9

Controlling Microbial Growth in the Environment

Terminology of Microbial Control

- Sterilization
- Aseptic
- Disinfection/disinfectants
- Antisepsis/antiseptic
- Degerming

Sanitization
- Pasteurization
- Suffix – stasis/static
- Suffix – cide/cidal
**Microbial Death Rates**

![Graph showing microbial death rates over time.](image)

*Figure 9.1*

**Action of Antimicrobial Agents**

- Many types of chemical and physical microbial controls
- Modes of action fall into two basic categories
  - Alteration of cell walls or cytoplasmic membranes
  - Interference with protein and nucleic acid structure

**Alteration of Cell Walls and Membranes**

- Cell wall maintains integrity of cell
  - When disrupted, cannot prevent cell from bursting due to osmotic effects
- Cytoplasmic membrane contains cytoplasm and controls passage of chemicals into and out of cell
  - When damaged, cellular contents leak out
- Viral envelope responsible for attachment of virus to target cell
  - Damage to envelope interrupts viral replication
- Nonenveloped viruses have greater tolerance of harsh conditions
**Damage to Proteins and Nucleic Acids**

- Protein function depends on 3-D shape
  - Extreme heat or certain chemicals denature proteins
- Chemicals, radiation, and heat can alter or destroy nucleic acids
  - Can produce fatal mutants
  - Can halt protein synthesis through action on RNA

**Selection of Microbial Control Methods**

- Ideally, agents should be:
  - Inexpensive
  - Fast-acting
  - Stable during storage
  - Control all microbial growth while being harmless to humans, animals, and objects

**Factors Affecting the Efficacy of Antimicrobial methods**

- Nature of site to be treated
- Degree of susceptibility of microbes involved
- Environmental conditions that pertain
Site to Be Treated

- Harsh chemicals and extreme heat cannot be used on humans, animals, and fragile objects
- Method and level of microbial control based on site of medical procedure

Relative Susceptibility of Microorganisms

**Most susceptible**
- Enveloped viruses
- Gram-positive bacteria
- Nonenveloped viruses
- Fungi
- Gram-negative bacteria
- Active stage protozoa (trophozoites)
- Cysts of protozoa
- Mycobacteria
- Bacterial endospores

**Most resistant**

Effectiveness of germicides classified as high, intermediate, or low

- High-level kill all pathogens, including endospores
- Intermediate-level kill fungal spores, protozoan cysts, viruses and pathogenic bacteria
- Low-level germicides kill vegetative bacteria, fungi, protozoa, and some viruses
Environmental Conditions

![Graph showing the number of living microbes over time at different temperatures (45°C and 20°C).](image)

Figure 9.3

Methods for Evaluating Disinfectants and Antiseptics

- Phenol coefficient
- Use-dilution test
- In-use test

Phenol Coefficient

- Evaluating the efficacy of disinfectants and antiseptics by determining the ratio of agent’s ability to control microbes to that of phenol
- Greater than 1.0 indicates that agent is more effective than phenol
- Has been replaced by newer methods
**Use-Dilution Test**

- Metal cylinders dipped into broth cultures of bacteria and dried
- Contaminated cylinder immersed into dilution of disinfectant for 10 minutes
- Cylinders removed, washed, and placed into tube of medium for 48 h
- Most effective agent entirely prevents growth at highest dilution
- New standard procedure being developed

**In-Use Test**

- Swabs taken from objects before and after application of disinfectant or antiseptic
- Swabs inoculated into growth medium and incubated
- Medium monitored for growth
- Accurate determination of proper strength and application procedure for each specific situation

**Physical Methods of Microbial Control**

- Exposure to extremes of heat
- Exposure to extremes of cold
- Desiccation
- Filtration
- Osmotic pressure
- Radiation
### Heat-Related Methods

- Effects of high temperatures
  - Denaturation of proteins
  - Interference with integrity of cytoplasmic membrane and cell walls
  - Disruption of structure and function of nucleic acids
- Thermal death point – lowest temperature that kills all cells in broth in 10 minutes
- Thermal death time – time to sterilize volume of liquid at set temperature

### Moist Heat

- Used to disinfect, sanitize, and sterilize
- Kills by denaturing proteins and destroying cytoplasmic membranes
- More effective than dry heat; water better conductor of heat than air
- Methods of microbial control using moist heat
  - Boiling
  - Autoclaving
  - Pasteurization
  - Ultrahigh-Temperature Sterilization

### Boiling

- Kills vegetative cells of bacteria and fungi, protozoan trophozoites, and most viruses within 10 minutes at sea level
- Temperature cannot exceed 100°C at sea level; steam carries some heat away
- Boiling time is critical
- Water boils at lower temperatures at higher elevations; requires longer boiling time
- Endospores, protozoan cysts, and some viruses can survive boiling
**Autoclaving**

- Pressure applied to boiling water prevents steam from escaping
- Boiling temperature increases as pressure increases
- Autoclave conditions – 121ºC, 15 psi, 15 minutes

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**Autoclave**

![Autoclave diagram](image)

**Figure 9.6a**

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**Autoclave**

![Autoclave diagram with labels](image)

**Figure 9.6b**
**Pasteurization**

- Pasteur’s method
- Today, also used for milk, ice cream, yogurt, and fruit juices
- Not sterilization; heat-tolerant and heat-loving microbes survive
  - These do not cause spoilage prior to consumption
  - These are generally not pathogenic

**Pasteurization**

- Milk
  - Batch method – 30 minutes at 63°C
  - Flash pasteurization – 72°C for 15 seconds
  - Ultrahigh-temperature pasteurization – 134°C for 1 second

**Ultrahigh-Temperature Sterilization**

- 140°C for 1 second, then rapid cooling
- Treated liquids can be stored at room temperature
Dry Heat

- Used for materials that cannot be sterilized with or are damaged by moist heat
- Denatures proteins and oxidizes metabolic and structural chemicals
- Requires higher temperatures for longer time than moist heat
- Incineration – ultimate means of sterilization

Refrigeration and Freezing

- Decrease microbial metabolism, growth, and reproduction
  - Chemical reactions occur slower at low temperatures
  - Liquid water not available
- Psychrophilic microbes can multiply in refrigerated foods
- Refrigeration halts growth of most pathogens
- Slow freezing more effective than quick freezing
- Organisms vary in susceptibility to freezing

Desiccation and Lyophilization

- Drying inhibits growth due to removal of water; only microbiostatic
- Lyophilization used for long term preservation of microbial cultures
  - Prevents formation of damaging ice crystals
Filtration

- Nonsterile medium
- Membrane filter
- To vacuum pump
- Sterile medium

**Figure 9.9a**

Filtration

**Figure 9.9b**

Osmotic Pressure

- High concentrations of salt or sugar in foods to inhibit growth
- Cells in a hypertonic solution of salt or sugar lose water; cell desiccates
- Fungi have greater ability than bacteria to survive hypertonic environments
Radiation

- Shorter wavelength equals more energy and greater penetration
- Radiation described as ionizing or nonionizing according to effects on cellular chemicals

Ionizing Radiation

- Wavelengths shorter than 1 nm – electron beams, gamma rays, and X rays
- Eject electrons from atoms to create ions
- Ions disrupt hydrogen bonding, oxidize double covalent bonds, and create hydroxide ions; hydroxide ions denature other molecules (DNA)

Ionizing Radiation

- Electron beams – effective at killing but do not penetrate well
  - Used to sterilize spices, meats, microbiological plastic ware, and medical and dental supplies
- Gamma rays – penetrate well but require hours to kill microbes
  - Used to sterilize meats, spices, and fresh fruits and vegetables
- X-rays require too much time to be practical for growth control
Nonionizing Radiation

- Wavelengths greater than 1 nm
- Excites electrons and causes them to make new covalent bonds
  - Affects 3-D structure of proteins and nucleic acids
- UV light causes pyrimidine dimers in DNA
- UV light does not penetrate well
- Suitable for disinfecting air, transparent fluids, and surfaces of objects

Chemical Methods of Microbial Control

- Affect microbes’ cell walls, cytoplasmic membranes, proteins, or DNA
- Effect varies with temperature, length of exposure, and amount of organic matter
- Also varies with pH, concentration, and age of chemical
- Tend to be more effective against enveloped viruses and vegetative cells of bacteria, fungi, and protozoa

Chemical Methods of Microbial Control

- Major Categories
  - Phenols
  - Alcohols
  - Halogens
  - Oxidizing agents
  - Surfactants
  - Heavy Metals
  - Aldehydes
  - Gaseous Agents
  - Antimicrobics
Phenol and Phenolics

- Intermediate- to low-level disinfectants
- Denature proteins and disrupt cell membranes
- Effective in presence of organic matter and remain active for prolonged time
- Commonly used in health care settings, labs, and homes (Lysol, triclosan)
- Have disagreeable odor and possible side effects
**Phenol coefficient**

- The phenol coefficient sets the chemical phenol (very nasty stuff in its pure form) as equal to 1.0 when used as a control agent against S. chloraeuis, S. aureus and P. aeruginosa.
- The time required to kill these bacteria by using dilutions of the test disinfects, compared to dilution of phenol, give a relative strength of the test disinfectant.
- Thus an agent with a phenol coefficient of 3.0 is three times as efficient as phenol as a control agent.
- Flaw, some chemical agents do not tolerate dilution well. Example: alcohol at 80% is a superior disinfectant, but a low concentration, it has a lower phenol coefficient than QUATS.

**Dilution method**

- The same bacteria in the phenol coefficient test that are dried on a small steel penicylinders are left in tubes containing dilution of the test disinfectant for 10 min. Microbes on the steel cylinders are cultured to determine survival.
- Demonstration of killing of 59/60 replicates for each organism on three different lots of product meets FDA and EPA criteria for hospital disinfectants.
- A key feature of the use dilution test is that it can allow determination of whether the agent killed the bacteria (bactericidal) or did not kill them but did not let them grow (bacteriostatic) - this is an important bit of information.

**Alcohols**

- Intermediate-level disinfectants
- Denature proteins and disrupt cytoplasmic membranes
- Evaporate rapidly – both advantageous and disadvantageous
- Swabbing of skin with 70% ethanol prior to injection.
### Halogens

- Intermediate-level antimicrobial chemicals
- Believed that they damage enzymes via oxidation or by denaturing them
- Iodine tablets, iodophores (Betadine®), chlorine treatment of drinking water, bleach, chloramines in wound dressings, and bromine disinfection of hot tubs

### Oxidizing Agents

- Peroxides, ozone, and peracetic acid kill by oxidation of microbial enzymes
- High-level disinfectants and antiseptics
- Hydrogen peroxide can disinfect and sterilize surfaces of objects
  - Catalase neutralizes; not useful for treating open wounds
- Ozone treatment of drinking water
- Peracetic acid – effective sporocide used to sterilize equipment

### Surfactants

- “Surface active” chemicals that reduce surface tension of solvents to make them more effective at dissolving solutes
- Soaps and detergents
  - Soaps have hydrophilic and hydrophobic ends; good degreasing agents but not antimicrobial
  - Detergents are positively charged organic surfactants
- Quats – colorless, tasteless, harmless to humans, and antimicrobial; ideal for many medical and industrial application
  - Low-level disinfectants
Heavy Metals

- Ions are antimicrobial because they alter the 3-D shape of proteins, inhibiting or eliminating their function
- Low-level bacteriostatic and fungistatic agents
- 1% silver nitrate to prevent blindness caused by *N. gonorrhoeae*
- Thimerosal used to preserve vaccines
- Copper controls algal growth in reservoirs, fish tanks, swimming pools, and water storage tanks; interferes with chlorophyll

Aldehydes

- Compounds containing terminal –CHO groups
- Cross-link with amino, hydroxyl, sulfhydryl, and carboxyl groups to denature proteins and inactivate nucleic acids
- Glutaraldehyde both disinfects (short exposure) and sterilizes (long exposure)
- Formalin used in embalming and disinfection of rooms and instruments

Gaseous Agents

- Ethylene oxide, propylene oxide, and beta-propiolactone used in closed chambers to sterilize items
- Denature proteins and DNA by cross-linking functional groups
- Used in hospitals and dental offices
- Can be hazardous to people, often highly explosive, extremely poisonous, and are potentially carcinogenic
Antimicrobials

- Antibiotics, semisynthetic, and synthetic chemicals
- Typically used for treatment of disease
- Some are used for antimicrobial control outside the body

Development of Resistant Microbes

- Little evidence that extensive use of products containing antiseptic and disinfecting chemicals adds to human or animal health
- The use of such products promotes the development of resistant microbes

Potency of antimicrobial chemical agents

1. **Sterilants** destroy everything, including endospores
   - for sterilizing scalpels, respiratory therapy equipment, proctoscopes, plastic Petri dishes, endoscopes
     - e.g. gluteraldehde, hydrogen peroxide
2. **High level** disinfectants do not reliably destroy endospores
   - e.g. iodine, phenol, chlorhexidine, heavy metals such as silver nitrate
3. **Intermediate level** disinfectants will kill *Mycobacterium*, but do not destroy all viruses or endospores, even with prolonged exposure
   - e.g. alcohols: ethyl alcohol, isopropyl
4. **Low level** disinfectants will not kill *Mycobacterium*
   - e.g. soaps, detergents