Ch 9

Physical and Chemical Control of Microbes
SLOs

• Define sterilization, disinfection, decontamination, antisepsis, degermation.
• List microbial agents from most resistant to least resistant to control.
• Compare the action of microbicidal and microbistatic agents.
• Name four categories of cellular targets for physical and chemical agents.
• Explain the various methods of physical control of microorganisms.
  ➢ Compare and contrast moist and dry heat sterilization methods.
  ➢ Define thermal death time and thermal death point.
  ➢ Identify advantages and disadvantages of cold and desiccation methods.
  ➢ Differentiate between the two modes of radiation control methods.
  ➢ Explain how filtration and osmotic pressure function as control methods.
• Name the desirable characteristics of chemical control agents.
• List and explain the chemical control agents covered in lecture:
  ➢ Halogens
  ➢ Phenolic compounds
  ➢ Alcohol
  ➢ Hydrogen peroxide
  ➢ Aldehydes and ethylene oxide
  ➢ Heavy metals
  ➢ Food preservatives
Controlling Microorganisms

• Relevance of controlling our exposure to potentially harmful microbes!

• Outside of body, four possible outcomes:
  - Sterilization
  - Disinfection
  - Antisepsis
  - Decontamination (also called sanitization)

Review Table 9.1
Microbial Control Methods

Physical agents
- Heat
  - Dry
    - Incineration
    - Dry oven
    - Sterilization
  - Moist
    - Steam under pressure
    - Boiling water, hot water, pasteurization
    - Sterilization
  - Radiation
    - Disinfection
  - Ionizing
    - X ray, cathode, gamma
    - Sterilization
  - Nonionizing
    - UV
    - Disinfection

Chemical agents
- Gases
  - Sterilization
  - Disinfection
  - On animate objects
    - Antisepsis
    - Disinfection
    - Sterilization
  - On inanimate objects
    - Decontamination
    - Sanitization

Mechanical removal methods
- Filtration
  - Air
  - Liquids
  - Sterilization

Disinfection: The destruction or removal of vegetative pathogens but not bacterial endospores. Usually used only on inanimate objects.

Sterilization: The complete removal or destruction of all viable microorganisms. Used on inanimate objects.

Antisepsis/Degermation: Chemicals applied to body surfaces to destroy or inhibit vegetative pathogens.

Decontamination/Sanitization: The mechanical removal of most microbes.
Relative Resistance of Microbial Forms

- **1° target**: Microbial agents capable of causing infection or spoilage
  - Often mixtures of microbes with extreme differences in resistance and harmfulness.

- Goal of sterilization is destruction of ESs

Diagram:

- Most resistant
  - Prions
  - Endospores of bacteria
  - Mycobacteria
  - Cysts of protozoa
  - Vegetative protozoa
  - Gram-negative bacteria
  - Fungi, including most fungal spores
  - Viruses without envelopes
  - Gram-positive bacteria
  - Viruses with lipid envelopes

Least resistant
Fig 9.2
Table 9.2 Comparative Resistance of Bacterial Endospores and Vegetative Cells to Control Agents

<table>
<thead>
<tr>
<th>Method</th>
<th>Endospores</th>
<th>Vegetative Forms</th>
<th>Endospores Are ___× More Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat (moist)</td>
<td>120°C</td>
<td>80°C</td>
<td>1.53</td>
</tr>
<tr>
<td>Radiation (X-ray) dosage</td>
<td>4,000 Grays</td>
<td>1,000 Grays</td>
<td>43</td>
</tr>
<tr>
<td>Sterilizing gas</td>
<td>1,200 mg/L</td>
<td>700 mg/L</td>
<td>1.73</td>
</tr>
<tr>
<td>(ethylene oxide)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sporicidal liquid</td>
<td>3 h</td>
<td>10 min</td>
<td>183</td>
</tr>
<tr>
<td>(2% glutaraldehyde)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Agents vs. Processes - Vocabulary

• Sterilization and disinfection: processes

• -cide = able to kill: Bactericide, fungicide, virucide, sporicide, germicide/microbicide

• -static = stand still: Bacteriostatic ....

• Disinfectants vs. antiseptics

• Sepsis: the growth of microorganisms in the blood and other tissues

• Asepsis: prevents the entry of infectious agents into sterile tissues.

Aseptic techniques
Practical Matters in Microbial Control

• Critical medical devices:
  – Expected to come into contact with sterile tissues, ⇒ must be ______________

• Semicritical devices:
  – Come into contact with Mucosa, ⇒ high-level disinfection

• Noncritical devices:
  – Only touch intact skin ⇒ low-level disinfection
What is Microbial Death?

• Permanent termination of all vital processes:
  "For microbes = Permanent loss of reproductive capability."

• **Death rate is logarithmic.** *(not all at once!!)*
  Active die more quickly than less metabolically active cells.

• **Factors Affecting Death Rate:**
  • # of microbes
  • Types of the microbial agents
  • Temperature and pH of environment
Factors Affecting Death Rate, cont.

- Concentration (dose, intensity) of agent:
  - UV radiation is most effective at 260 nm
  - Most disinfectants are more active at higher concentrations.

- Mode of action of the agent

- Presence of solvents and interfering organic matter: Saliva, blood, feces can inhibit action of disinfectants and action of heat
Rate of Microbial Death

Bacterial populations subjected to heat or antimicrobial chemicals die at a constant rate.

Microbial Death Curve, plotted logarithmically, shows this constant death rate as a straight line.
Modes of Action of Antimicrobial Agents

- **Least selective** agents tend to be effective against the widest range of microbes: **Heat and radiation**.

- **Selective** agents target only a single cellular component: **Drugs**.

**Cellular Targets:**

- Cell wall
- Membrane and its permeability
- Cellular synthetic processes (NA)
- Proteins

**Check your understanding:**
Would a chemical microbial agent that affects plasma membranes affect humans?
Methods of Physical Control: Heat

- High temp.: Microbicidal
- Low temp.: Microbistatic
- Moist heat vs. dry heat:
- Heat ____________ proteins

Table 9.4 Comparison of Times and Temperatures to Achieve Sterilization with Moist and Dry Heat

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Moist heat</th>
<th>Dry heat</th>
</tr>
</thead>
<tbody>
<tr>
<td>121</td>
<td>15</td>
<td>600</td>
</tr>
<tr>
<td>125</td>
<td>10</td>
<td>180</td>
</tr>
<tr>
<td>134</td>
<td>3</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
</tr>
</tbody>
</table>
Moist Heat Sterilization: Autoclaving

- **Autoclave**: Steam under pressure
- Most dependable sterilization method
- **Steam must directly contact** material to be sterilized.
- Pressurized steam reaches higher temperatures.
- Normal autoclave conditions at 15 psi: 
  ______ °C for ___ min.
- **Prion destruction**: 
  132°C for 4.5 hours
- Limitations of the autoclave
Thermal Death Measurements

• **Thermal death time** (TDT): shortest length of time required to kill all test microbes at a specified temperature

• **Thermal death point** (TDP): lowest temperature required to kill all microbes in a sample in 10 minutes

• What microbial agents are most heat resistant?
**Pasteurization**

- Significant number reduction (esp. spoilage and pathogenic organisms) → does not sterilize!
- Historical goal: destruction of *M. tuberculosis*
- **Classic holding (batch) method:** 63°C for 30 min
- **Flash pasteurization (HTST):** 72°C for 15 sec. Most common in US. Thermoduric organisms survive
- **Ultra High Temperature (UHT):**
  - 134°C for 1-2 secs
  - 140°C for < 1 sec.
  - Technically not pasteurization because it sterilizes.
Dry Heat Sterilization Kills by Oxidation

- Flaming of loop
- Incineration of carcasses
  - Anthrax
  - Foot and mouth disease
  - Bird flu
- Hot-air sterilization

<table>
<thead>
<tr>
<th>Equivalent treatments</th>
<th>Hot-air</th>
<th>Autoclave</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>170°C, 2 hr</td>
<td>121°C, 15 min</td>
</tr>
</tbody>
</table>

Hot-air Autoclave Equivalent treatments 170°C, 2 hr 121°C, 15 min
Effects of Cold and Desiccation

- Slows enzymatic reactions ⇒ inhibits microbial growth.
- When storing food in refrigerator, watch out for ________________!
- Freezing, used for long term storage of microbial agents, however....... 
- Deep freezing (~−80°C) preserves bacteria, viruses, and fungi for long periods.
- Desiccation = _______________. Some microbes are killed by desiccation; on others desiccation has no effect.
- Lyophilization: ____________________________
  For long term storage: Pure cultures frozen instantaneously, then exposed to vacuum to remove water (advantage?)
Radiation

- Energy emitted from atomic activities:
  - Gamma rays
  - X rays
  - Ultraviolet radiation

![Fig. 9.4](image-url)
Ionizing Radiation

• X-rays, $\gamma$-rays have short wave length → dislodge e- from atoms → production of free radicals and other highly reactive molecules

• Used for sterilization of heat sensitive materials: drugs, vitamins, herbs, suture material

• Also as “cold pasteurization” of food ⇒ Consumer fears!? 
Effect: thymine dimers

- Actively dividing organisms are more sensitive because thymine dimers cause ______________?

- Used to fight air and surface contamination. Only kills at close range and directly exposed microbial agents

  - E.g.: germicidal lamps in OR, cafeteria, and our lab ??
Filtration

- Effective to remove microbes from **air and liquids**
- **Membrane filters** for **fluids** (e.g. cellulose acetate).
  - Pore size for bacteria: 0.2 – 0.4 μm
  - Pore size for viruses: 0.01 μm

- **Charcoal**, diatomaceous earth, unglazed porcelain

- Sterilization possible

- Used for?

  - Unable to remove soluble molecules (toxins)

- **Air filtration** using **high efficiency particulate air (HEPA) filters**.
  Effective to 0.3 μm

- HEPA filters used in hospital rooms and sterile rooms.
Osmotic Pressure

- What happens when adding large amounts of salt or sugar to foods?
- Pickling, smoking, and drying foods used for centuries to preserve foods.
- Osmotic pressure is never a sterilizing technique.
Chemical Agents in Microbial Control

- Occur in the liquid, gaseous, or solid state
- Range from disinfectants and antiseptics to sterilants and preservatives
  - **Disinfectants** regulated by EPA
  - **Antiseptics** regulated by FDA
- **Aqueous solutions**: chemicals dissolved in pure water as the solvent
- **Tinctures**: chemicals dissolved in pure alcohol or water-alcohol mixtures
- Few chemical agents achieve sterility
Selecting a Microbicidal Chemical

1. Rapid action, even in low concentrations
2. Solubility and stability
3. Broad-spectrum action without toxicity
4. Penetration of inanimate surfaces to sustain persistent action
5. Resistance to inactivation by organic matter
6. Affordability and ready availability

No chemical can completely fulfill all requirements: **Glutaraldehyde** and **hydrogen peroxide** approach this ideal.
Levels of Chemical Decontamination

- **High-level germicides**
  - kill endospores
  - used as sterilants for heat sensitive devices

- **Intermediate-level germicides**
  - kill fungal spores, *M. tuberculosis*, and viruses

- **Low-level germicides**
  - eliminate only vegetative bacteria, vegetative fungal cells, and some viruses.
  - Used for cleaning surfaces that touch skin but not mm
Factors Affecting the Germicidal Activity of Chemicals

• **Type of microbial agent** being treated

• **Composition of material being treated**
  - Smooth, solid objects are more reliably disinfected than those with pores or pockets.
  - Organic material can hinder penetration ⇒ adequate cleaning must precede the use of a germicide or sterilant!

• **Degree of contamination**

• **Time of exposure:** Most compounds require adequate contact time to allow the chemical to penetrate and act on microbes present.

• **Strength and chemical action** of germicide
Factors Affecting the Germicidal Activity of Chemicals (cont’d)

• Chemical Strength or Concentration
  - *Dilutions*: a small volume of the liquid chemical is diluted in a larger volume of solvent to achieve a certain ratio
  - *Parts per million*: used for solutions such as chlorine that are effective in very diluted concentrations
  - *Percentage solutions*: solute is added to water by weight or volume
Modes of action affecting protein function  

Fig 9.7

- **Native State**
  - Substrate
  - Enzyme

- **Different Shape**
- **Complete Denaturation**

- **Blocked Active Site**
  - Agents

Active site can no longer accept the substrate, and the enzyme is inactive.
# Germicidal Chemical Categories

Compare the following 6 slides to your Table 9.9

## Table 9.9 Germicidal Categories According to Chemical Group

Techniques and chemicals that are capable of sterilizing are highlighted with a pink background.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target Microbes</th>
<th>Form(s)</th>
<th>Mode of Action</th>
<th>Indications for Use</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Halogens: chlorine</td>
<td>Can kill endospores (slowly); all other microbes</td>
<td>Liquid/gaseous chlorine (Cl₂), hypochlorites (OCl⁻), chloramines (NH₂Cl)</td>
<td>In solution, these compounds combine with water and release hypochlorous acid (HOCI); denature enzymes permanently and suspend metabolic reactions</td>
<td>Chlorine kills bacteria, endospores, fungi, and viruses; gaseous/liquid chlorine used to disinfect drinking water, sewage and waste water; hypochlorites used in healthcare to treat wounds, disinfect bedding and instruments, sanitize food equipment and in restaurants, pools, and spas; chloramines alternate to pure chlorine in treating drinking water; also used to treat wounds and skin surfaces</td>
<td>Less effective if exposed to light, alkaline pH, and excess organic matter</td>
</tr>
<tr>
<td>Halogens: iodine</td>
<td>Can kill endospores (slowly); all other microbes</td>
<td>Free iodine in solution (I₂) lodothors (complexes of iodine and alcohol)</td>
<td>Penetrates cells of microorganisms where it interferes with a variety of metabolic functions; interferes with the hydrogen and disulfide bonds</td>
<td>2% iodine, 2.4% sodium iodide (aqueous iodine) used as a topical antiseptic 5% iodine, 10% potassium iodide used as a disinfectant for plastic and rubber instruments</td>
<td>Can be extremely irritating to the skin and is toxic when absorbed</td>
</tr>
</tbody>
</table>
Halogen

Chlorine
- Oxidizing agent
- Widely used as disinfectant
- Forms **bleach** (hypochlorous acid) when added to water.
- Broad spectrum, sporicidal (pools, drinking water)

Iodine
More reactive, more germicidal. Alters protein synthesis and membranes.

**Tincture of iodine** (solution with alcohol) → wound antiseptic

Hydrogen Peroxide: Oxidizing agent

Inactivated by catalase ⇒

Not good for open wounds

Good for inanimate objects; packaging for food industry (containers etc.)

3% solution (higher conc. available)

Especially effective against anaerobic bacteria (e.g.:

Effervescent action, may be useful for wound cleansing through removal of tissue debris
Aldehydes (alkylating agents)

- **Inactivate proteins** by cross-linking with functional groups (–NH₂, –OH, –COOH, –SH)

- **Formaldehyde**:  
  - Embalming ⇒ Formalin  
  - Virus inactivation for vaccines

- **Glutaraldehyde**: Liquid Sterilant for delicate surgical instruments (Kills *S. aureus* in 5, *M. tuberculosis* in 10 min, ES in 3 – 10h)

- **Ethylene oxide**: Gaseous Sterilant
Phenol and Phenolic Compounds

- Phenol = carbolic acid (historic importance)
- Who used first?
- Many derivatives today:
  - Phenolics, *e.g.*: Lysol
  - Bisphenols, *e.g.:
    - Hexachlorophene (in pHisoHex used in hospitals)
    - Triclosan (toothpaste, antibacterial soaps, etc.)

Phenol and derivatives disrupt plasma membranes (lipids!) and lipid rich cell walls (???)

Remain active in presence of organic compounds
• **Ethyl** (60 – 80% solutions) and **isopropyl alcohol**

• Denature proteins, dissolve lipids

• No activity against spores and poorly effective against viruses and fungi

• Easily inactivated by organic debris

• Also used in hand sanitizers and cosmetics

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

<table>
<thead>
<tr>
<th>Concentration of Ethanol (%)</th>
<th>Time of Exposure (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>100</td>
<td>G</td>
</tr>
<tr>
<td>95</td>
<td>NG</td>
</tr>
<tr>
<td>90</td>
<td>NG</td>
</tr>
<tr>
<td>80</td>
<td>NG</td>
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<td>70</td>
<td>NG</td>
</tr>
<tr>
<td>60</td>
<td>NG</td>
</tr>
<tr>
<td>50</td>
<td>G</td>
</tr>
<tr>
<td>40</td>
<td>G</td>
</tr>
</tbody>
</table>

**Note:**

G = growth
NG = no growth

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**Heavy Metals**

**Oligodynamic action**: toxic effect due to metal ions combining with sulfhydryl (—SH) and other functional groups ⇒ proteins are denatured.

- **Silver** (1% AgNO₃): Antiseptic for eyes of newborns

- **Copper** against chlorophyll containing organisms → **Algicides**; also X-gel **hand sanitizer**

- **Zinc** (ZnCl₂) in mouthwashes, ZnO as antifungal in paint
Sodium nitrate and nitrite prevent endospore germination. Prevents ES germination. Used in meats. Conversion to nitrosamines: Carcinogenic!

Organic acids
Inhibit metabolism
E.g.: Sorbic acid, benzoic acid, etc.
In foods and cosmetics

Sulfur dioxide
In wine
Soaps and Detergents
Major purpose is mechanical removal and use as wetting agent to reduce surface tension and make antiseptic or disinfectant more effective in spreading over and penetrating surfaces.
Case File: Preparing the Skin

Inside the Clinic: Fresh Air and Sunshine: Low-Tech is Cutting Edge Again.