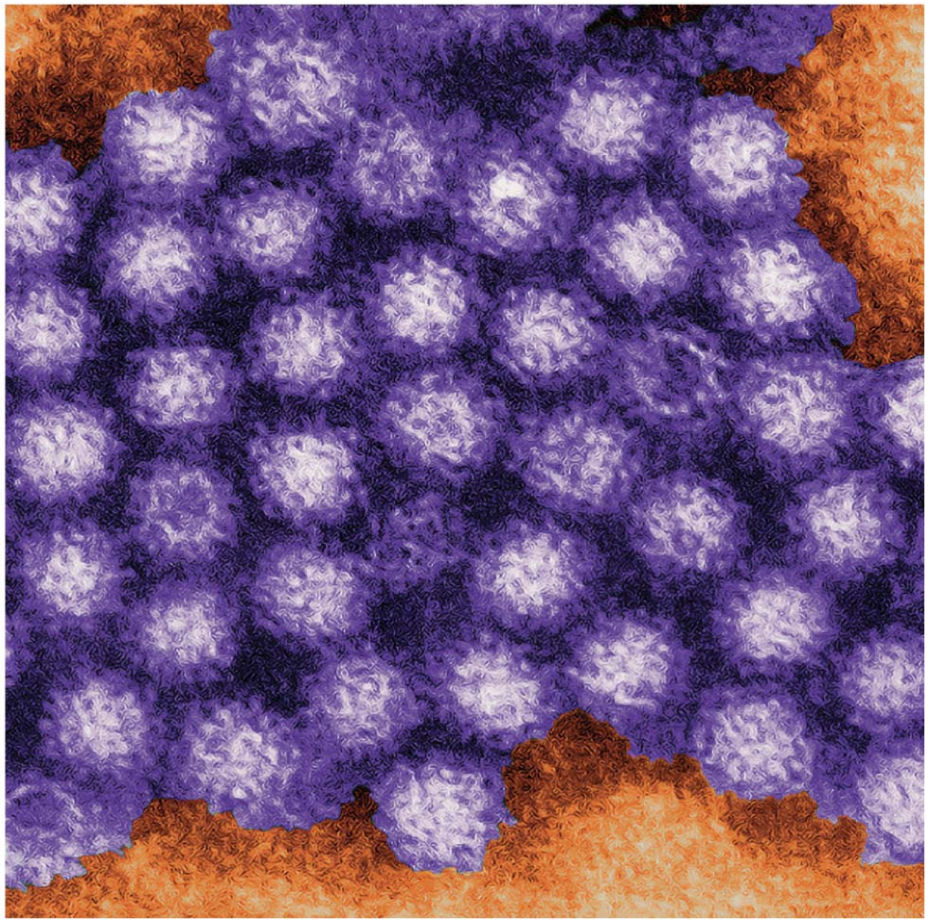
Chapter 7

The Control of Microbial Growth



The Terminology of Microbial Control

- **Sepsis** refers to microbial contamination / microbial growth
- **Asepsis** is the absence of significant contamination
- **Antisepsis**: removing pathogens from living tissue
- **Aseptic Surgery** - techniques that prevent microbial contamination of wounds

The Terminology of Microbial Control

- **Sterilization**: removing all microbial life // vegetative and spores

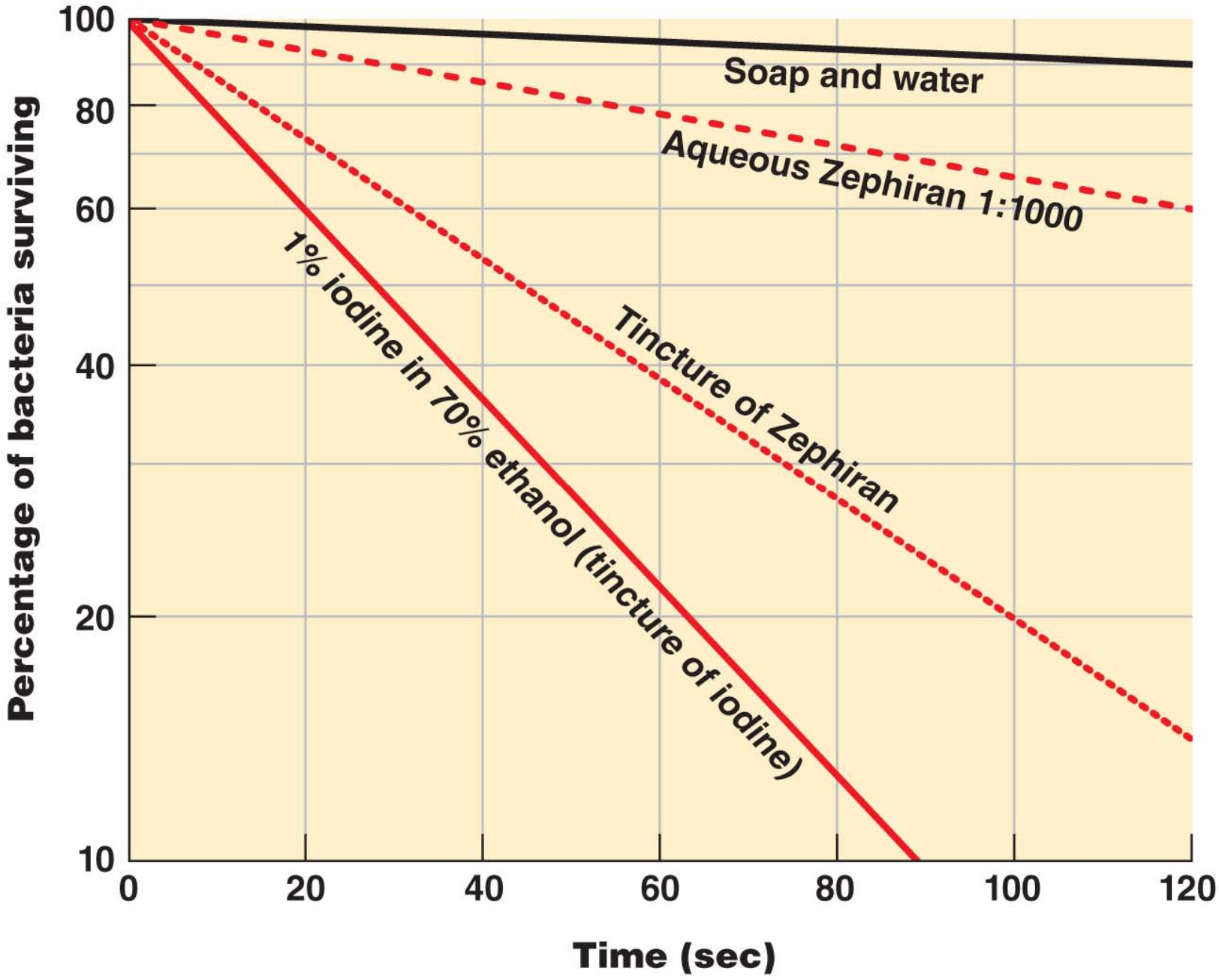
- **Commercial sterilization**: degree of sterilization to ensure killing *C. botulinum* endospores // more resistant microbes may still be viable but less likely to be pathogenic

- **Disinfection**: removing vegetative microbes / reduce number as in sanitize but does not sterilize! / does not eliminate not spores

The Terminology of Microbial Control

- **Degerming**: removing most microbes from a limited area
- **Sanitization**: lowering microbial counts on eating utensils // washing dishes is an example of sanitization
- **Biocide / germicide**: killing microbes
- **Bacteriostasis**: inhibiting but not killing, microbes

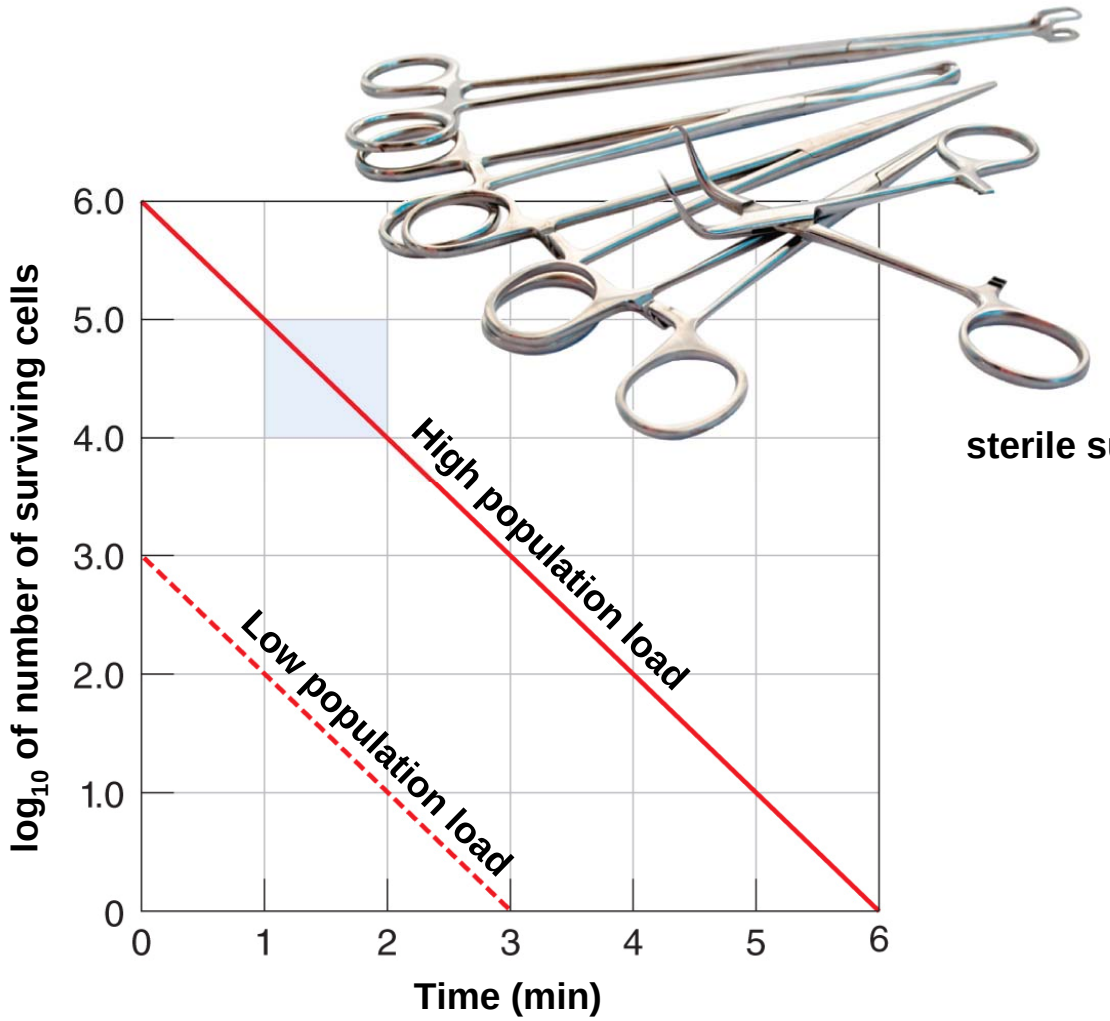
A comparison of the effectiveness of various antiseptics.



Effectiveness of Treatment

- Depends on:
  - Number of microbes present at start of treatment
  - Environment (organic matter, temperature, biofilms vs colony)
  - Time of exposure
  - Microbial characteristics

Understanding the Microbial Death Curve.



sterile surgical equipment

(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
- Damage to proteins
- Damage to nucleic acids

Physical Methods of Microbial Control Agents

- Heat
- Pasteurization
- Filtration
- Low Temperatures
- Desiccation
- Osmotic Pressure
- Radiation

Heat

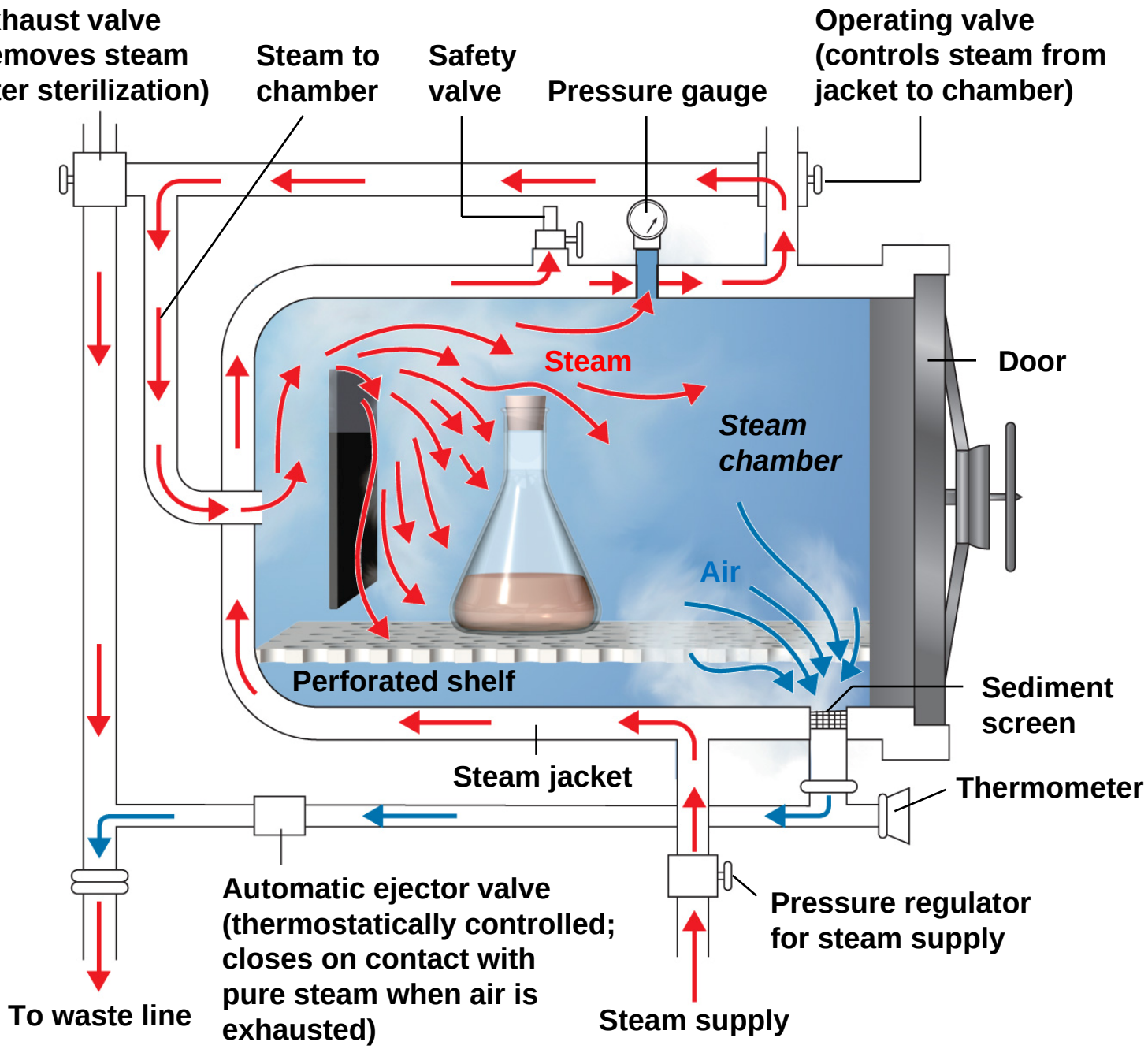
- **Thermal death point (TDP)**: lowest temperature at which all cells in a culture are killed in 10 min

- **Thermal death time (TDT)**: time during which all cells in a culture are killed

- Decimal Reduction Time (DTR) : Minutes to kill 90% of a population at a given temperature

An Autoclave

- Moist heat denatures proteins
- **Autoclave**: steam under pressure
- Steam must contact surface



Pasteurization

- Reduces spoilage by lowering the number of organisms and pathogens
- Does not sterilize the “food”
- Equivalent treatments
  - 63°C for 30 min
  - **High-temperature short-time**: 72°C for 15 sec
  - **Ultra-high-temperature**: 140°C for <1 sec
  - Thermoduric organisms survive

Used to Preserve Foods

- Nisin
- Potassium sorbate
- Sodium nitrite
- Calcium propionate

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

Requires high temperature and longer exposure

Physical Methods of Microbial Control

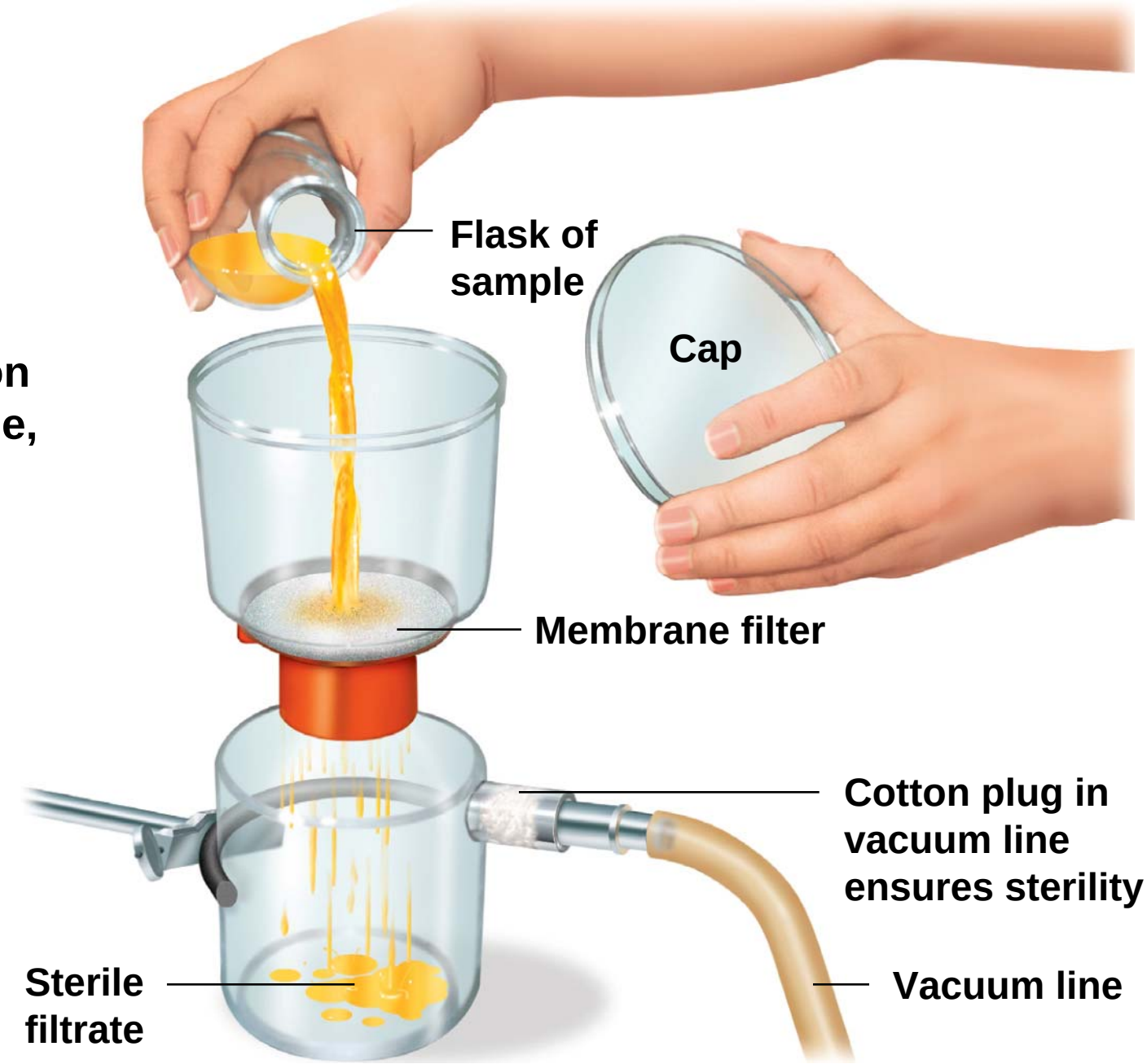
- **Low temperature** inhibits microbial growth
  - Refrigeration
  - Deep-freezing
  - Lyophilization
- **High pressure** denatures proteins
- **Desiccation** prevents metabolism
- **Osmotic pressure** causes plasmolysis

Examples of sterilization indicators.

Filtration

- **HEPA** removes microbes >0.3 µm
- **Membrane filtration** removes microbes >0.22 µm

Filter sterilization with a disposable, pre-sterilized plastic unit.



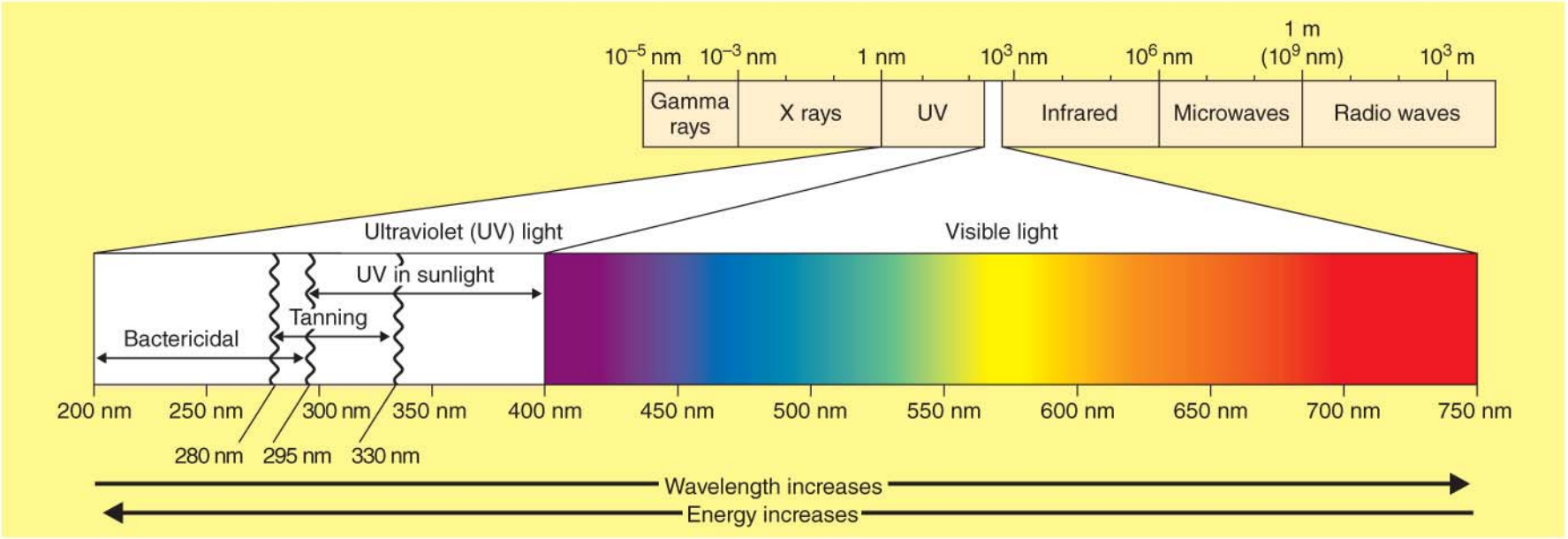
Radiation

- **Ionizing radiation** (X rays, gamma rays, electron beams) /// Ionizes water to release OH• // Damages DNA and other molecules

- **Nonionizing radiation** (UV, 260 nm) /// Damages DNA

- **Microwaves** kill by heat // not especially antimicrobial

The radiant energy spectrum.



Chemical Methods of Microbial Control Agents
Types of Disinfectants

- Phenols / Phenolics
- Bisphenols *
- Biguanides
- Halogens
- Alcohols *
- Heavy Metals
- Quaternary Ammonium Compounds (Quats) *
- Aldehydes
- Peroxygens
- Ethylene oxide *

Principles of Effective Disinfection

Important Variables

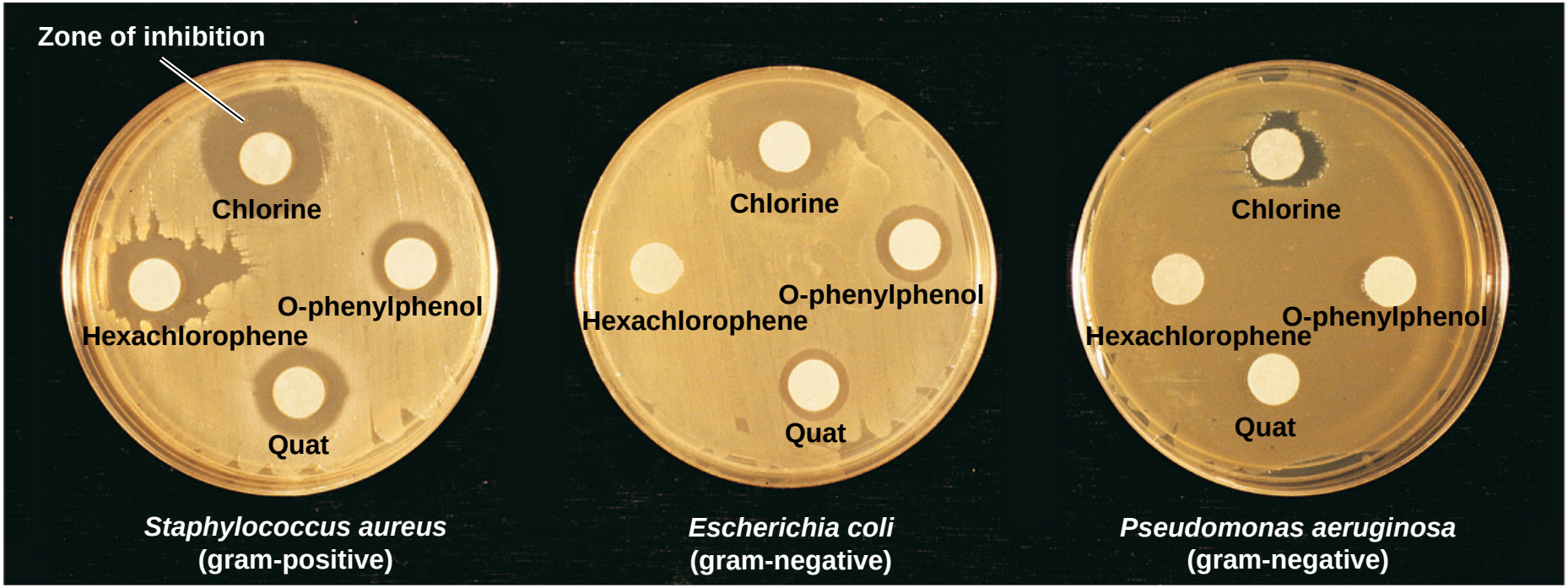
- Concentration of disinfectant
- Organic matter
- pH
- Time // Exposure

Note: the typical pattern of microbial death occurs within a population of bacteria at a constant rate

Effectiveness of Antimicrobial Agents // Use-Dilution Test

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

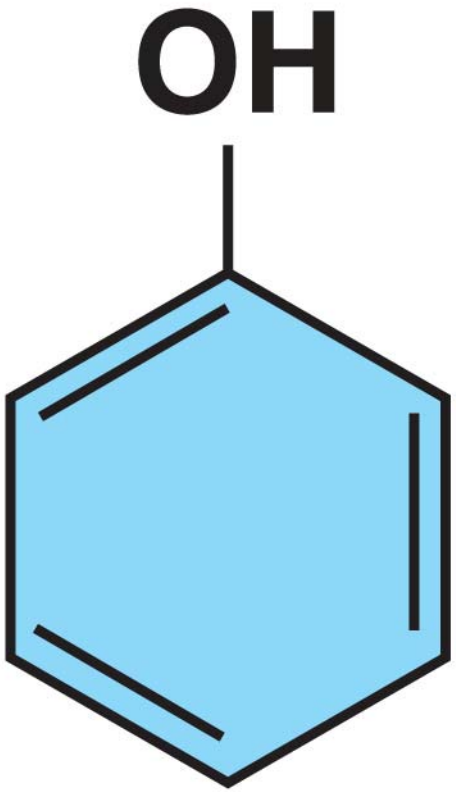
Effectiveness of Antimicrobial Agents // Disk-Diffusion Test



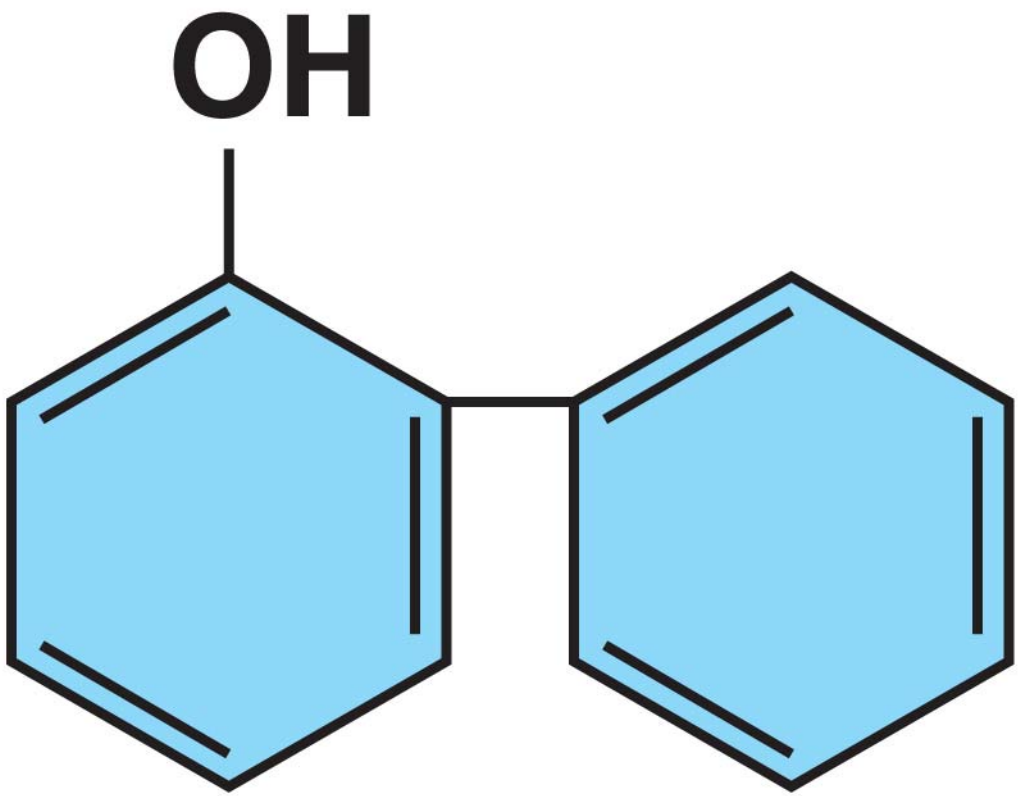
Phenol and Phenolics

- Disrupt plasma membranes
- Lister first used phenol in late 1800 to reduce infections during surgery
- Rarely used today
- Common phenolic still used is cresols
- Main ingredient of Lysol // good surface disinfectant

The structure of phenolics and bisphenols.



(a) Phenol

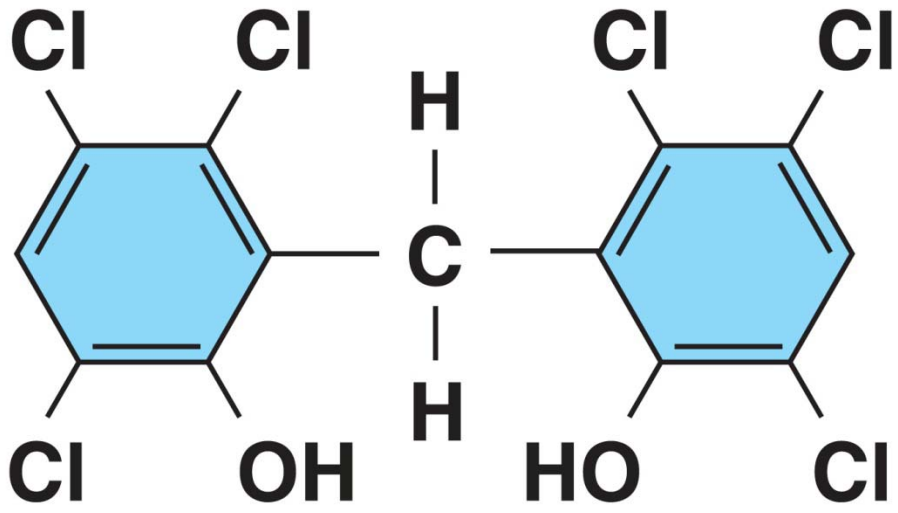


(b) O-phenylphenol

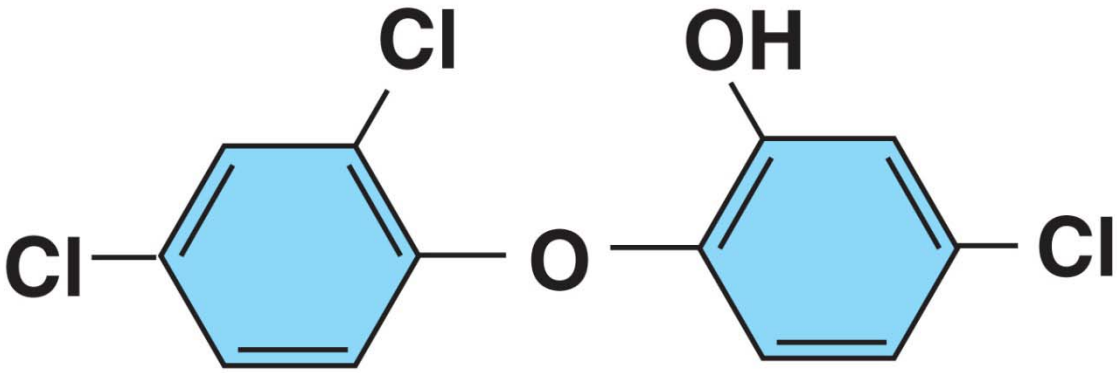
Bisphenols

- Hexachlorophene & triclosan // Disrupt plasma membranes
- Very effective against gram positive bacteria // staphylococci and streptococci
- Hexachlorophene ingredient in prescription lotion (pHisoHex) – used for surgical and hospital microbial control procedures
- Triclosan – used in antimicrobial soaps // even been incorporated into cutting boards!

The structure of phenolics and bisphenols.



(c) Hexachlorophene (a bisphenol)



(d) Triclosan (a bisphenol)

Biguanides

- Chlorhexidine // Disrupts plasma membranes
- Broad spectrum // best against gram positive bacteria

Halogens

- Iodine (I_2) – one of oldest and most effective antiseptics
  - **Tinctures**: in aqueous alcohol
  - **Iodophors**: in organic molecules
  - Alter protein synthesis and membranes
- Chlorine
  - Bleach: hypochlorous acid (HOCl)
  - Chloramine: chlorine + ammonia
  - Oxidizing agents

Alcohols

- Ethanol, isopropanol
  - Denature proteins & dissolve lipids
  - Require water // water needed to denature proteins!
  - 100% alcohol will not disinfect
  - Alcohol used on open wound will coagulate surface tissue and allow deeper tissue to harbor vegetative bacteria

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

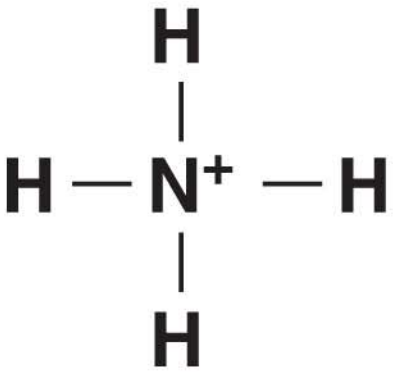
Note:
G = growth
NG = no growth

Heavy Metals

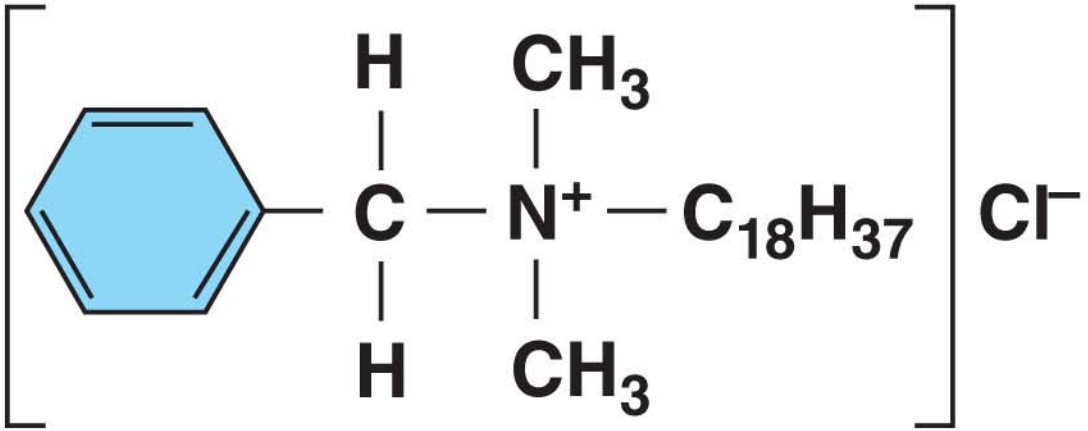
- Ag, Hg, and Cu
  - Silver nitrate may be used to prevent gonorrheal ophthalmia neonatorum
  - Silver sulfadiazine used as a topical cream on burns
  - Copper sulfate is an algicide
- Oligodynamic action
  - Denature proteins

Oligodynamic action of heavy metals.

The ammonium ion and a quaternary ammonium compound, benzalkonium chloride (Zephiran).



Ammonium ion



Benzalkonium chloride

Surface-Active Agents, or Surfactants

Soap	Degerming
Acid-anionic detergents	Sanitizing
Quaternary ammonium compounds (cationic detergents)	Bactericidal, denature proteins, disrupt plasma membrane

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

Aldehydes

- Inactivate proteins by cross-linking with functional groups ($–NH_2$, –OH, –COOH, –SH)
- Used to <u>disinfect medical equipment</u>
  - Glutaraldehyde, formaldehyde, and ortho-phthalaldehyde
  - Will not kill endospores!

Gaseous Sterilants

- Ethylene oxide
- Denature proteins
- Requires a closed chamber
- Kills all vegetative bacteria and endospores
- Used for sterilization of heat-sensitive material

Plasma

- Free radicals destroy microbes
- Use: tubular instruments

Supercritical Fluids

- CO_2 with gaseous and liquid properties
- Used with medical implants
- Used to decontaminate bone and tendons

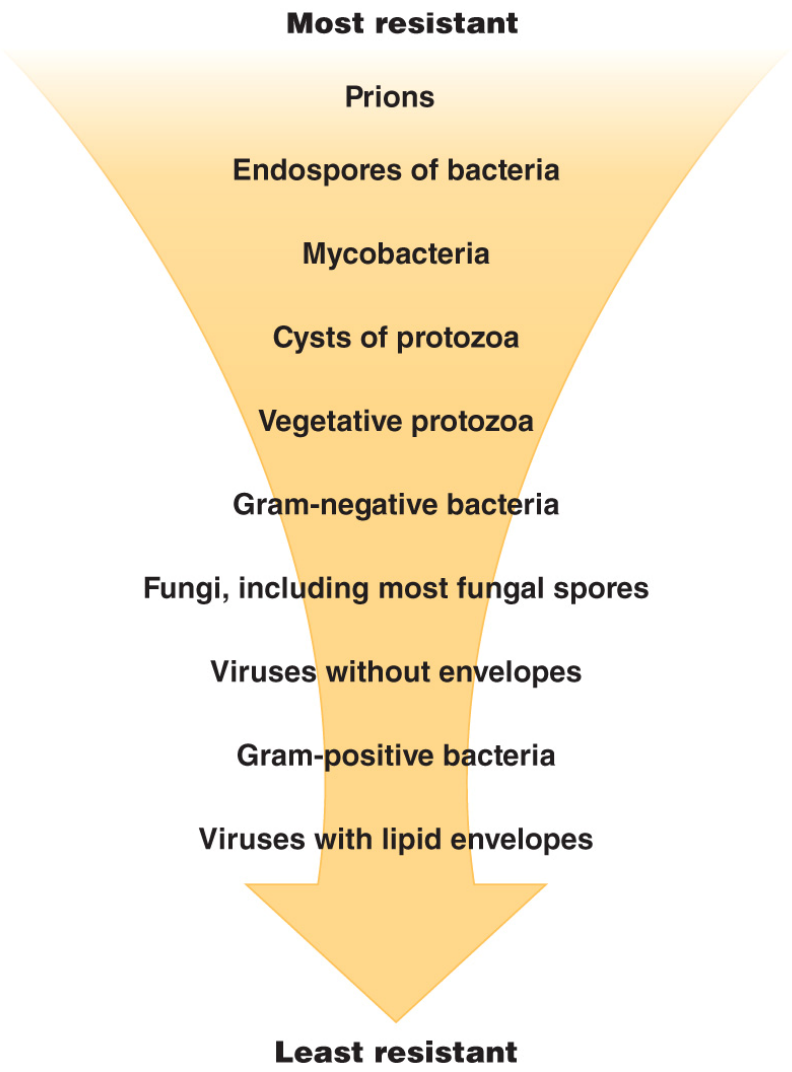
Peroxygens

- Oxidizing agents
- Useed on contaminated surfaces
  - O_3, H_2O_2, peracetic acid

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

Decreasing order of resistance of microorganisms to chemical biocides.



Note: boiling will not "deactivate" prions

Only incineration, proteases, or autoclaving with NaOH will deactivate prions