram-negative bacteria

Fungi

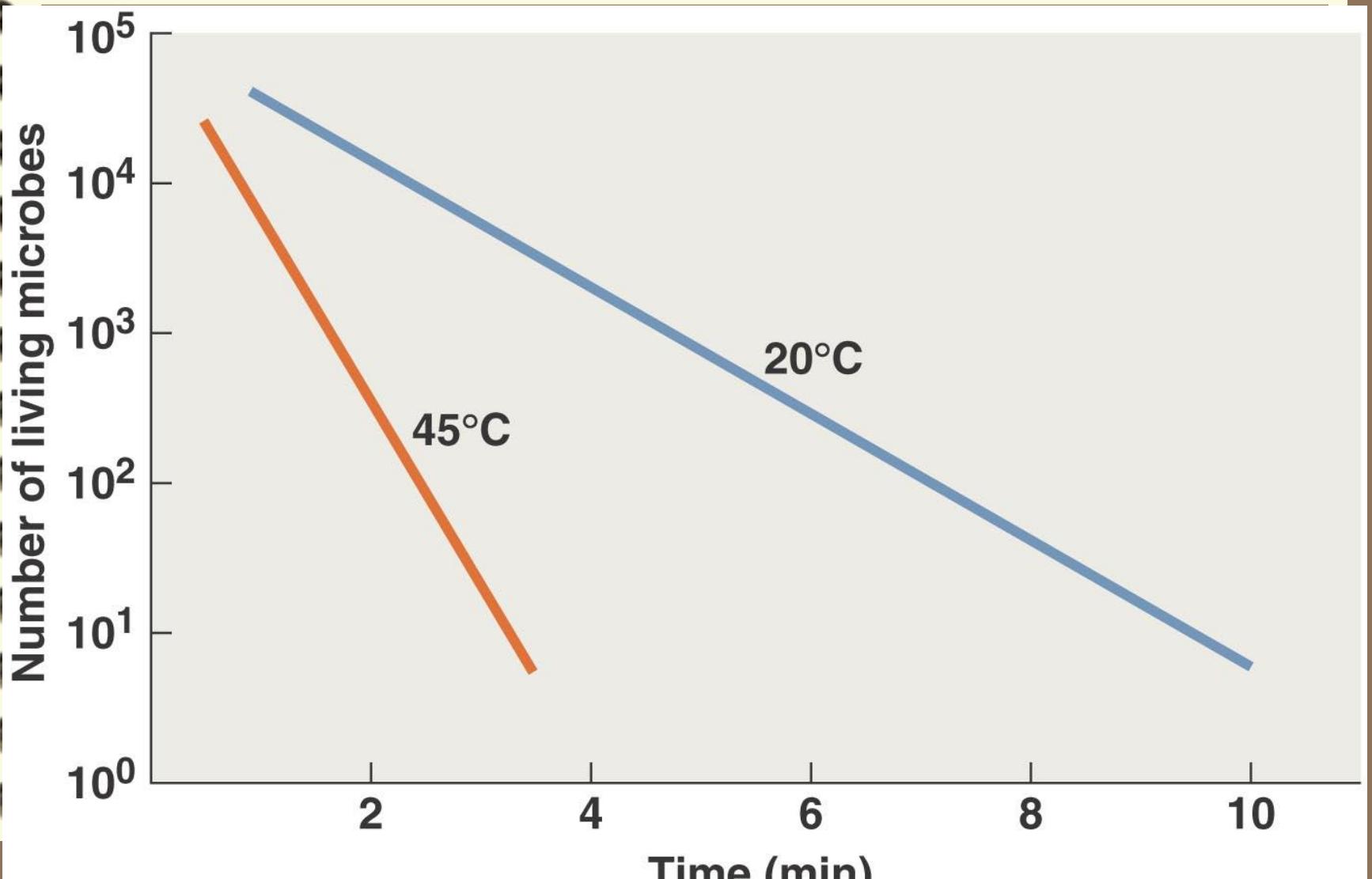
Nonenveloped viruses

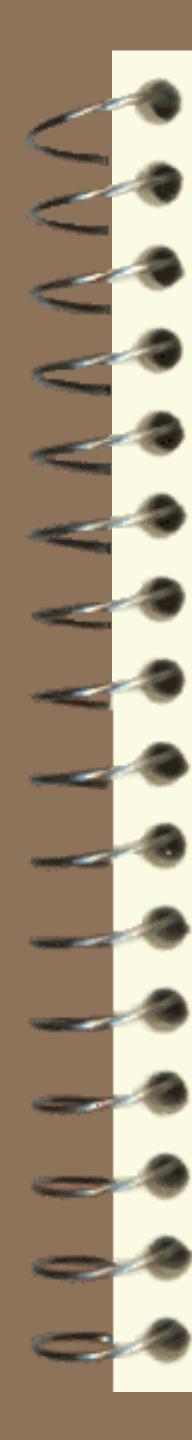
Gram-positive bacteria

Enveloped viruses

Most susceptible

Effect of Temperature on Efficacy of Antimicrobial





Physical Methods of Microbial Control:

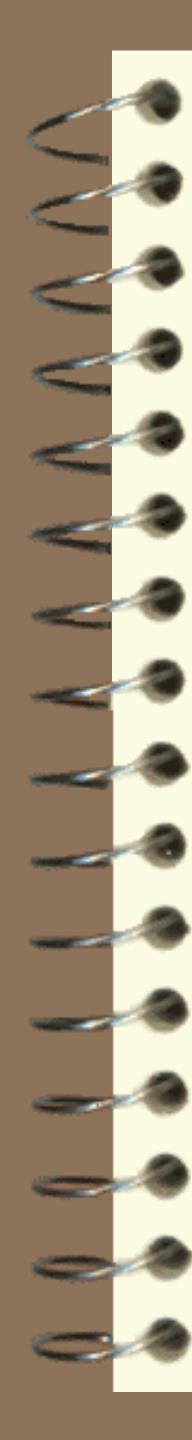
Heat: Kills microorganisms by denaturing their enzymes and other proteins and destroying membranes. Heat resistance varies widely among microbes.

- ◆ **Thermal Death Point (TDP):** Lowest temperature at which all of the microbes in a liquid suspension will be killed in ten minutes.
- ◆ **Thermal Death Time (TDT):** Minimal length of time in which all bacteria will be killed at a given temperature.
- ◆ **Decimal Reduction Time (DRT):** Time in minutes at which 90% of bacteria at a given temperature will be killed. Used in canning industry.

TABLE 7.2

Microbial 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



Physical Methods of Microbial Control:

Moist Heat: Kills microorganisms by **coagulating** their proteins.

In general, moist heat is much more effective than dry heat.

◆ **Boiling:** Heat to 100°C or more at sea level. Kills vegetative forms of bacterial pathogens, almost all viruses, and fungi and their spores within 10 minutes or less. Endospores and some viruses are not destroyed this quickly. However brief boiling will kill most pathogens.

◆ **Hepatitis virus:** Can survive up to 30 minutes of boiling.

◆ **Endospores:** Can survive up to 20 hours or more of boiling.

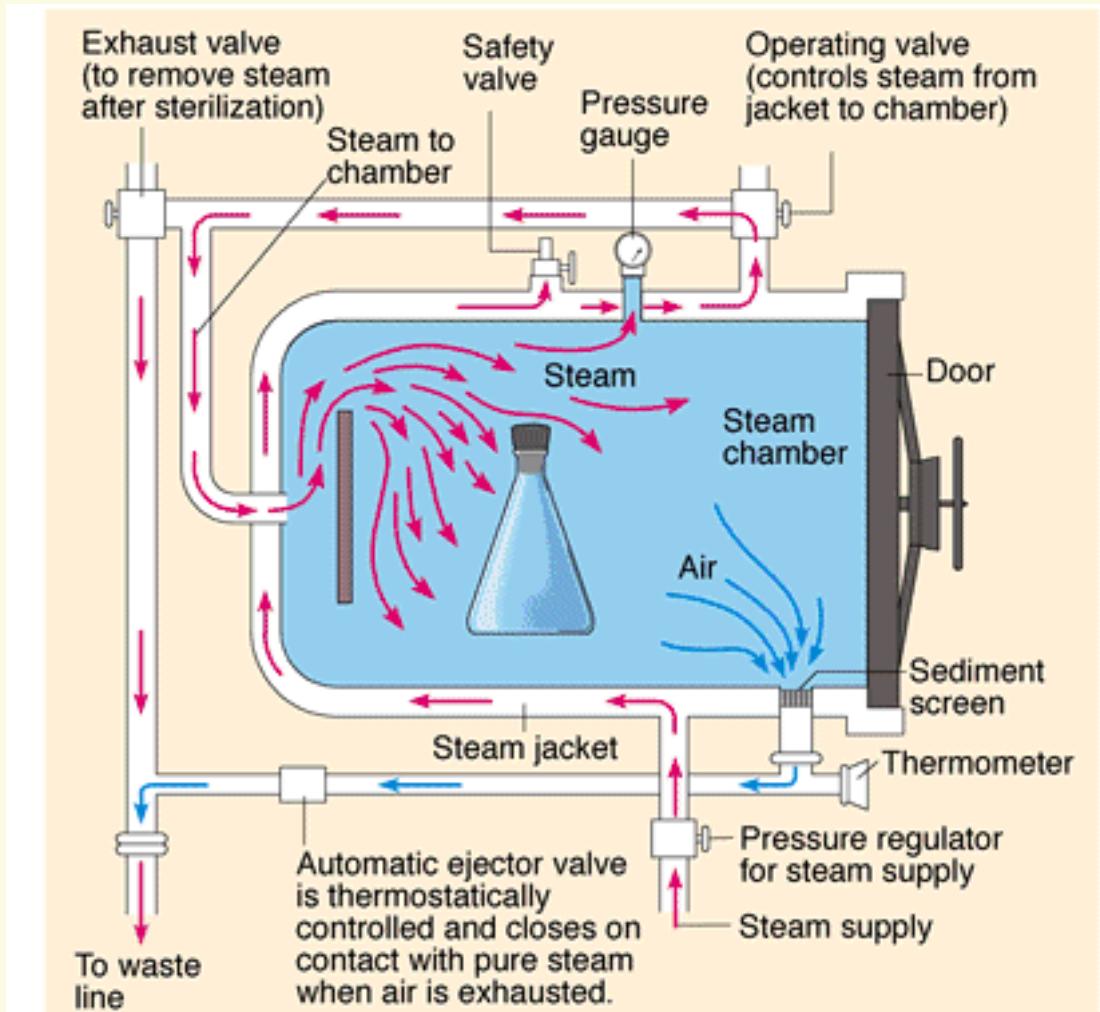
Physical Methods of Microbial Control:

Moist Heat (Continued):

Reliable sterilization with moist heat requires temperatures above that of boiling water.

- ◆ **Autoclave:** Chamber which is filled with hot steam under pressure. Preferred method of sterilization, unless material is damaged by heat, moisture, or high pressure.
 - ◆ Temperature of steam reaches **121°C** at twice atmospheric pressure.
 - ◆ Most effective when organisms contact steam directly or are contained in a small volume of liquid.
 - ◆ **All organisms and endospores are killed within 15 minutes.**
 - ◆ Require more time to reach center of solid or large volumes of liquid.

Autoclave: Closed Chamber with High Temperature and Pressure



Sterilization Indicators

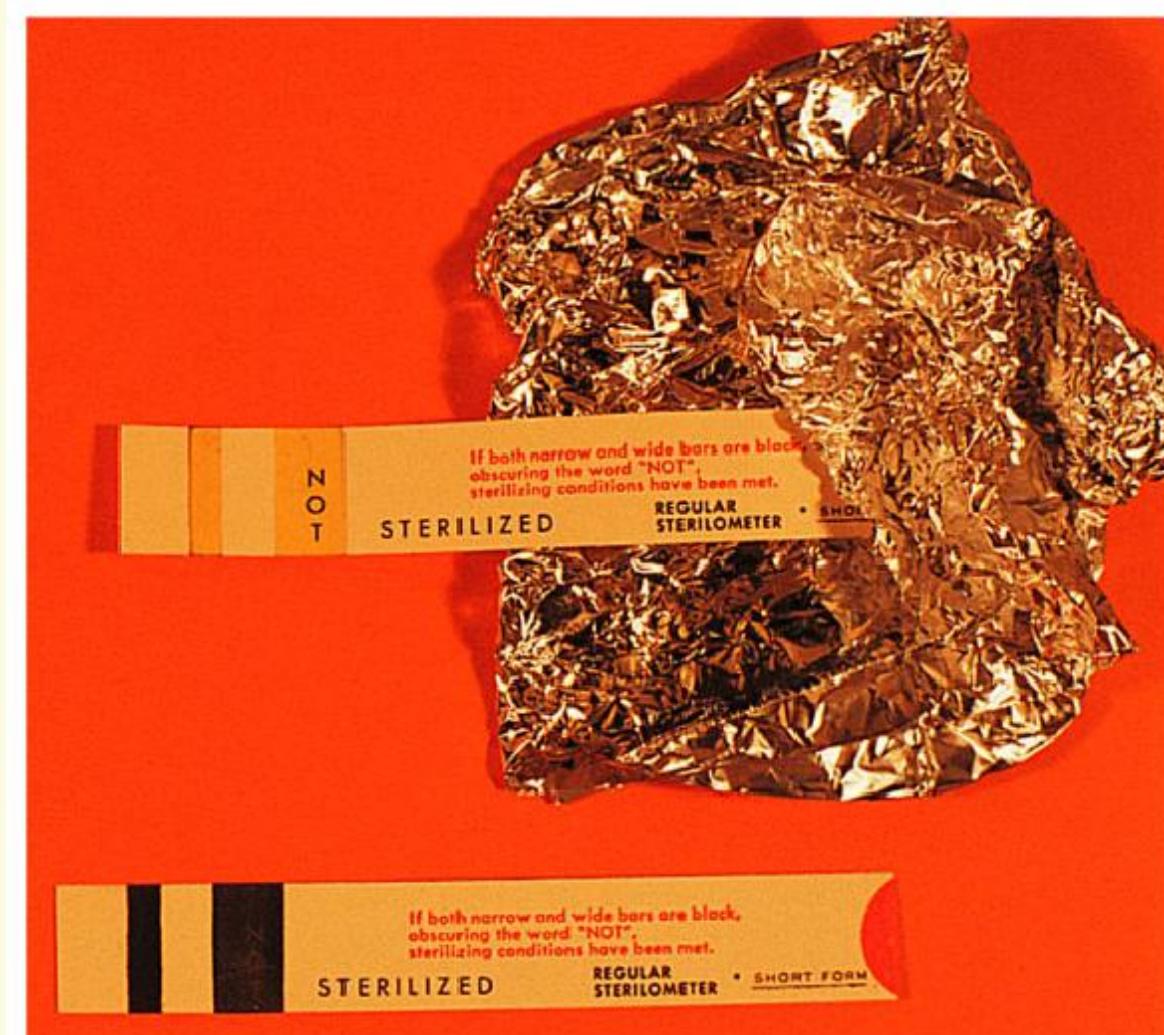


TABLE 7.4**The Effect of Container Size
on Autoclave Sterilization
Times for Liquid Solutions***

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. A container is usually not filled past 75% of its capacity.

Physical Methods of Microbial Control:

- ◆ **Pasteurization:** Developed by Louis Pasteur to prevent the spoilage of beverages by reducing the number of microbes in milk, wine, juices, beer, etc.
 - ◆ **Classic Method of Pasteurization:** Milk was exposed to 65°C for 30 minutes.
 - ◆ **Flash (High Temperature Short Time) Pasteurization:** Used today. Milk is exposed to 72°C for 15 seconds.
 - ◆ **Ultrahigh Temperature Pasteurization (UHT):** Milk is treated at 134°C for 1 second. May affect taste of beverage.
 - ◆ **Ultrahigh temperature sterilization:** 140°C for 1-3 seconds and then cooled very quickly in a vacuum chamber.
- Advantage:** Milk can be stored at room temperature for several months.

Physical Methods of Microbial Control:

Dry Heat: Kills by oxidation effects.

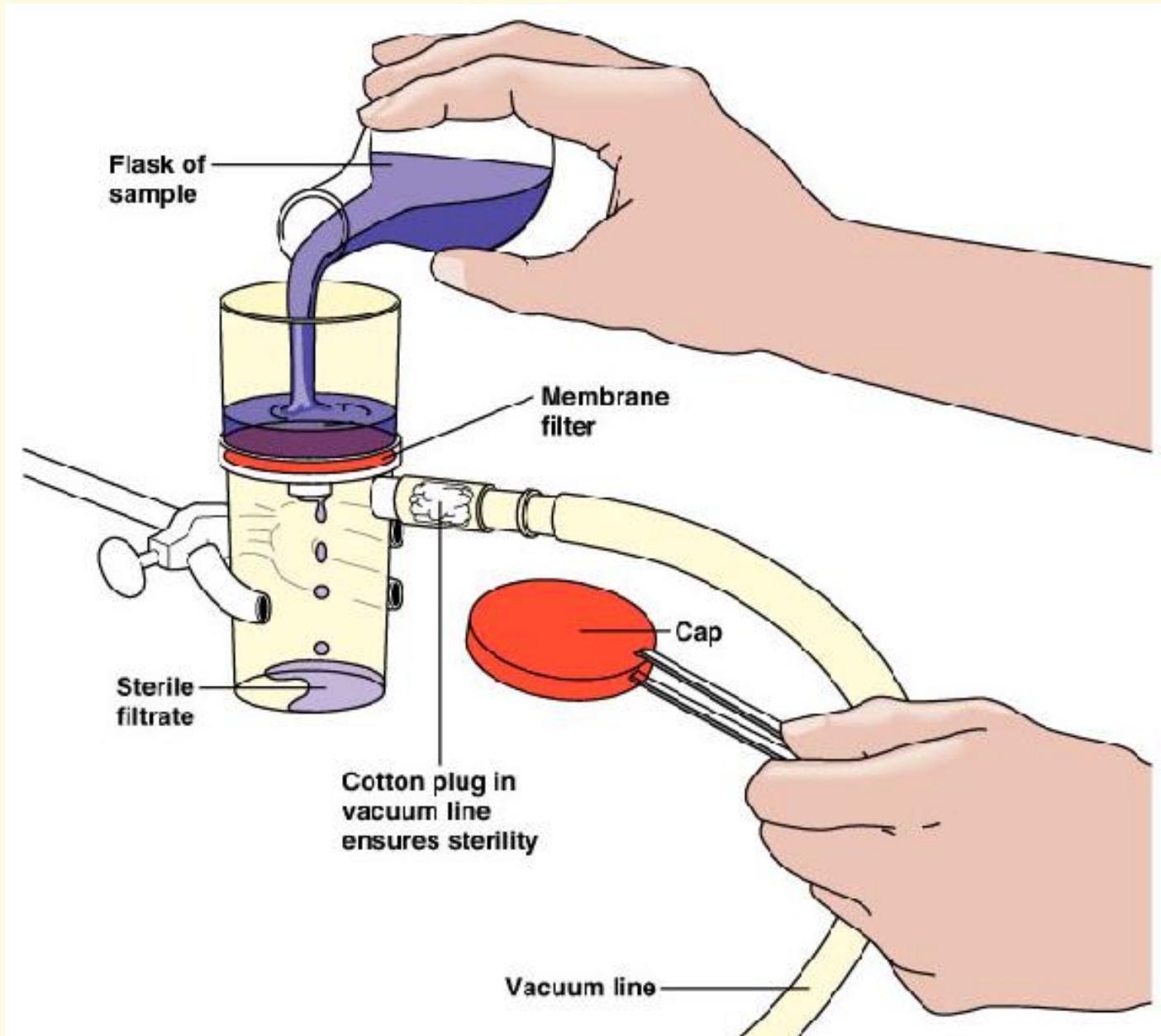
- ◆ **Direct Flaming:** Used to sterilize inoculating loops and needles. Heat metal until it has a red glow.
- ◆ **Incineration:** Effective way to sterilize disposable items (paper cups, dressings) and biological waste.
- ◆ **Hot Air Sterilization:** Place objects in an oven. Require 2 hours at 170°C for sterilization. Dry heat transfers heat less effectively to a cool body, than moist heat.

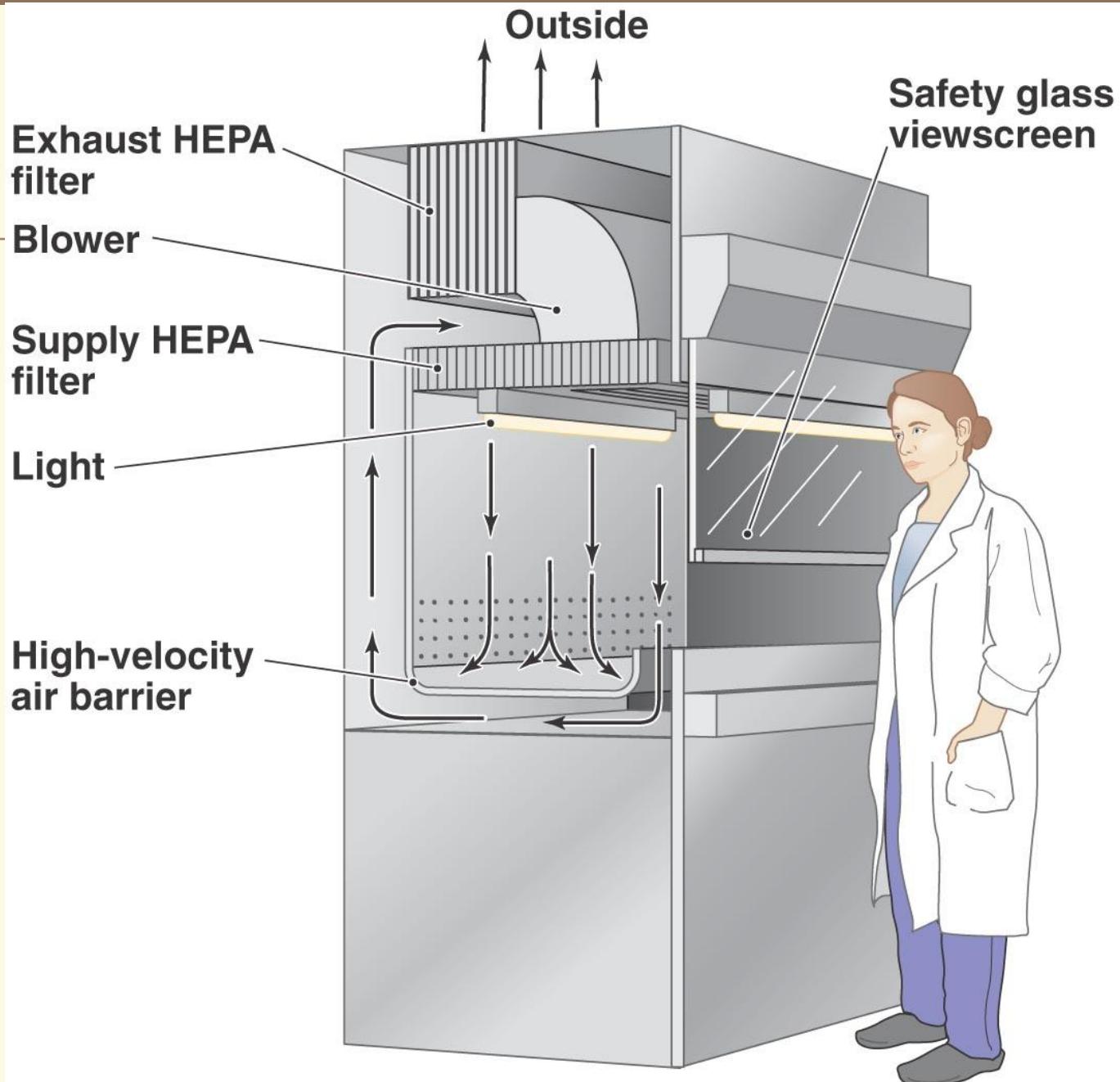
Physical Methods of Microbial Control:

Filtration: Removal of microbes by passage of a liquid or gas through a screen like material with small pores. Used to sterilize heat sensitive materials like vaccines, enzymes, antibiotics, and some culture media.

- ◆ **High Efficiency Particulate Air Filters (HEPA):** Used in operating rooms and burn units to remove bacteria from air.
- ◆ **Membrane Filters:** Uniform pore size. Used in industry and research. Different sizes:
 - ◆ **0.22 and 0.45um Pores:** Used to filter most bacteria. Don't retain spirochetes, mycoplasmas and viruses.
 - ◆ **0.01 um Pores:** Retain all viruses and some large proteins.

Filter Sterilization with Disposable Unit





Physical Methods of Microbial Control:

Low Temperature: Effect depends on microbe and treatment applied.

◆ **Refrigeration:** Temperatures from 0 to 7°C. **Bacteriostatic** effect. Reduces metabolic rate of most microbes so they cannot reproduce or produce toxins.

◆ **Freezing:** Temperatures below 0°C.

◆ **Flash Freezing:** Does not kill most microbes.

◆ **Slow Freezing:** More harmful because ice crystals disrupt cell structure.

◆ Over a third of vegetative bacteria may survive 1 year.

◆ Most parasites are killed by a few days of freezing.

Physical Methods of Microbial Control:

Dessication: In the absence of water, microbes cannot grow or reproduce, but some may remain viable for years. After water becomes available, they start growing again. Susceptibility to dessication varies widely:

- ◆ *Neisseria gonorrhoea*: Only survives about one hour.
- ◆ *Mycobacterium tuberculosis*: May survive several months.
- ◆ Viruses are fairly resistant to dessication.
- ◆ *Clostridium spp.* and *Bacillus spp.*: May survive decades.

Lyophilization: Combines freezing and drying with a vacuum, to preserve cells for many years. Use liquid nitrogen or dry ice.

Physical Methods of Microbial Control:

Osmotic Pressure: The use of high concentrations of salts and sugars in foods is used to increase the osmotic pressure and create a **hypertonic** environment.

Plasmolysis: As water leaves the cell, plasma membrane shrinks away from cell wall. Cell may not die, but usually stops growing.

- ◆ **Yeasts and molds:** More resistant to high osmotic pressures.
- ◆ *Staphylococci spp.* that live on skin are fairly resistant to high osmotic pressure.

Physical Methods of Microbial Control:

Radiation: Three types of radiation kill microbes:

1. Ionizing Radiation: Gamma rays, X rays, electron beams, or higher energy rays. Have short wavelengths (less than 1 nanometer).

Dislodge electrons from atoms and form ions.

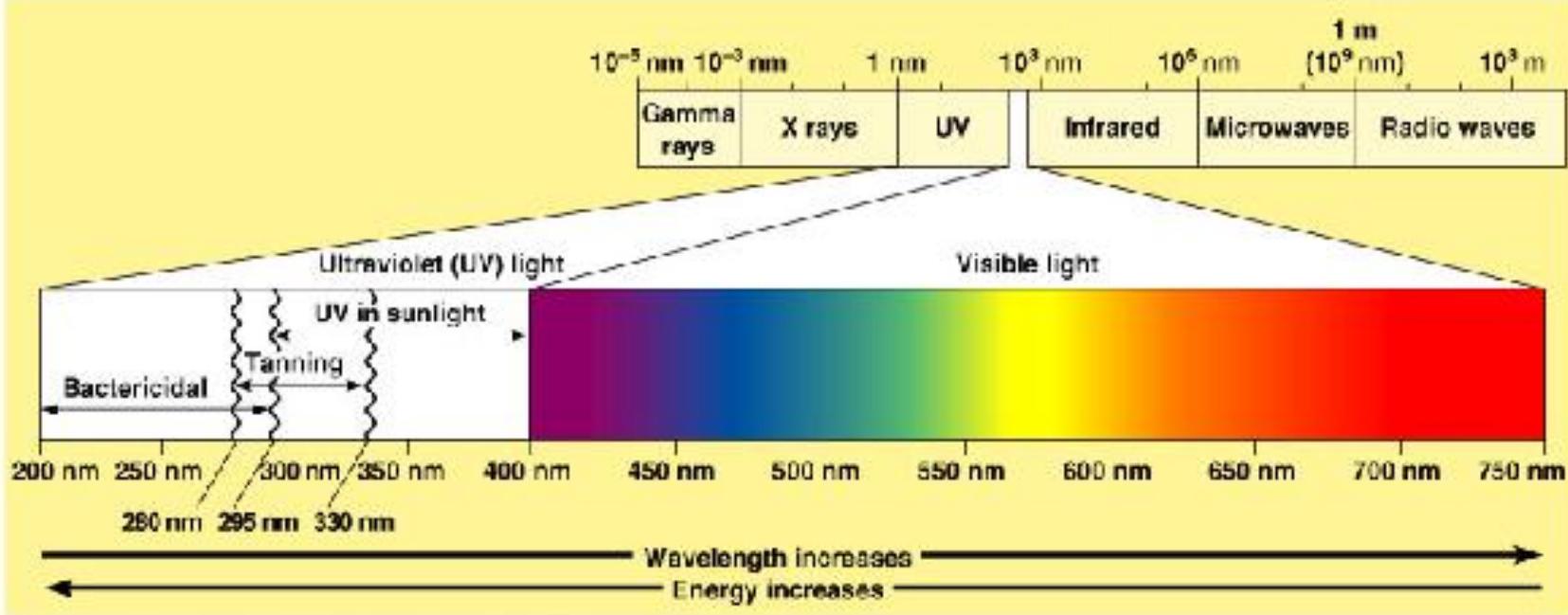
Action: Cause mutations in DNA and produce peroxides.

Used to sterilize pharmaceuticals and disposable medical supplies.

The FDA has approved the use of gamma rays for meats, spices, fruits, and vegetables. Kills microbes, insects, and fruit/vegetable cells which prevents both spoilage and overripening.

Disadvantages: Penetrates human tissues. May cause genetic mutations in humans and microbes. Concerns about nutritional value of food.

Forms of Radiation



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Physical Methods of Microbial Control:

Radiation: Three types of radiation kill microbes:

2. Ultraviolet light (Nonionizing Radiation):

Wavelength is longer than 1 nanometer.

Action: Damages DNA by producing thymine dimers, which cause mutations. Inhibits DNA transcription and replication,

Used to disinfect operating rooms, nurseries, cafeterias.

Disadvantages: Damages skin, eyes. Doesn't penetrate paper, glass, and cloth.

Physical Methods of Microbial Control:

▪ **Radiation:** Three types of radiation kill microbes:

3. **Microwave Radiation:** Wavelength ranges from 1 millimeter to 1 meter.

Heat is absorbed by water molecules.

May kill vegetative cells in moist foods.

Bacterial endospores, which do not contain water, are not damaged by microwave radiation.

Solid foods are unevenly penetrated by microwaves.

Trichinosis outbreaks have been associated with pork cooked in microwaves.

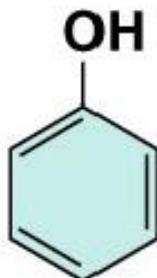
Chemical Methods of Microbial Control

Types of Disinfectants

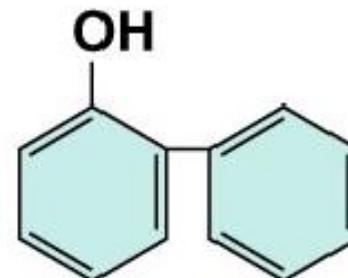
1. Phenols and Phenolics:

- ◆ **Phenol** (carbolic acid) was first used by Lister as a disinfectant.
 - ◆ Rarely used today because it is a skin irritant and has strong odor.
 - ◆ Used in some throat sprays and lozenges.
 - ◆ Acts as local anesthetic.
- ◆ **Phenolics** are chemical derivatives of phenol
 - ◆ **Cresols**: Derived from coal tar (**Lysol**).
 - ◆ **Biphenols (pHisoHex)**: Effective against gram-positive staphylococci and streptococci. Used in nurseries. Excessive use in infants may cause neurological damage.
- ◆ **Action**: Destroy plasma membranes and denature proteins.
- ◆ **Advantages**: Stable, persist for long times after applied, and remain active in the presence of organic compounds.

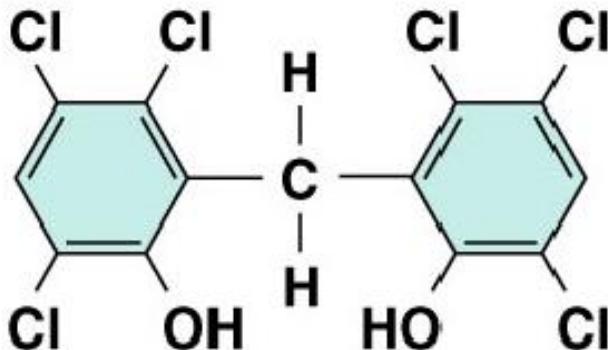
Phenolics and Bisphenols



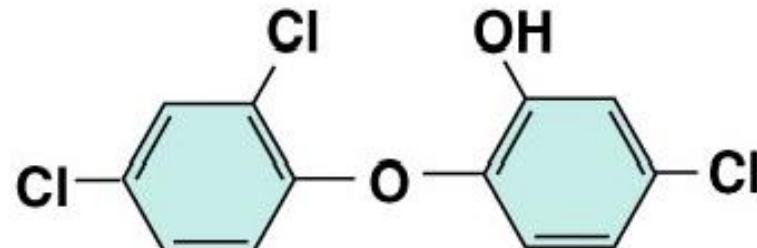
(a) Phenol



(b) O-phenylphenol



(c) Hexachlorophene (a bisphenol)



(d) Triclosan (a bisphenol)

Chemical Methods of Microbial Control

Types of Disinfectants

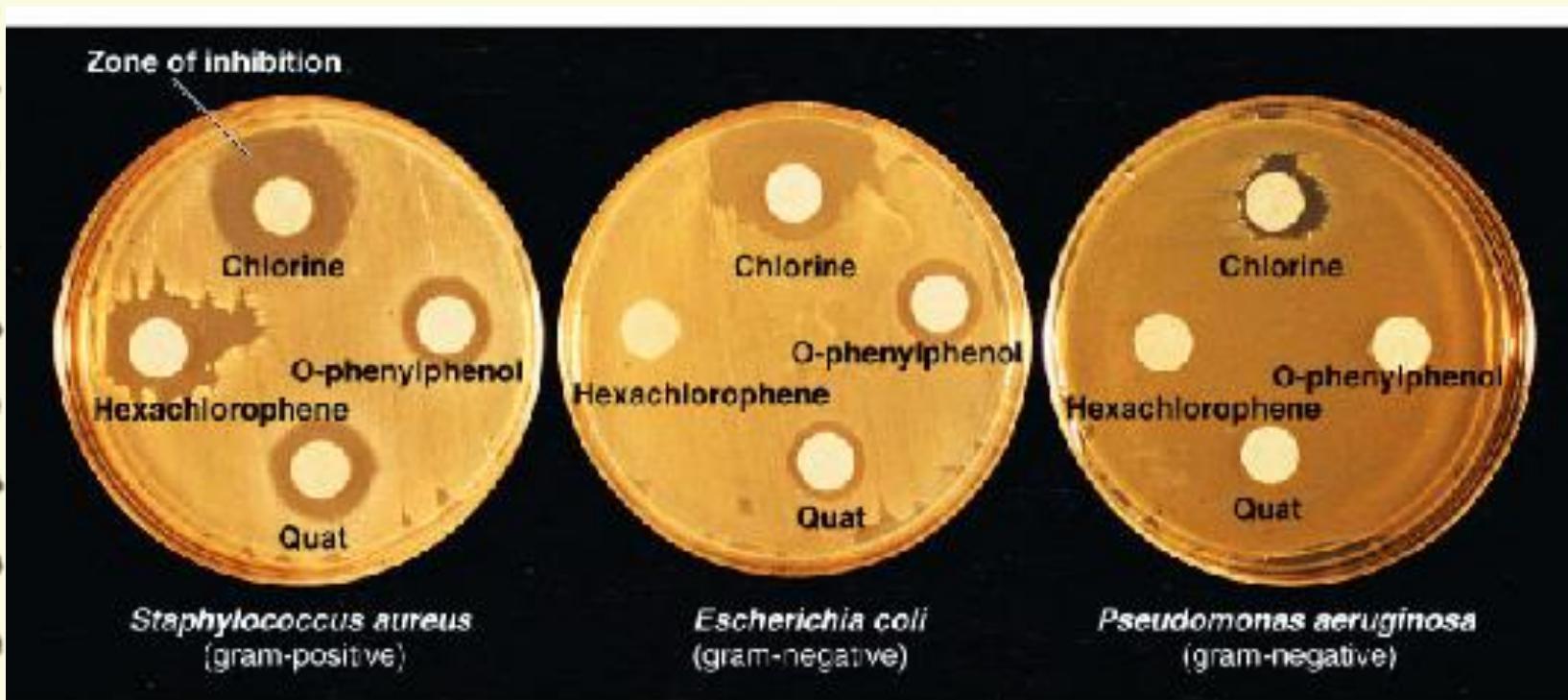
2. Halogens: Effective alone or in compounds.

Action: Denature proteins

A. Iodine:

- ◆ **Tincture of iodine** (alcohol solution) was one of first antiseptics used.
- ◆ Combines with amino acid tyrosine in proteins and denatures proteins.
- ◆ Stains skin and clothes, somewhat irritating.
- ◆ **Iodophors:** Compounds with iodine that are **slow releasing**, take several minutes to act. Used as skin antiseptic in surgery. Not effective against bacterial endospores.
 - ◆ Betadine
 - ◆ Isodine

Comparison of Disinfectants by Disk Diffusion Method



Chemical Methods of Microbial Control

Types of Disinfectants

2. Halogens: Effective alone or in compounds.

B. Chlorine:

- When mixed in water forms **hypochlorous acid**:



Hypochlorous acid

- Used to disinfect drinking water, pools, and sewage.
- Chlorine is easily inactivated by organic materials.
- Sodium hypochlorite (NaOCl):** Is active ingredient of bleach.
- Chloramines:** Consist of chlorine and ammonia. Less effective as germicides.

Chemical Methods of Control

Types of Disinfectants

3. Alcohols:

- ◆ Kill bacteria, fungi, but not endospores or naked viruses.
- ◆ **Action:** Denature proteins and disrupt cell membranes.
- ◆ Evaporate, leaving no residue.
- ◆ Used to mechanically wipe microbes off skin before injections or blood drawing.
- ◆ Not good for open wounds, because cause proteins to coagulate.
 - ◆ **Ethanol:** Drinking alcohol. Optimum concentration is 70%.
 - ◆ **Isopropanol:** Rubbing alcohol. Better disinfectant than ethanol. Also cheaper and less volatile.

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.

Chemical Methods of Control

Types of Disinfectants

4. Heavy Metals:

- ◆ Include copper, selenium, mercury, silver, and zinc.
- ◆ **Oligodynamic action:** Denature proteins. Very tiny amounts are effective.

A. Silver:

- ◆ 1% silver nitrate used to protect infants against gonorrheal eye infections until recently.

B. Mercury

- ◆ Organic mercury compounds like merthiolate and mercurochrome are used to disinfect skin wounds.
- ◆ Thimerosal is used in some vaccines (flu), recently removed from most pediatric vaccines..

C. Copper

- ◆ Copper sulfate is used to kill algae in pools and fish tanks.

Oligodynamic Action of Heavy Metals



Chemical Methods of Control

Types of Disinfectants

4. Heavy Metals:

D. Selenium

- ◆ Kills fungi and their spores. Used for fungal infections.
- ◆ Also used in dandruff shampoos.

E. Zinc

- ◆ Zinc chloride is used in mouthwashes.
- ◆ Zinc oxide is used as antifungal agent in paints and diaper rash cream.

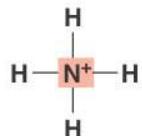
Chemical Methods of Control

Types of Disinfectants

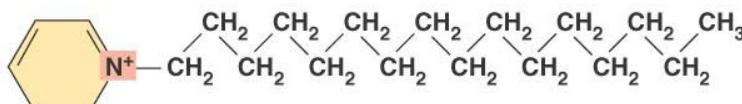
5. Quaternary Ammonium Compounds (Quats):

- ◆ Widely used surface active agents.
- ◆ Cationic (positively charged) detergents.
- ◆ Effective against gram positive bacteria, less effective against gram-negative bacteria.
- ◆ Also destroy fungi, amoebas, and enveloped viruses.
- ◆ **Zephiran**, Cepacol, also found in our lab spray bottles.
- ◆ ***Pseudomonas*** strains that are resistant and can grow in presence of Quats are a big concern in hospitals.
- ◆ **Advantages:** Strong antimicrobial action, colorless, odorless, tasteless, stable, and nontoxic.
- ◆ **Diasadvantages:** Form foam. Organic matter interferes with effectiveness. Neutralized by soaps and anionic detergents.

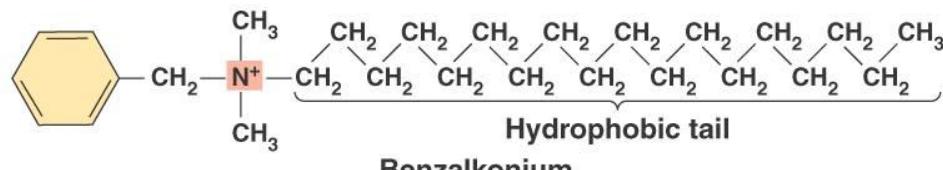
Quaternary Ammonium Compounds



(a) Ammonium ion



Cetylpyridinium



Benzalkonium

(b) Quaternary ammonium ions

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Chemical Methods of Control

Types of Disinfectants

6. Aldehydes:

- ◆ Include some of the most effective antimicrobials.
- ◆ **Action:** Denature proteins by forming covalent crosslinks with several functional groups.

A. Formaldehyde gas ($\text{H}_2\text{C=O}$):

- ◆ Excellent disinfectant.
- ◆ Commonly used as formalin, a 37% aqueous solution.
- ◆ Formalin was used extensively to preserve biological specimens and inactivate viruses and bacteria in vaccines.
- ◆ Irritates mucous membranes, strong odor.
- ◆ Also used in mortuaries for embalming.

Chemical Methods of Control

Types of Disinfectants

6. Aldehydes:

B. Glutaraldehyde:

- ◆ Less irritating and more effective than formaldehyde.
- ◆ One of the few chemical disinfectants that is a sterilizing agent.
- ◆ A 2% solution of glutaraldehyde (Cidex) is:
 - ◆ Bactericidal, tuberculocidal, and viricidal in 10 minutes.
 - ◆ Sporicidal in 3 to 10 hours.
- ◆ Commonly used to disinfect hospital instruments.
- ◆ Also used in mortuaries for embalming.

Chemical Methods of Control

Types of Disinfectants

7. Gaseous Sterilizers:

- ◆ Chemicals that sterilize in a chamber similar to an autoclave.
- ◆ **Action:** Denature proteins, by replacing functional groups with alkyl groups.

A. Ethylene Oxide:

- ◆ Kills all microbes and endospores, but requires exposure of 4 to 18 hours.
- ◆ Toxic and explosive in pure form.
- ◆ Highly penetrating.
- ◆ Most hospitals have ethylene oxide chambers to sterilize mattresses and large equipment.

Chemical Methods of Control

Types of Disinfectants

8. Peroxygens (Oxidizing Agents):

- ◆ Oxidize cellular components of treated microbes.
- ◆ **Action:** Disrupt membranes and proteins.

A. Ozone:

- ◆ Used along with chlorine to disinfect water.
- ◆ Helps neutralize unpleasant tastes and odors.
- ◆ More effective killing agent than chlorine, but less stable and more expensive.
- ◆ Highly reactive form of oxygen.
- ◆ Made by exposing oxygen to electricity or UV light.

Chemical Methods of Control

Types of Disinfectants

8. Peroxygens (Oxidizing Agents):

B. Hydrogen Peroxide:

- ◆ Used as an antiseptic.
- ◆ Not good for open wounds because quickly broken down by catalase present in human cells.
- ◆ Effective in disinfection of inanimate objects.
- ◆ Sporicidal at higher temperatures.
- ◆ Used by food industry and to disinfect contact lenses.

C. Benzoyl Peroxide:

- ◆ Used in acne medications.

Chemical Methods of Control

Types of Disinfectants

8. Peroxygens (Oxidizing Agents):

D. Peracetic Acid:

- ◆ One of the most effective liquid sporicides available.
- ◆ **Sterilant :**
 - ◆ Kills bacteria and fungi in less than 5 minutes.
 - ◆ Kills endospores and viruses within 30 minutes.
- ◆ Used widely in disinfection of food and medical instruments because it does not leave toxic residues.

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
Quaternary ammonium compounds	No activity	No activity
Chlorines	Fair	Fair
Iodine	Poor	Good
Alcohols	Poor	Good
Glutaraldehyde	Fair	Good
Chlorhexidine	No activity	Fair

Efficiency of Different Chemical Antimicrobial Agents

