



## Techniques & Applications

### 7.2 Universal Precautions for Microbiology Laboratories

Blood and other body fluids from all patients should be considered infective.

1. All specimens of blood and body fluids should be put in a well-constructed container with a secure lid to prevent leaking during transport. Care should be taken when collecting each specimen to avoid contaminating the outside of the container and of the laboratory form accompanying the specimen.
2. All persons processing blood and body-fluid specimens should wear gloves. Masks and protective eyewear should be worn if mucous membrane contact with blood or body fluids is anticipated. Gloves should be changed and hands washed after completion of specimen processing.
3. For routine procedures, such as histologic and pathological studies or microbiologic culturing, a biological safety cabinet is not necessary. However, biological safety cabinets should be used whenever procedures are conducted that have a high potential for generating droplets. These include activities such as blending, sonicating, and vigorous mixing.
4. Mechanical pipetting devices should be used for manipulating all liquids in the laboratory. Mouth pipetting must not be done.
5. Use of needles and syringes should be limited to situations in

which there is no alternative, and the recommendations for preventing injuries with needles outlined under universal precautions should be followed. [Techniques & Applications 35.1: Standard microbiological practices](#)

6. Laboratory work surfaces should be decontaminated with an appropriate chemical germicide after a spill of blood or other body fluids and when work activities are completed.
7. Contaminated materials used in laboratory tests should be decontaminated before reprocessing or be placed in bags and disposed of in accordance with institutional policies for disposal of infective waste.
8. Scientific equipment that has been contaminated with blood or other body fluids should be decontaminated and cleaned before being repaired in the laboratory or transported to the manufacturer.
9. All persons should wash their hands after completing laboratory activities and should remove protective clothing before leaving the laboratory.
10. There should be no eating, drinking, or smoking in the work area.

Source: Adapted from *Morbidity and Mortality Weekly Report*, 36 (Suppl. 2S) 5S–10S, 1987, the Centers for Disease Control and Prevention Guidelines.

**Table 7.4** Activity Levels of Selected Germicides

Class	Use Concentration of Active Ingredient	Activity Level <sup>a</sup>
<b>Gas</b>		
Ethylene oxide	450–500 mg/liter <sup>b</sup>	High
<b>Liquid</b>		
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 <sup>c</sup>	High to intermediate
Iodophors	75–150 mg/liter <sup>c</sup>	Intermediate to low
Iodine + alcohol	0.5 + 70%	Intermediate
Chlorine compounds	0.1–0.5% <sup>d</sup>	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. Reprinted by permission.

<sup>a</sup>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).

<sup>b</sup>In autoclave-type equipment at 55 to 60°C.

<sup>c</sup>Available iodine.

<sup>d</sup>Free chlorine.

**Table 7.5** Relative Efficacy of Commonly Used Disinfectants and Antiseptics

Class	Disinfectant	Antiseptic	Comment
<b>Gas</b>			
Ethylene oxide	3–4 <sup>a</sup>	0 <sup>a</sup>	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
<b>Liquid</b>			
Glutaraldehyde, aqueous	3	0	Sporicidal; active solution unstable; toxic
Stabilized hydrogen peroxide	3	0	Sporicidal; solution stable up to 6 weeks; toxic orally and to eyes; mildly skin toxic; little inactivation 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; greatly inactivated by organic matter; weakly bactericidal

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

<sup>a</sup>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.

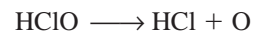
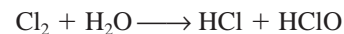
about 70 to 80% concentration. They act by denaturing proteins and possibly by dissolving membrane lipids. A 10 to 15 minute soaking is sufficient to disinfect thermometers and small instruments.

## Halogens

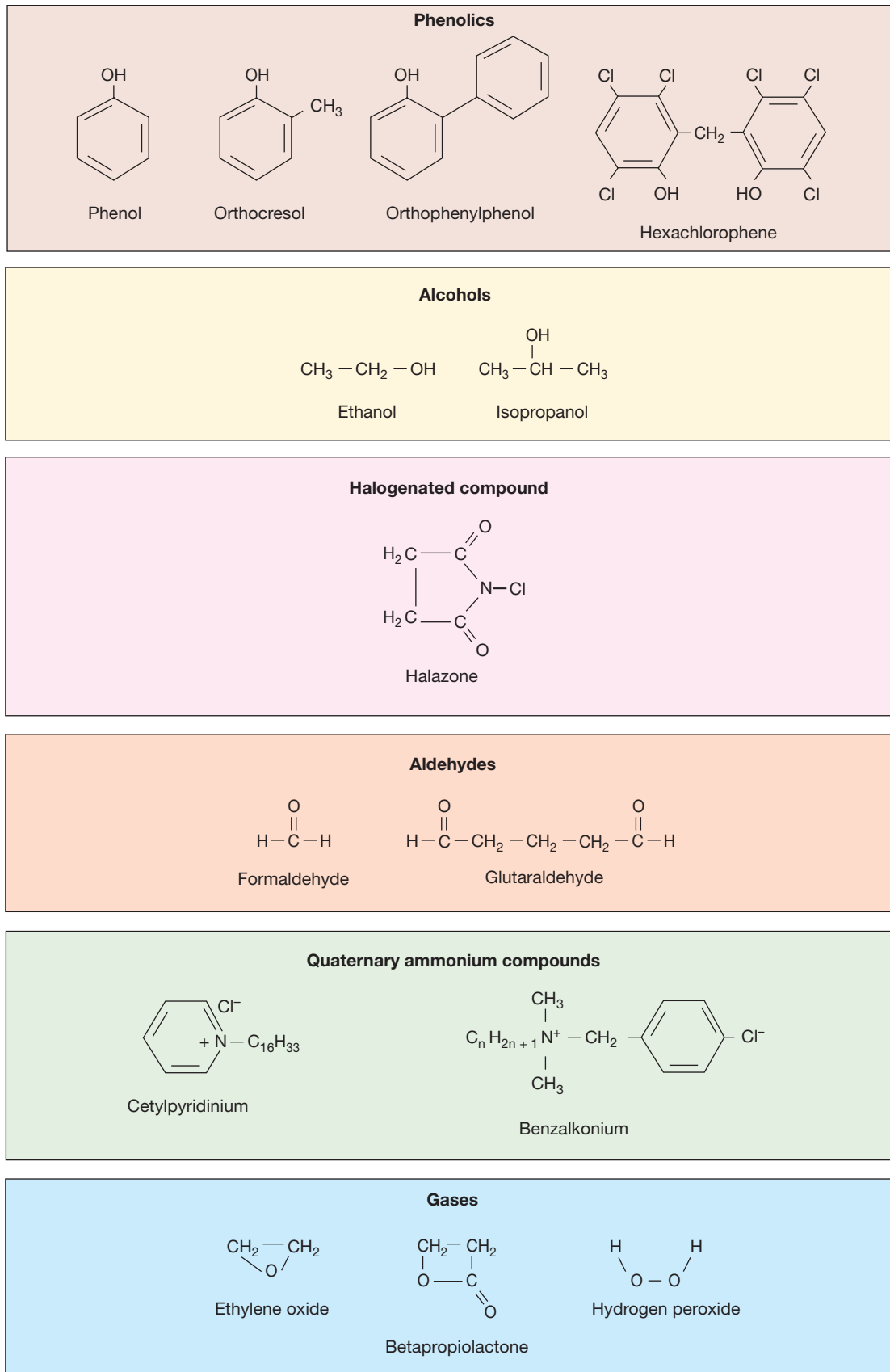
A halogen is any of the five elements (fluorine, chlorine, bromine, iodine, and astatine) in group VIIA of the periodic table. They exist as diatomic molecules in the free state and form saltlike compounds with sodium and most other metals. The halogens iodine and chlorine are important antimicrobial agents. **Iodine** is used as a skin antiseptic and kills by oxidizing cell constituents and iodinating cell proteins. At higher concentrations, it may even kill some spores. Iodine often has been applied as tincture of iodine, 2% or more iodine in a water-ethanol solution of potassium iodide. Although it is an effective antiseptic, the skin may be damaged, a stain is left, and iodine allergies can result. More recently iodine has been complexed with an organic carrier to form an **iodophor**. Iodophors are water soluble, stable, and nonstaining, and release iodine slowly to minimize skin burns and irritation. They are used in hospitals for preoperative skin degerming and in hospitals and

laboratories for disinfecting. Some popular brands are Wescodyne for skin and laboratory disinfection and Betadine for wounds.

**Chlorine** is the usual disinfectant for municipal water supplies and swimming pools and is also employed in the dairy and food industries. It may be applied as chlorine gas, sodium hypochlorite (bleach), or calcium hypochlorite, all of which yield hypochlorous acid (HClO) and then atomic oxygen. The result is oxidation of cellular materials and destruction of vegetative bacteria and fungi, although not spores.



Death of almost all microorganisms usually occurs within 30 minutes. Since organic material interferes with chlorine action by reacting with chlorine and its products, an excess of chlorine is added to ensure microbial destruction. One potential problem is that chlorine reacts with organic compounds to form carcinogenic trihalomethanes, which must be monitored in drinking water. Ozone



**Figure 7.11 Disinfectants and Antiseptics.** The structures of some frequently used disinfectants and antiseptics.

sometimes has been used successfully as an alternative to chlorination in Europe and Canada.

Chlorine is also an excellent disinfectant for individual use because it is effective, inexpensive, and easy to employ. Small quantities of drinking water can be disinfected with halazone tablets. Halazone (parasulfone dichloramidobenzoic acid) slowly releases chloride when added to water and disinfects it in about a half hour. It is frequently used by campers lacking access to uncontaminated drinking water.

Chlorine solutions make very effective laboratory and household disinfectants. An excellent disinfectant-detergent combination can be prepared if a 1/40 dilution of household bleach is combined with a nonionic detergent, such as a dishwashing detergent, to give a 0.8% detergent concentration. This mixture will remove both dirt and bacteria.

### Heavy Metals

For many years the ions of heavy metals such as mercury, silver, arsenic, zinc, and copper were used as germicides. These have now been superseded by other less toxic and more effective germicides (many heavy metals are more bacteriostatic than bactericidal). There are a few exceptions. In some hospitals, a 1% solution of silver nitrate is added to the eyes of infants to prevent ophthalmic gonorrhea. Silver sulfadiazine is used on burns. Copper sulfate is an effective algicide in lakes and swimming pools.

Heavy metals combine with proteins, often with their sulfhydryl groups, and inactivate them. They may also precipitate cell proteins.

### Quaternary Ammonium Compounds

Quaternary ammonium compounds are detergents that have antimicrobial activity and are effective disinfectants. **Detergents** [Latin *detergere*, to wipe away] are organic cleansing agents that are amphipathic, having both polar hydrophilic and nonpolar hydrophobic components. The hydrophilic portion of a quaternary ammonium compound is a positively charged quaternary nitrogen; thus quaternary ammonium compounds are cationic detergents. Their antimicrobial activity is the result of their ability to disrupt microbial membranes; they may also denature proteins.

Cationic detergents like benzalkonium chloride and cetylpyridinium chloride kill most bacteria but not *M. tuberculosis* or endospores. They have the advantages of being stable and nontoxic but they are inactivated by hard water and soap. Cationic detergents are often used as disinfectants for food utensils and small instruments and as skin antiseptics. Several brands are on the market. Zephiran contains benzalkonium chloride and Ceepryn, cetylpyridinium chloride.

### Aldehydes

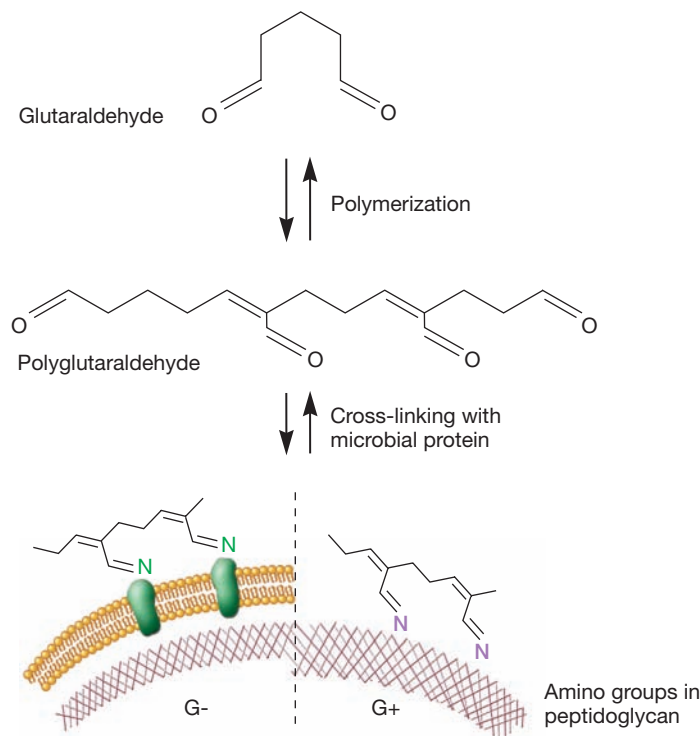
Both of the commonly used aldehydes, formaldehyde and glutaraldehyde (figure 7.11), are highly reactive molecules that combine with nucleic acids and proteins and inactivate them, probably by cross-linking and alkylating molecules (figure 7.12). They are sporicidal and can be used as chemical sterilants. Formaldehyde is usually dissolved in water or alcohol before use.

A 2% buffered solution of glutaraldehyde is an effective disinfectant. It is less irritating than formaldehyde and is used to disinfect hospital and laboratory equipment. Glutaraldehyde usually disinfects objects within about 10 minutes but may require as long as 12 hours to destroy all spores.

### Sterilizing Gases

Many heat-sensitive items such as disposable plastic petri dishes and syringes, heart-lung machine components, sutures, and catheters are sterilized with ethylene oxide gas (figure 7.11). **Ethylene oxide** (EtO) is both microbicidal and sporicidal and kills by combining with cell proteins. It is a particularly effective sterilizing agent because it rapidly penetrates packing materials, even plastic wraps.

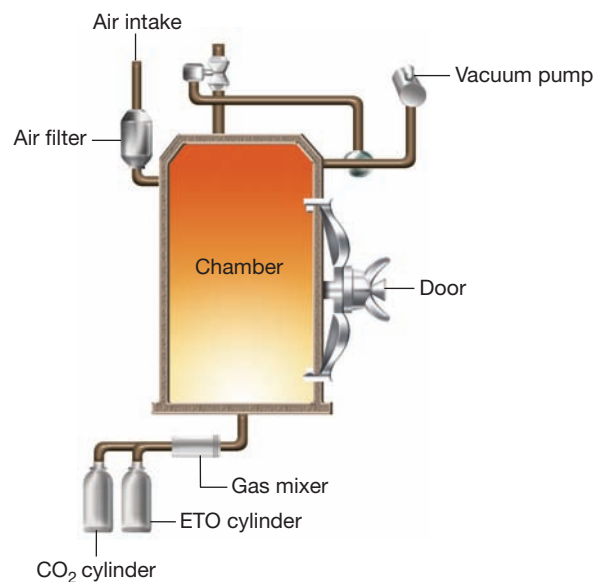
Sterilization is carried out in a special ethylene oxide sterilizer, very much resembling an autoclave in appearance, that controls the EtO concentration, temperature, and humidity (figure 7.13). Because pure EtO is explosive, it is usually supplied in a 10 to 20% concentration mixed with either CO<sub>2</sub> or dichlorodifluoromethane. The ethylene oxide concentration, humidity, and temperature influence the rate of sterilization. A clean object can be sterilized if treated for 5 to 8 hours at 38°C or 3 to 4 hours at 54°C when the relative humidity is maintained



**Figure 7.12 Effects of Glutaraldehyde.** Glutaraldehyde polymerizes and then interacts with amino acids in proteins (left) or in peptidoglycan (right). As a result, the proteins are alkylated and cross-linked to other proteins, which inactivates them. The amino groups in peptidoglycan are also alkylated and cross-linked, which prevents them from participating in other chemical reactions such as those involved in peptidoglycan synthesis.



(a)



(b)

**Figure 7.13 An Ethylene Oxide Sterilizer.** (a) An automatic ethylene oxide (EtO) sterilizer. (b) Schematic of an EtO sterilizer. Items to be sterilized are placed in the chamber and EtO and carbon dioxide are introduced. After the sterilization procedure is completed, the EtO and carbon dioxide are pumped out of the chamber and air enters.

at 40 to 50% and the EtO concentration at 700 mg/liter. Extensive aeration of the sterilized materials is necessary to remove residual EtO because it is so toxic.

**Betapropiolactone** (BPL) is occasionally employed as a sterilizing gas. In the liquid form it has been used to sterilize vaccines and sera. BPL decomposes to an inactive form after several hours and is therefore not as difficult to eliminate as EtO. It also destroys microorganisms more readily than ethylene oxide but does not penetrate materials well and may be carcinogenic. For these reasons, BPL has not been used as extensively as EtO.

**Vaporized hydrogen peroxide** can be used to decontaminate biological safety cabinets, operating rooms, and other large facilities. These systems introduce vaporized hydrogen peroxide into the enclosure for some time, depending on the size of the enclosure and the materials within. Hydrogen peroxide is toxic and kills a wide variety of microorganisms. However, during the course of the decontamination process, it breaks down to water and oxygen, both of which are harmless. Other advantages of these systems are that they can be used at a wide range of temperatures (4 to 80°C) and they do not damage most materials.

### Chemotherapeutic Agents

The chemicals discussed thus far are appropriate for use either on inanimate objects or external host tissues. **Chemotherapeutic agents** are chemicals that can be used internally to kill or inhibit the growth of microbes within host tissues. They can be used internally because they have **selective toxicity**; that is, they target the microbe and do relatively little if any harm to the host. Most chemotherapeutic agents are **antibiotics**—chemicals synthesized by microbes that are effective in controlling the growth of bacteria. Since the dis-

covery of the first antibiotics, pharmaceutical companies have developed numerous derivatives and many synthetic antibiotics. Chemotherapeutic agents for treating diseases caused by fungi, protists, and viruses have also been developed. Chemotherapeutic agents are described in more detail in chapter 34.

1. Why are most antimicrobial chemical agents disinfectants rather than sterilants? What general characteristics should one look for in a disinfectant?
2. Describe each of the following agents in terms of its chemical nature, mechanism of action, mode of application, common uses and effectiveness, and advantages and disadvantages: phenolics, alcohols, halogens, heavy metals, quaternary ammonium compounds, aldehydes, and ethylene oxide.
3. Which disinfectants or antiseptics would be used to treat the following: oral thermometer, laboratory bench top, drinking water, patch of skin before surgery, small medical instruments (probes, forceps, etc.)? Explain your choices.
4. How do chemotherapeutic agents differ from the other chemical control agents described in this chapter?
5. Which physical or chemical agent would be the best choice for sterilizing the following items: glass pipettes, tryptic soy broth tubes, nutrient agar, antibiotic solution, interior of a biological safety cabinet, wrapped package of plastic petri plates? Explain your choices.

## 7.6 EVALUATION OF ANTIMICROBIAL AGENT EFFECTIVENESS

Testing of antimicrobial agents is a complex process regulated by two different federal agencies. The U.S. Environmental Protection Agency regulates disinfectants, whereas agents used on humans and animals are under the control of the Food and Drug

Administration. Testing of antimicrobial agents often begins with an initial screening test to see if they are effective and at what concentrations. This may be followed by more realistic in-use testing.

The best-known disinfectant screening test is the **phenol coefficient test** in which the potency of a disinfectant is compared with that of phenol. A series of dilutions of phenol and the disinfectant being tested are prepared. A standard amount of *Salmonella typhi* and *Staphylococcus aureus* are added to each dilution; the dilutions are then placed in a 20 or 37°C water bath. At 5-minute intervals, samples are withdrawn from each dilution and used to inoculate a growth medium, which is incubated for two or more days and then examined for growth. If there is no growth in the growth medium, the dilution at that particular time of sampling killed the bacteria. The highest dilution (i.e., the lowest concentration) that kills the bacteria after a 10-minute exposure, but not after 5 minutes, is used to calculate the phenol coefficient. This is done by dividing the reciprocal of the appropriate dilution for the disinfectant being tested by the reciprocal of the appropriate phenol dilution. For instance, if the phenol dilution was 1/90 and maximum effective dilution for disinfectant X was 1/450, then the phenol coefficient of X would be 5. The higher the phenol coefficient value, the more effective the disinfectant under these test conditions. A value greater than 1 means that the disinfectant is more effective than phenol. A few representative phenol coefficient values are given in **table 7.6**.

The phenol coefficient test is a useful initial screening procedure, but the phenol coefficient can be misleading if taken as a direct indication of disinfectant potency during normal use. This is because the phenol coefficient is determined under carefully controlled conditions with pure bacterial strains, whereas disinfectants are normally used on complex populations in the presence of organic matter and with significant variations in environmental factors like pH, temperature, and presence of salts.

To more realistically estimate disinfectant effectiveness, other tests are often used. The rates at which selected bacteria are destroyed with various chemical agents may be experimentally

**Table 7.6** Phenol Coefficients for Some Disinfectants

Disinfectant	Phenol Coefficients <sup>a</sup>	
	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>
Phenol	1	1
Cetylpyridinium chloride	228	337
<i>O</i> -phenylphenol	5.6 (20°C)	4.0
<i>p</i> -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 <sub>2</sub> solution in EtOH	4.1–5.2 (20°C)	4.1–5.2 (20°C)

<sup>a</sup>All values were determined at 37°C except where indicated.

determined and compared. A **use dilution test** can also be carried out. Stainless steel cylinders are contaminated with specific bacterial species under carefully controlled conditions. The cylinders are dried briefly, immersed in the test disinfectants for 10 minutes, transferred to culture media, and incubated for two days. The disinfectant concentration that kills the organisms in the sample with a 95% level of confidence under these conditions is determined. Disinfectants also can be tested under conditions designed to simulate normal in-use situations. In-use testing techniques allow a more accurate determination of the proper disinfectant concentration for a particular situation.

1. Briefly describe the phenol coefficient test.
2. Why might it be necessary to employ procedures like the use dilution and in-use tests?

## Summary

### 7.1 Definitions of Frequently Used Terms

- a. Sterilization is the process by which all living cells, viable spores, viruses, and viroids are either destroyed or removed from an object or habitat. Disinfection is the killing, inhibition, or removal of microorganisms (but not necessarily endospores) that can cause disease.
- b. The main goal of disinfection and antisepsis is the removal, inhibition, or killing of pathogenic microbes. Both processes also reduce the total number of microbes. Disinfectants are chemicals used to disinfect inanimate objects; antiseptics are used on living tissue.
- c. Antimicrobial agents that kill organisms often have the suffix *-cide*, whereas agents that prevent growth and reproduction have the suffix *-static*.

### 7.2 The Pattern of Microbial Death

- a. Microbial death is usually exponential or logarithmic (**figure 7.2**).

### 7.3 Conditions Influencing the Effectiveness of Antimicrobial Agents

- a. The effectiveness of a disinfectant or sterilizing agent is influenced by population size, population composition, concentration or intensity of the agent, exposure duration, temperature, and nature of the local environment.

### 7.4 The Use of Physical Methods in Control

- a. Moist heat kills by degrading nucleic acids, denaturing enzymes and other proteins, and disrupting cell membranes.
- b. Although treatment with boiling water for 10 minutes kills vegetative forms, an autoclave must be used to destroy endospores by heating at 121°C and 15 pounds of pressure (**figure 7.3**).
- c. Glassware and other heat-stable items may be sterilized by dry heat at 160 to 170°C for 2 to 3 hours.
- d. The efficiency of heat killing is often indicated by the thermal death time or the decimal reduction time.
- e. Refrigeration and freezing can be used to control microbial growth and reproduction.
- f. Microorganisms can be efficiently removed by filtration with either depth filters or membrane filters (**figure 7.6**).
- g. Biological safety cabinets with high-efficiency particulate filters sterilize air by filtration (**figure 7.8**).
- h. Radiation of short wavelength or high-energy ultraviolet and ionizing radiation can be used to sterilize objects (**figures 7.9 and 7.10**).