# Chapter 9 Control Microbial Growth

## I. Definition And General Terms

### General considerations for effective control

A. Sterilization

1. the process of killing (or removing) all microorganisms on an object or in a material (e.g., liquid media).

B. Disinfection

1. the process of reducing the numbers of or inhibiting the growth of microorganisms, especially pathogens, to the point where they no longer pose a threat of disease.

C. Disinfectant

1. a chemical agent used to destroy microorganisms on inanimate objects such as dishes, tables, and floors. Disinfectants are not safe for living tissues.

D. Antiseptic

1. a chemical agent that can be administered safely to external body surfaces or mucous membranes to decrease microbial numbers. Antiseptics cannot be taken internally.

## I. Chemical agents that prevent or inhibit microbial growth

A. Definition of an antimicrobial agent

1. Cidal agents - Cidal drugs cause irreversible damage or death the target microbe independent of the host.

2. Bacteria that are treated with physical methods of microbial control or antimicrobial chemicals tend to die at a constant rate. When plotted on semi-logarithmic graph paper the number of surviving microbes versus time, the result will be a straight line. Therefore, bacterial death is constant.

3. Static agent

a. Static drugs inhibit growth or reproduction are dependent on the immune system of the human host for elimination of the pathogens from the body.

b. bacteriostatic

An antibacterial compound is an agent that kills microbes or inhibits their growth. A graphical representation show the number of cells decreases with time once the

antimicrobial agent is added to the culture. A bacteriostatic compound inhibits with growth and reproduction of microbes, bu does not kill them. That is why a graphical representation of the cell number does not decrease after the bacteriostatic compound added, but remains constant over time.

B. Examples of Food preservatives

1. Organic acids
   a. Inhibit metabolism
      1) Sorbic acid, Benzoic acid, calcium propionate
      2) Control molds and bacteria in foods and cosmetics

2. Nitrite
   a. Prevents endospore germination

3. Antibiotics
   a. Nisin and natamycin prevent spoilage of cheese

## II. Definitions Physical Methods

Heat is an economical and simple way to destroy microbes
Heat methods work by denaturing proteins.

A. Thermal death point (TDP)
   1. The lowest temperature at which all bacteria in a liquid culture are killed within 10 minutes.

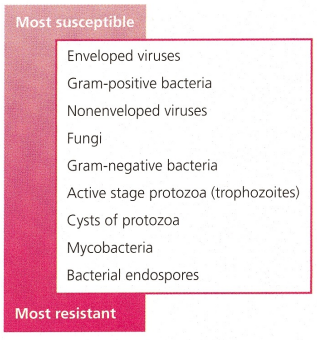
B. Thermal death time (TDT)
   1. The time required to kill all bacteria in a liquid culture at a given temperature.

C. Decimal reduction time (DRT)
   1. The time required to kill 90% of the bacteria in a liquid culture at a given temperature.

## III. Factors Influencing Disinfectant Activity

A. Disinfectant activity
   1. the number of microorganisms,

   2. Types of microbes (some are more resistant than others, e.g., gram-positive bacteria are generally more sensitive to antibiotics than are gram-negative ones)




▲ *Figure 9.2*
Relative susceptibilities of microbes to antimicrobial agents.

3. physiology of organisms (growing organisms are more susceptible than dormant ones)

4. environment (pH. presence or absence of organic matter)

5. Mode action of the agent used. How does the agent kill or inhibit the microbe.

6. Temperature (increased temperatures generally enhance disinfectant activity).
   a. Most antimicrobial agents exert their effect by damaging either the plasma membrane or proteins or nucleic acids

7. Some desirable qualities of a good germicidal agent include
   a. The agent most be rapid in action (even at low concentrations)
   b. The agent should be soluble in water or alcohol and have long-term stability
   c. The agent should be broad spectrum without being toxic to humans and animals tissues
   d. The agent should be able to penetration of inanimate surfaces to sustain a cumulative and persistent action
   e. The agent should be resistance to becoming inactivated by organic matter
   f. The agent should have the qualities of being non-corrosive or have non-staining properties
   g. The agent should be able to sanitize and deodorize
   h. The agent should be affordable and readily available.

**IV. Control Methods**

The factors one must take into account for a good germicidal agent to work:
1) The nature of the material being treated
2) the degree of contamination
3) The length of exposure time needed
4) The strength of the germicidal agent require upon the target microbe.

## Major Methods for Microbial Control

V. Physical Agents
VI. Mechanical Agents
VII. Chemical Agents

**V. Physical Agents**

A. Moist heat
1. Methods include boiling, pasteurization, and autoclaving.

   a. Very inexpensive and readily available; usually 100°C for 15 minutes-many vegetative cells and viruses are killed/inactivated within 10 minutes at 100°C.

B. Pasteurization

2. Primarily used to decrease the number of pathogenic organisms in food without adversely affecting the flavor;
   a) Ultra pasteurization of milk is 130°C - 140°C for 1 - 2 seconds
   b) flash pasteurization is 72°C for 15 seconds
   c) normal pasteurization at 63°C for 30 minutes.

C. Autoclaving

a. Steam under pressure-the most effective moist heat method; usually 121.5°C at 15 psi for 15 minutes.

D. Dry heat

1. Direct flaming or incineration and hot air (160°C-170°C).
   160°C for 1.5 to 2 hours

   170°C for 1 hour

E. Freezing

1. Temperatures between 0° and 7°C may inhibit the reproduction of certain organisms or the production of toxins.

2. Rarely bactericidal

3. Not be an effective method of disinfection
   a. Quick freezing is often used to store microorganisms for long periods of time
   b. Slow freezing causes severe damage to cellular constituents and may be bactericidal.

F. Desiccation

Drying or freeze-drying can be used to inhibit growth (via inhibition of enzymes); organisms remain viable.

G. Irradiation & its Effects

a. Dependents on wavelength and on intensity and duration of exposure.

b. **Ionizing radiation** (alpha, beta, gamma, and x-rays, cathode rays, high-energy protons and neutrons) exhibits a high degree of penetration. It creates free radicals in the medium, leading to the denaturation of proteins and nucleic acids. It can result in mutations.
   1) Used in food preservation processes
   2) Viruses and spores are somewhat resistant.
   3) Gram-negative bacteria are more sensitive to ionizing radiation than are gram-positive organisms.

c. Ultraviolet radiation is a form of non-ionizing radiation.
   1)Low degree of penetration.

a) Results in thymine dimers (cross-linkages) in DNA that interfere with replication.

d. Microwaves

*Microwaves usually do not kill directly organisms*

1) Kills. indirectly from heat generated in micro waved materials.
2) Visible light can cause oxidation of some light-sensitive materials.

**VI. Mechanical Agents**

A. Filtration

1. Mechanical means of removing microorganisms. The liquid or gas is passed through a filter with pores small enough to prevent passage of microbes. This method can be used for substances that are sensitive to heat.

a. HEPA filters: removes at least 99.97% of airborne particles 0.3 micrometers (μm) in diameter.

b. ULPA filter: remove from the air at least 99.999% of dust, pollen, mold, bacteria and any airborne particles with a size of 120 nanometers or larger.

B. Osmotic pressure

1. Extremely hypertonic conditions can cause plasmolysis (i.e., contraction of all the cell membrane away from the cell wall).

**VII. Chemical and gas**

1. Referred to as cold sterilization (See Table: Chemical Disinfectants next page)
Halogens agents (chlorine, iodine), Quaternary ammonium compounds, Heavy metals (Ag, Cu, Hg, Zn), Oxidizing agents, Aldehydes. Alcohols. Phenolics, Ethylene Oxide

2. Chlorhexidine is similar to hexachlorophene.
a) Chlorhexidine is often combined with detergents or alcohol as a disinfectant of skin.
b) Effective disinfectant of most vegetative bacteria and enveloped viruses.
c) Used in surgical hand scrubs.

3. Quaternary ammonium compounds or quats are cationic detergents
a)Bactericidal against gram-positive bacteria, less effective against gram-negative bacteria.

b)Quats are also fungicidal, amoebicidal, and are effective against enveloped viruses.
a) Mechanism of action of detergents - unknown.
1) The permeability of the membrane is probably affected denaturing proteins

**VIII. Method for the evaluation of the effectiveness of an antimicrobial agent**

A. Evaluating the Potency of a Disinfectant

1. Disinfectant quickly kills microorganisms without causing damage to the contaminated material

a) Potency is affected by

1) Concentration of the agent
2) Length of exposure
3)Temperature
4) pH
5) Interfering organic matter

2. Kirby-Bauer disk diffusion method

a. Disk impregnated with chemical placed onto a bacterial media culture

b. Zone of clearing measured around disk and information used to determine effectiveness of chemical compound

3. Use dilution test

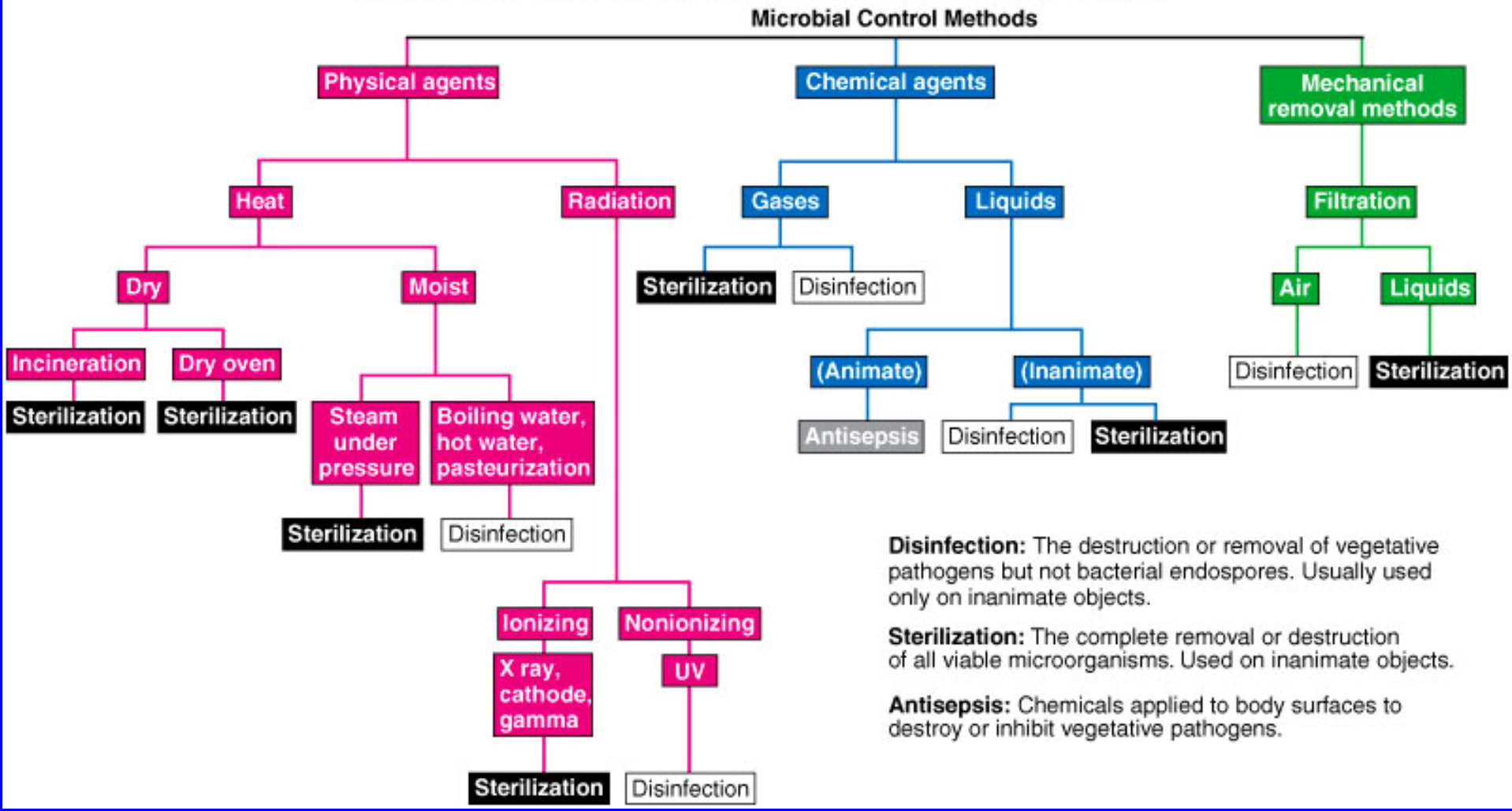
a. Diluted rates of agents used to determine chemical strength. A chemical that can be greatly diluted and still be effective gets a high rating for effectiveness. Ideally a use dilution rate is one that kills microorganisms at the 95% level of confidence.

b. Direct spray method

1) Used to test chemicals that are not water soluble.

c. Tissue toxicity text

1) Tests antiseptic quality through exposure of tissue culture systems to dilutions of the agent.



## Recommended use of heat to control bacterial growth

| Treatment | Temperature | Effectiveness |
|---|---|---|
| Incineration | > 500° | Vaporizes organic material on nonflammable surfaces but may destroy many substances in the process |
| Boiling | 100° | 30 minutes of boiling kills microbial pathogens and vegetative forms of bacteria but my not kill bacterial endospores |
| Intermittent Boiling | 100° | Three 30 minute intervals of boiling, followed by periods of cooling kills bacterial endospores |
| Autoclave and pressure cooker (steam pressure) | 121°/15 minutes at 15 PSI | Kills all forms of life including bacterial endospores. The substance being sterilized must be maintained at the effective Temp for the full time. |
| Dry heat (hot air oven) | 160°/2 hours | For materials that must remain dry and which are not destroyed at Temp between 121°and 170°. Good for glassware, metal, not plastic or rubber items. |
| Dry heat (hot air oven) | 170°/1 hour | Same as above. Note increasing Temp by 10° shortens the sterilizing time by 50% |
| Pasteurization (batch method) | 63°/30 min | Kills most vegetative bacterial cells including pathogens such as streptococci, staphylococci and Mycobacterium tuberculosis |
| Pasteurization (Flash method) | 72°/15 seconds | Effect on bacterial cells similar to batch method; for milk, this method is more conducive to industry and has fewer undesirable effects on quality or taste |
| Ultra Pasteurization | 132/1 second | 99.9% bacterial kill |

# Chemical Disinfectants

| Chemical Agent | Mode of Action | Example |
|---|---|---|
| Phenolics | Very toxic, disrupt cell membranes and denature proteins | Phenol, cresol, hexachlorophene |
| Alcohols | Disrupt membranes and denature proteins | Ethanol, methanol, ispropanol |
| Aldehydes (alkylating agents) | Very effective, denature proteins | Formaldehyde, glutaraldehyde |
| Oxidizing agents | Very toxic to humans, oxidize molecules within cells, generate oxygen gas | Ozone, peroxide |
| Halogens | Negatively affected by presence of organic matter, oxidize cell components, disrupt membrane | Iodine, chlorine, Fluorine |
| Heavy metals | inactivated by organic compounds, combine with sulfhydryl groups, denature proteins | Silver, mercury (very toxic) copper, zinc, selenium, arsenic |
| Surface-acting agents | Vary in degree, can simply reduce surface tension allowing organism to be washed away, or may disrupt membranes and denature proteins | Soaps, detergents (including quaternanry ammonium salts), surfactants |
| Organic acids | Inhibit fungal metabolism (used as food preservative) | Benzoic acid, propionic acid, sorbic acid |
| Gases | Denature proteins | Ethylene oxid (very toxic), vapors from formaldehyde, methyl bromide |
| Antiseptic dyes | Block cell wall synthesis, interfere with DNA replication | Acriflavine, crystal violet |