Chapter 7

Control of Microorganisms by Physical and Chemical Agents

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Definition of Frequently Used Terms

- **sterilization**
  - **destruction or removal of all viable organisms**
- **disinfection**
  - **killing, inhibition, or removal of pathogenic organisms**
  - **disinfectants**
    - **agents, usually chemical, used for disinfection**
    - **usually used on inanimate objects**

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More definitions…

- **sanitization**
  - **reduction of microbial population to levels deemed safe (based on public health standards)**
- **antisepsis**
  - **prevention of infection of living tissue by microorganisms**
  - **antiseptics**
    - **chemical agents that kill or inhibit growth of microorganisms when applied to tissue**

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Antimicrobial agents

- **agents that kill microorganisms or inhibit their growth**
- **-cidal agents kill**
- **-static agents inhibit growth**

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-cidal agents

-cide

- **suffix indicating that agent kills**

- **germicide**
  - **kills pathogens and many nonpathogens but not necessarily endospores**
- **include bactericides, fungicides, algicides, and viricides**

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-static agents

-static

- **suffix indicating that agent inhibits growth**
- **include bacteriostatic and fungistatic**

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The Pattern of Microbial Death

- **microorganisms are not killed instantly**
- **population death usually occurs exponentially**
- **microorganisms are considered to be dead when they are unable to reproduce in conditions that normally support their reproduction**

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Table 7.1 A Theoretical Microbial Heat-Killing Experiment

Minute	Microbial Number at Start of Minute[a]	Microorganisms Killed in 1 Minute (90% of total)[a]	Microorganisms at End of 1 Minute	Log_{10} of Survivors
1	10^6	9×10^5	10^5	5
2	10^5	9×10^4	10^4	4
3	10^4	9×10^3	10^3	3
4	10^3	9×10^2	10^2	2
5	10^2	9×10^1	10	1
6	10^1	9	1	0
7	1	0.9	0.1	−1

[a]Assume that the initial sample contains 10^6 vegetative microorganisms per ml and that 90% of the organisms are killed during each minute of exposure. The temperature is 121° C.

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Conditions Influencing the Effectiveness of Antimicrobial Agent Activity

- **population size**
  - **larger populations take longer to kill than smaller populations**
- **population composition**
  - **microorganisms differ markedly in their sensitivity to antimicrobial agents**

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More conditions…

- **concentration or intensity of an antimicrobial agent**
  - **usually higher concentrations or intensities kill more rapidly**
  - **relationship is not linear**
- **duration of exposure**

  longer exposure ⇒ more organisms killed

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More conditions…

- **temperature**
  - **higher temperatures usually increase amount of killing**
- **local environment**
  - **many factors (e.g., pH, viscosity and concentration of organic matter) can profoundly impact effectiveness**

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The Use of Physical Methods in Control

- **heat**
- **low temperatures**
- **filtration**
- **radiation**

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Heat

- **moist heat**
  - **effective against all types of microorganisms**
  - **degrades nucleic acids, denatures proteins, and disrupts membranes**
- **dry heat sterilization**
  - **less effective, requiring higher temperatures and longer exposure times**
  - **oxidizes cell constituents and denatures proteins**

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Measuring heat-killing efficiency

- **thermal death time (TDT)**
  - **shortest time needed to kill all microorganisms in a suspension at a specific temperature and under defined conditions**
- **decimal reduction time (*D* or *D value*)**
  - **time required to kill 90% of microorganisms or spores in a sample at a specific temperature**

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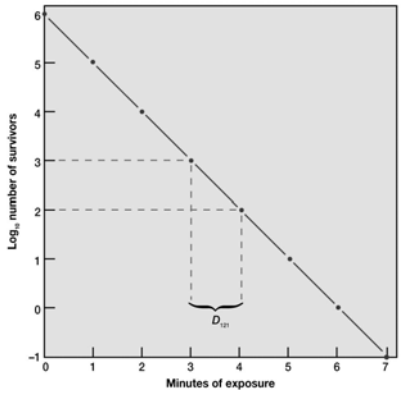

Figure 7.1

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Other measures…

- ***Z* value**
  - **increase in temperature required to reduce *D* by 1/10**
- ***F* value**
  - **time in minutes at a specific temperature needed to kill a population of cells or spores**

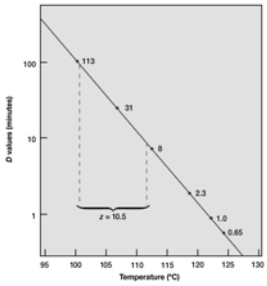

Figure 7.2

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Table 7.3 *D* Values and *z* Values for Some Food-Borne Pathogens

Organism	Substrate	*D* Value (°C) in Minutes	*z* Value (°C)
Clostridium botulinum	Phosphate buffer	$D_{121} = 0.204$	10
Clostridium perfringens (heat-resistant strain)	Culture media	$D_{90} = 3–5$	6–8
Salmonella	Chicken à la king	$D_{60} = 0.39–0.40$	4.9–5.1
Staphylococcus aureus	Chicken à la king	$D_{60} = 5.17–5.37$	5.2–5.8
	Turkey stuffing	$D_{60} = 15.4$	6.8
	0.5% NaCl	$D_{60} = 2.0–2.5$	5.6

Values taken from F.L. Bryan, 1979, "Processes That Affect Survival and Growth of Microorganisms," *Time-Temperature Control of Foodborne Pathogens*, 1979. Atlanta: Centers for Disease Control and Prevention, Atlanta, GA.

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Moist heat

Table 7.2 Approximate Conditions for Moist Heat Killing

Organism	Vegetative Cells	Spores
Yeasts	5 minutes at 50–60°C	5 minutes at 70–80°C
Molds	30 minutes at 62°C	30 minutes at 80°C
Bacteria[a]	10 minutes at 60–70°C	2 to over 800 minutes at 100°C 0.5–12 minutes at 121°C
Viruses	30 minutes at 60°C	

[a]Conditions for mesophilic bacteria.

autoclaves
– used to kill endospores efficiently
– use saturated steam under pressure to reach temperatures above boiling

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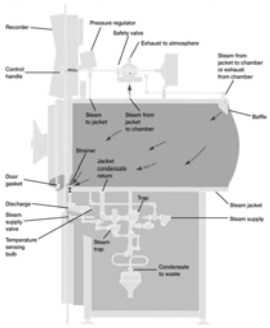

Figure 7.3

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Moist heat…

- **pasteurization**
  - **controlled heating at temperatures well below boiling**
  - **reduces total microbial population and thereby increases shelf life of treated material**

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Pasteurization of milk

- **flash pasteurization (high temperature short-term – HTST)**
  - **72°C for 15 seconds then rapid cooling**
- **ultrahigh-temperature (UHT) sterilization**
  - **140 to 150°C for 1 to 3 seconds**

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Low Temperatures

- **freezing**
  - **stops microbial reproduction due to lack of liquid water**
  - **some microorganisms killed by ice crystal disruption of cell membranes**
- **refrigeration**
  - **slows microbial growth and reproduction**

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Filtration

- **reduces microbial population or sterilizes solutions of heat-sensitive materials by removing microorganisms**
- **also used to reduce microbial populations in air**

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Filtering liquids

- **depth filters**
  - **thick fibrous or granular filters that remove microorganisms by physical screening, entrapment, and/or adsorption**
- **membrane filters**
  - **porous membranes with defined pore sizes that remove microorganisms primarily by physical screening**

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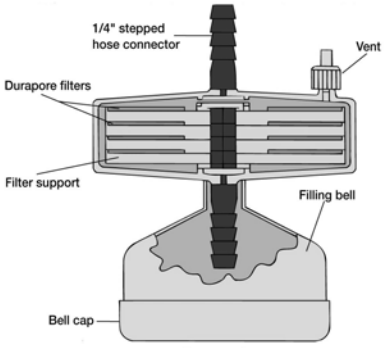

Figure 7.4a

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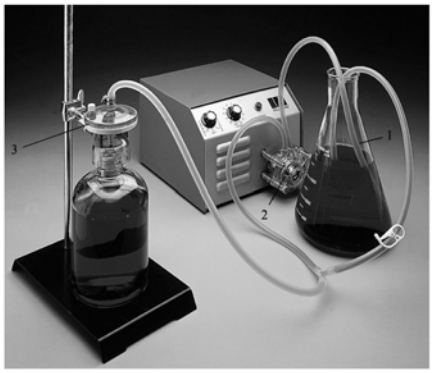

Figure 7.4b

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Figure 7.5a nylon membrane with bacterial removal rating of 0.2 µm

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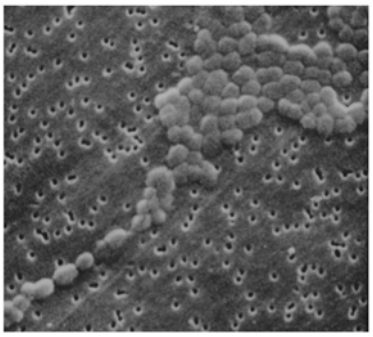

Figure 7.5b

polycarbonate membrane with 0.4 μm pores

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Filtering air

- **surgical masks**
- **cotton plugs on culture vessels**
- **high-efficiency particulate air (HEPA) filters**
  - **used in laminar flow biological safety cabinets**

Figure 7.6a

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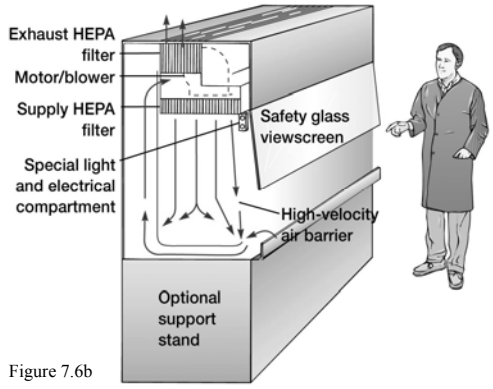

Figure 7.6b

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Radiation

- **ultraviolet (UV) radiation**
  - **limited to surface sterilization because UV radiation does not penetrate glass, dirt films, water, and other substances**
- **ionizing radiation**
  - **penetrates deep into objects**
  - **destroys bacterial endospores; not always effective against viruses**
  - **used for sterilization and pasteurization of antibiotics, hormones, sutures, plastic disposable supplies, and food**

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The Use of Chemical Agents in Control

Table 7.5 Relative Efficacy of Commonly Used Disinfectants and Antiseptics

Class	Disinfectant	Antiseptic	Comment
Gas			
Ethylene oxide	3–4[a]	0[a]	Sporicidal; toxic; good penetration; requires relative humidity of 30% or more; microbicidal activity varies with apparatus used; absorbed by porous material; dry spores highly resistant; moisture must be present, and presoaking is most desirable
Liquid			
Glutaraldehyde, aqueous	3	0	Sporicidal; active solution unstable; toxic
Stabilized hydrogen peroxide	3	0	Sporicidal; use solution stable up to 6 weeks; toxic orally and to eyes; mildly skin toxic; little inactivated by organic matter
Formaldehyde + alcohol	3	0	Sporicidal; noxious fumes; toxic; volatile
Formaldehyde, aqueous	1–2	0	Sporicidal; noxious fumes; toxic
Phenolic compounds	3	0	Stable, corrosive, little inactivation by organic matter; irritates skin
Chlorine compounds	1–2	0	Fast action; inactivation by organic matter; corrosive; irritates skin
Alcohol	1	3	Rapidly microbicidal except for bacterial spores and some viruses; volatile; flammable; dries and irritates skin
Iodine + alcohol	0	4	Corrosive; very rapidly microbicidal; causes staining; irritates skin; flammable
Iodophors	1–2	3	Somewhat unstable; relatively bland; staining temporary; corrosive
Iodine, aqueous	0	2	Rapidly microbicidal; corrosive; stains fabrics; stains and irritates skin
Quaternary ammonium compounds	1	0	Bland; inactivated by soap and anionics; compounds absorbed by fabrics; old or dilute solution can support growth of gram-negative bacteria
Hexachlorophene	0	2	Bland; insoluble in water, soluble in alcohol; not inactivated by soap; weakly bactericidal
Chlorhexidine	0	3	Bland; soluble in water and alcohol; weakly bactericidal
Mercurial compounds	0	±	Bland; much inactivated by organic matter; weakly bactericidal

Source: From Seymour S. Block, *Disinfection, Sterilization and Preservation*, Copyright © 1983 Lea & Febiger, Malvern, Pa. 1983. Reprinted by permission.
[a]Subjective ratings of practical usefulness in a hospital environment—4 is maximal usefulness; 0 is little or no usefulness; ± signifies that the substance is sometimes useful but not always.

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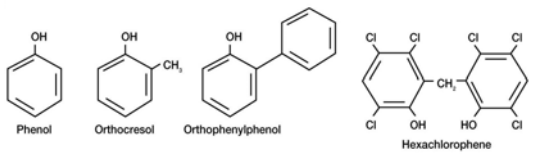
Phenolics



Hexachlorophene

Figure 7.7

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Phenolics

- **commonly used as laboratory and hospital disinfectants**
- **act by denaturing proteins and disrupting cell membranes**
- **tuberculocidal, effective in presence of organic material, and long lasting**
- **disagreeable odor and can cause skin irritation**

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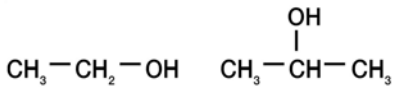
Alcohols



Ethanol Isopropanol

Figure 7.7

- **bactericidal, fungicidal, but not sporicidal**
- **inactivate some viruses**
- **denature proteins and possibly dissolve membrane lipids**

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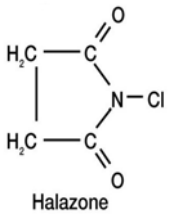
Halogens

- **e.g., iodine**
  - **skin antiseptic**
  - **oxidizes cell constituents and iodinates proteins**
  - **at high concentrations may kill spores**
  - **skin damage, staining, and allergies can be a problem**
  - **iodophore**
    - **iodine complexed with organic carrier**



Halazone

Figure 7.7

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Halogens…

- **e.g., chlorine**
  - **oxidizes cell constituents**
  - **important in disinfection of water supplies and swimming pools, used in dairy and food industries, effective household disinfectant**
  - **destroys vegetative bacteria and fungi, but not spores**
  - **can react with organic matter to form carcinogenic compounds**

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Heavy Metals

- **e.g., ions of mercury, silver, arsenic, zinc, and copper**
- **effective but usually toxic**
- **combine with and inactivate proteins; may also precipitate proteins**

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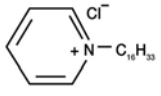
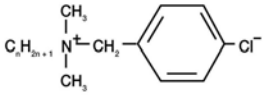
Quaternary Ammonium Compounds



Cetylpyridinium chloride



Benzalkonium chloride

Figure 7.7

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Quaternary Ammonium Compounds

- **detergents**
  - **organic molecules with hydrophilic and hydrophobic ends**
  - **act as wetting agents and emulsifiers**
- **cationic detergents are effective disinfectants**
  - **kill most bacteria, but not *Mycobacterium tuberculosis* or endospores**
  - **safe and easy to use, but inactivated by hard water and soap**

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Aldehydes

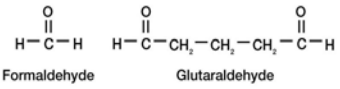

Figure 7.7

- **highly reactive molecules**
- **sporicidal and can be used as chemical sterilants**
- **combine with and inactivate nucleic acids and proteins**

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Sterilizing Gases

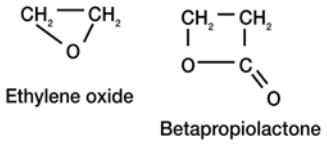

Figure 7.7

- **used to sterilize heat-sensitive materials**
- **microbicidal and sporicidal**
- **combine with and inactivate proteins**

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Evaluation of Antimicrobial Agent Effectiveness

- **complex process regulated by US federal agencies**
  - **Environmental Protection Agency**
  - **Food and Drug Administration**

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Phenol coefficient test

- **potency of a disinfectant is compared to that of phenol**

Table 7.6 Phenol Coefficients for Some Disinfectants

	Phenol Coefficients[a]	
Disinfectant	***Salmonella typhi***	***Staphylococcus aureus***
Phenol	1	1
Cetylpyridinium chloride	228	337
O-phenylphenol	5.6 (20°C)	4.0
p-cresol	2.0–2.3	2.3
Hexachlorophene	5–15	15–40
Merthiolate	600	62.5
Mercurochrome	2.7	5.3
Lysol	1.9	3.5
Isopropyl alcohol	0.6	0.5
Ethanol	0.04	0.04
2% I_2 solution in EtOH	4.1–5.2 (20°C)	4.1–5.2 (20°C)

[a]All values were determined at 37°C except where indicated.

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Other evaluation methods

- **use dilution test**
  - **determines rate at which selected bacteria are destroyed by various chemical agents**
- **in-use testing**
  - **testing done using conditions that approximate normal use of disinfectant**

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Table 7.4 Activity Levels of Selected Germicides

Class	Use Concentration of Active Ingredient	Activity Level[a]
Gas		
Ethylene oxide	450–500 mg/liter [b]	High
Liquid		
Glutaraldehyde, aqueous	2%	High to intermediate
Formaldehyde + alcohol	8 + 70%	High
Stabilized hydrogen peroxide	6–30%	High to intermediate
Formaldehyde, aqueous	6–8%	High to intermediate
Iodophors	750–5,000 mg/liter[c]	High to intermediate
Iodophors	75–150 mg/liter[c]	Intermediate to low
Iodine + alcohol	0.5 + 70%	Intermediate
Chlorine compounds	0.1–0.5%[d]	Intermediate
Phenolic compounds, aqueous	0.5–3%	Intermediate to low
Iodine, aqueous	1%	Intermediate
Alcohols (ethyl, isopropyl)	70%	Intermediate
Quaternary ammonium compounds	0.1–0.2% aqueous	Low
Chlorhexidine	0.75–4%	Low
Hexachlorophene	1–3%	Low
Mercurial compounds	0.1–0.2%	Low

Source: From Seymour S. Block, *Disinfection, Sterilization and Preservation*. Copyright © 1983 Lea & Febiger, Malvern, Pa. 1983. Reprinted by permission.

[a]High-level disinfectants destroy vegetative bacterial cells including *M. tuberculosis*, bacterial endospores, fungi, and viruses. Intermediate-level disinfectants destroy all of the above except endospores. Low-level agents kill bacterial vegetative cells except for *M. tuberculosis*, fungi, and medium-sized lipid-containing viruses (but not bacterial endospores or small, nonlipid viruses).

[b]In autoclave-type equipment at 55 to 60°C.

[c]Available iodine.

[d]Free chlorine.