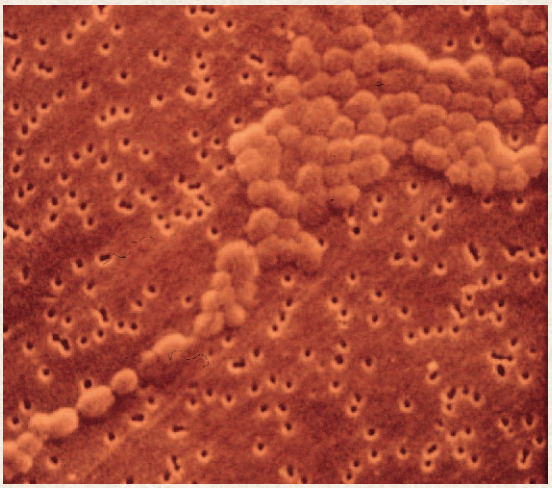
CHAPTER 7

# Control of Microorganisms by Physical and Chemical Agents



Bacteria are trapped on the surface of a membrane filter used to remove microorganisms from fluids.

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## Concepts

1. Microbial population death is exponential, and the effectiveness of an agent is not fixed but influenced by many environmental factors.
2. Solid objects can be sterilized by physical agents such as heat and radiation; liquids and gases are sterilized by heat, radiation, and filtration through the proper filter.
3. Most chemical agents do not readily destroy bacterial endospores and therefore cannot sterilize objects; they are used as disinfectants, sanitizers, and antiseptics. Objects can be sterilized by gases like ethylene oxide that destroy endospores.
4. A knowledge of methods used for microbial control is essential for personal and public safety.

*We all labour against our own cure, for death is the cure of all diseases.*

—*Sir Thomas Browne*

The chapters in Part II are concerned with the nutrition, growth, and control of microorganisms. This chapter addresses the subject of the nonspecific control and destruction of microorganisms, a topic of immense practical importance. Although many microorganisms are beneficial and necessary for human well-being, microbial activities may have undesirable consequences, such as food spoilage and disease. Therefore it is essential to be able to kill a wide variety of microorganisms or inhibit their growth to minimize their destructive effects. The goal is twofold: (1) to destroy pathogens and prevent their transmission, and (2) to reduce or eliminate microorganisms responsible for the contamination of water, food, and other substances.

This chapter focuses on the control of microorganisms by nonspecific physical and chemical agents. Chapter 35 introduces the use of antimicrobial chemotherapy to control microbial disease.

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From the beginning of recorded history, people have practiced disinfection and sterilization, even though the existence of microorganisms was long unsuspected. The Egyptians used fire to sterilize infectious material and disinfectants to embalm bodies, and the Greeks burned sulfur to fumigate buildings. Mosaic law commanded the Hebrews to burn any clothing suspected of being contaminated with the leprosy bacterium. Today the ability to destroy microorganisms is no less important: it makes possible the aseptic techniques used in microbiological research, the preservation of food, and the prevention of disease. The techniques described in this chapter are also essential to personal safety in both the laboratory and hospital (**Box 7.1**).

There are several ways to control microbial growth that have not been included in this chapter, but they should be considered for a more complete picture of how microorganisms are controlled. Chapter 6 describes the effects of osmotic activity, pH, temperature, $O_2$, and radiation on microbial growth and survival (*see pp. 121–31*). Chapter 41 discusses the use of physical and chemical agents in food preservation (*see pp. 970–73*).

## 7.1 Definition of Frequently Used Terms

Terminology is especially important when the control of microorganisms is discussed because words like disinfectant and antiseptic often are used loosely. The situation is even more confusing because a particular treatment can either inhibit growth or kill depending on the conditions.

The ability to control microbial populations on inanimate objects, like eating utensils and surgical instruments, is of considerable practical importance. Sometimes it is necessary to eliminate all microorganisms from an object, whereas only partial destruction of the microbial population may be required in other situations. **Sterilization** [Latin *sterilis,* unable to produce offspring or barren] is the process by which all living cells, viable spores, viruses, and viroids (*see chapter 18*) are either destroyed or removed from an object or habitat. A sterile object is totally free of viable microorganisms, spores, and other infectious agents. When sterilization is achieved by a chemical agent, the chemical is called a sterilant. In

## Box 7.1

### Safety in the Microbiology Laboratory

Personnel safety should be of major concern in all microbiology laboratories. It has been estimated that thousands of infections have been acquired in the laboratory, and many persons have died because of such infections. The two most common laboratory-acquired bacterial diseases are typhoid fever and brucellosis. Most deaths have come from typhoid fever (20 deaths) and Rocky Mountain spotted fever (13 deaths). Infections by fungi (histoplasmosis) and viruses (Venezuelan equine encephalitis and hepatitis B virus from monkeys) are also not uncommon. Hepatitis is the most frequently reported laboratory-acquired viral infection, especially in people working in clinical laboratories and with blood. In a survey of 426 U.S. hospital workers, 40% of those in clinical chemistry and 21% in microbiology had antibodies to hepatitis B virus, indicating their previous exposure (though only about 19% of these had disease symptoms).

Efforts have been made to determine the causes of these infections in order to enhance the development of better preventive measures. Although often it is not possible to determine the direct cause of infection, some major potential hazards are clear. One of the most frequent causes of disease is the inhalation of an infectious aerosol. An aerosol is a gaseous suspension of liquid or solid particles that may be generated by accidents and laboratory operations such as spills, centrifuge accidents, removal of closures from shaken culture tubes, and plunging of contaminated loops into a flame. Accidents with hypodermic syringes and needles, such as self-inoculation and spraying solutions from the needle, also are common. Hypodermics should be employed only when necessary and then with care. Pipette accidents involving the mouth are another major source of infection; pipettes should be filled with the use of pipette aids and operated in such a way as to avoid creating aerosols.

People must exercise care and common sense when working with microorganisms. Operations that might generate infectious aerosols should be carried out in a biological safety cabinet. Bench tops and incubators should be disinfected regularly. Autoclaves must be maintained and operated properly to ensure adequate sterilization. Laboratory personnel should wash their hands thoroughly before and after finishing work.

contrast, **disinfection** is the killing, inhibition, or removal of microorganisms that may cause disease. The primary goal is to destroy potential pathogens, but disinfection also substantially reduces the total microbial population. **Disinfectants** are agents, usually chemical, used to carry out disinfection and are normally used only on inanimate objects. A disinfectant does not necessarily sterilize an object because viable spores and a few microorganisms may remain. **Sanitization** is closely related to disinfection. In sanitization, the microbial population is reduced to levels that are considered safe by public health standards. The inanimate object is usually cleaned as well as partially disinfected. For example, sanitizers are used to clean eating utensils in restaurants.

It is frequently necessary to control microorganisms on living tissue with chemical agents. **Antisepsis** [Greek *anti,* against, and *sepsis,* putrefaction] is the prevention of infection or sepsis and is accomplished with **antiseptics.** These are chemical agents applied to tissue to prevent infection by killing or inhibiting pathogen growth; they also reduce the total microbial population. Because they must not destroy too much host tissue, antiseptics are generally not as toxic as disinfectants.

A suffix can be employed to denote the type of antimicrobial agent. Substances that kill organisms often have the suffix -cide [Latin *cida,* to kill]: a **germicide** kills pathogens (and many nonpathogens) but not necessarily endospores. A disinfectant or antiseptic can be particularly effective against a specific group, in which case it may be called a **bactericide, fungicide, algicide,** or **viricide.** Other chemicals do not kill, but they do prevent growth. If these agents are removed, growth will resume. Their names end in -static [Greek *statikos,* causing to stand or stopping]—for example, **bacteriostatic** and **fungistatic.**

Although these agents have been described in terms of their effects on pathogens, it should be noted that they also kill or inhibit the growth of nonpathogens as well. Their ability to reduce the total microbial population, not just to affect pathogen levels, is quite important in many situations.

1. Define the following terms: sterilization, sterilant, disinfection, disinfectant, sanitization, antisepsis, antiseptic, germicide, bactericide, bacteriostatic.

## 7.2 The Pattern of Microbial Death

A microbial population is not killed instantly when exposed to a lethal agent. Population death, like population growth, is generally exponential or logarithmic—that is, the population will be reduced by the same fraction at constant intervals (**table 7.1**). If the logarithm of the population number remaining is plotted against the time of exposure of the microorganism to the agent, a straight line plot will result (compare **figure 7.1** with figure 6.2). When the population has been greatly reduced, the rate of killing may slow due to the survival of a more resistant strain of the microorganism.

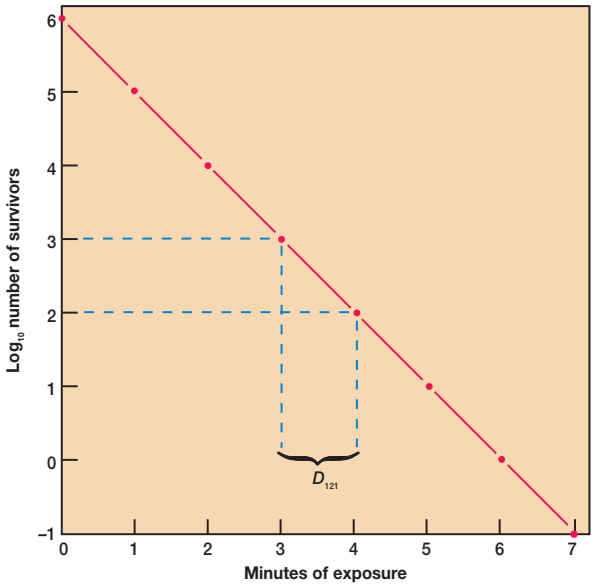



**Figure 7.1 The Pattern of Microbial Death.** An exponential plot of the survivors versus the minutes of exposure to heating at 121°C. In this example the $D_{121}$ value is 1 minute. The data are from table 7.1.

**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 | $10^6$ | $9 \times 10^5$ | $10^5$ | 5 |
| 2 | $10^5$ | $9 \times 10^4$ | $10^4$ | 4 |
| 3 | $10^4$ | $9 \times 10^3$ | $10^3$ | 3 |
| 4 | $10^3$ | $9 \times 10^2$ | $10^2$ | 2 |
| 5 | $10^2$ | $9 \times 10^1$ | 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.

To study the effectiveness of a lethal agent, one must be able to decide when microorganisms are dead, a task by no means as easy as with macroorganisms. It is hardly possible to take a bacterium's pulse. A bacterium is defined as dead if it does not grow and reproduce when inoculated into culture medium that would normally support its growth. In like manner an inactive virus cannot infect a suitable host.

1. Describe the pattern of microbial death and how one decides whether microorganisms are actually dead.

## 7.3 Conditions Influencing the Effectiveness of Antimicrobial Agent Activity

Destruction of microorganisms and inhibition of microbial growth are not simple matters because the efficiency of an **antimicrobial agent** (an agent that kills microorganisms or inhibits their growth) is affected by at least six factors.

1. Population size. Because an equal fraction of a microbial population is killed during each interval, a larger population requires a longer time to die than a smaller one. This can be seen in the theoretical heat-killing experiment shown in table 7.1 and figure 7.1. The same principle applies to chemical antimicrobial agents.
2. Population composition. The effectiveness of an agent varies greatly with the nature of the organisms being treated because microorganisms differ markedly in susceptibility. Bacterial endospores are much more resistant to most antimicrobial agents than are vegetative forms, and younger cells are usually more readily destroyed than mature organisms. Some species are able to withstand adverse conditions better than others. *Mycobacterium tuberculosis,* which causes tuberculosis, is much more resistant to antimicrobial agents than most other bacteria.
3. Concentration or intensity of an antimicrobial agent. Often, but not always, the more concentrated a chemical agent or intense a physical agent, the more rapidly microorganisms are destroyed. However, agent effectiveness usually is not directly related to concentration or intensity. Over a short range a small increase in concentration leads to an exponential rise in effectiveness; beyond a certain point, increases may not raise the killing rate much at all. Sometimes an agent is more effective at lower concentrations. For example, 70% ethanol is more effective than 95% ethanol because its activity is enhanced by the presence of water.
4. Duration of exposure. The longer a population is exposed to a microbicidal agent, the more organisms are killed (figure 7.1). To achieve sterilization, an exposure duration sufficient to reduce the probability of survival to $10^{-6}$ or less should be used.
5. Temperature. An increase in the temperature at which a chemical acts often enhances its activity. Frequently a lower concentration of disinfectant or sterilizing agent can be used at a higher temperature.
6. Local environment. The population to be controlled is not isolated but surrounded by environmental factors that may either offer protection or aid in its destruction. For example, because heat kills more readily at an acid pH, acid foods and beverages such as fruits and tomatoes are easier to pasteurize than foods with higher pHs like milk. A second important environmental factor is organic matter that can protect microorganisms against heating and chemical disinfectants. Biofilms are a good example. The organic matter in a surface biofilm will protect the biofilm's microorganisms; furthermore, the biofilm and its microbes often will be hard to remove. It may be necessary to clean an object before it is disinfected or sterilized. Syringes and medical or dental equipment should be cleaned before sterilization because the presence of too much organic matter could protect pathogens and increase the risk of infection. The same care must be taken when pathogens are destroyed during the preparation of drinking water. When a city's water supply has a high content of organic material, more chlorine must be added to disinfect it.

1. Briefly explain how the effectiveness of antimicrobial agents varies with population size, population composition, concentration or intensity of the agent, treatment duration, temperature, and local environmental conditions.

## 7.4 The Use of Physical Methods in Control

Heat and other physical agents are normally used to control microbial growth and sterilize objects, as can be seen from the continual operation of the autoclave in every microbiology laboratory. The four most frequently employed physical agents are heat, low temperatures, filtration, and radiation.

### Heat

Fire and boiling water have been used for sterilization and disinfection since the time of the Greeks, and heating is still one of the most popular ways to destroy microorganisms. Either moist or dry heat may be applied.

Moist heat readily kills viruses, bacteria, and fungi (**table 7.2**). Exposure to boiling water for 10 minutes is sufficient to destroy vegetative cells and eucaryotic spores. Unfortunately the temperature of boiling water (100°C or 212°F) is not high enough to destroy bacterial endospores that may survive hours of boiling. Therefore boiling can be used for disinfection of drinking water and objects not harmed by water, but boiling does not sterilize.

Because heat is so useful in controlling microorganisms, it is essential to have a precise measure of the heat-killing efficiency.

**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<br>0.5–12 minutes at 121°C |
| Viruses | 30 minutes at 60°C | |

[a]Conditions for mesophilic bacteria.

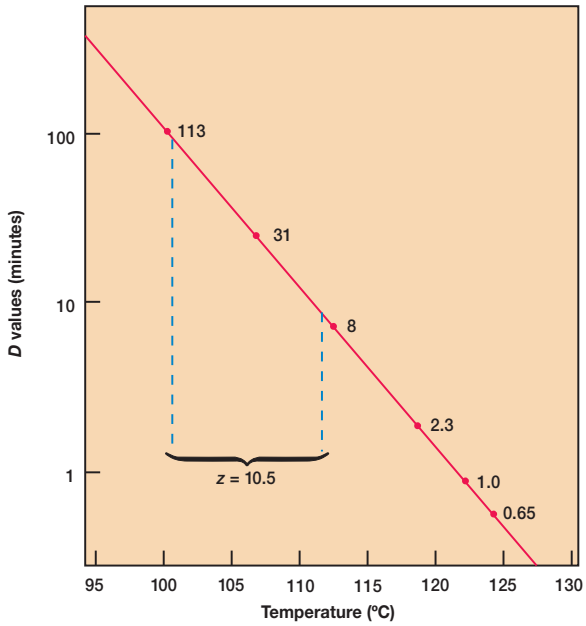



**Figure 7.2** ***z* Value Calculation.** The *z* value used in calculation of time-temperature relationships for survival of a test microorganism, based on *D* value responses at various temperatures. The *z* value is the increase in temperature needed to reduce the decimal reduction time (*D*) to 10% of the original value. For this homogeneous sample of a test microorganism the *z* value is 10.5°. The *D* values are plotted on a logarithmic scale.

Initially effectiveness was expressed in terms of thermal death point (TDP), the lowest temperature at which a microbial suspension is killed in 10 minutes. Because TDP implies that a certain temperature is immediately lethal despite the conditions, **thermal death time (TDT)** is now more commonly used. This is the shortest time needed to kill all organisms in a microbial suspension at a specific temperature and under defined conditions. However, such destruction is logarithmic, and it is theoretically not possible to "completely destroy" microorganisms in a sample, even with extended heating. Therefore an even more precise figure, the **decimal reduction time (*D*)** or ***D* value** has gained wide acceptance. The decimal reduction time is the time required to kill 90% of the microorganisms or spores in a sample at a specified temperature. In a semilogarithmic plot of the population remaining versus the time of heating (figure 7.1), the *D* value is the time required for the line to drop by one log cycle or tenfold. The *D* value is usually written with a subscript, indicating the temperature for which it applies. *D* values are used to estimate the relative resistance of a microorganism to different temperatures through calculation of the ***z* value.** The *z* value is the increase in temperature required to reduce *D* to 1/10 its value or to reduce it by one log cycle when log *D* is plotted against temperature (**figure 7.2**). Another way to describe heating effectiveness is with the *F* value. The ***F* value** is the time in minutes at a specific temperature (usually 250°F or 121.1°C) needed to kill a population of cells or spores.

The food processing industry makes extensive use of *D* and *z* values. After a food has been canned, it must be heated to eliminate the risk of botulism arising from *Clostridium botulinum* spores. Heat treatment is carried out long enough to reduce a population of $10^{12}$ *C. botulinum* spores to $10^{0}$ (one spore); thus there is a very small chance of any can having a viable spore. The *D* value for these spores at 121°C is 0.204 minutes. Therefore it would take 12*D* or 2.5 minutes to reduce $10^{12}$ spores to one spore by heating at 121°C. The *z* value for *C. botulinum* spores is 10°C—that is, it takes a 10°C change in temperature to alter the *D* value tenfold. If the cans were to be processed at 111°C rather than at 121°C, the *D* value would increase by tenfold to 2.04 minutes and the 12*D* value to 24.5 minutes. *D* values and *z* values for some common food-borne pathogens are given in **table 7.3.** Three *D* values are included for *Staphylococcus aureus* to illustrate the variation of killing rate with environment and the protective effect of organic material. Food processing (pp. 970–73); Botulism (p. 929)

Moist heat sterilization must be carried out at temperatures above 100°C in order to destroy bacterial endospores, and this requires the use of saturated steam under pressure. Steam sterilization is carried out with an **autoclave** (**figure 7.3**), a device somewhat like a fancy pressure cooker. The development of the autoclave by Chamberland in 1884 tremendously stimulated the growth of microbiology. Water is boiled to produce steam, which is released through the jacket and into the autoclave's chamber. The air initially present in the chamber is forced out until the chamber is filled with saturated steam and the outlets are closed. Hot, saturated steam continues to enter until the chamber reaches the desired temperature and pressure, usually 121°C and 15 pounds of pressure. At this temperature saturated steam destroys all vegetative cells and endospores in a small volume of liquid within 10 to 12 minutes. Treatment is continued for about 15 minutes to provide a margin of safety. Of course, larger containers of liquid such as flasks and carboys will require much longer treatment times.

Moist heat is thought to kill so effectively by degrading nucleic acids and by denaturing enzymes and other essential proteins. It also may disrupt cell membranes.

Autoclaving must be carried out properly or the processed materials will not be sterile. If all air has not been flushed out of the chamber, it will not reach 121°C even though it may reach a

**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.

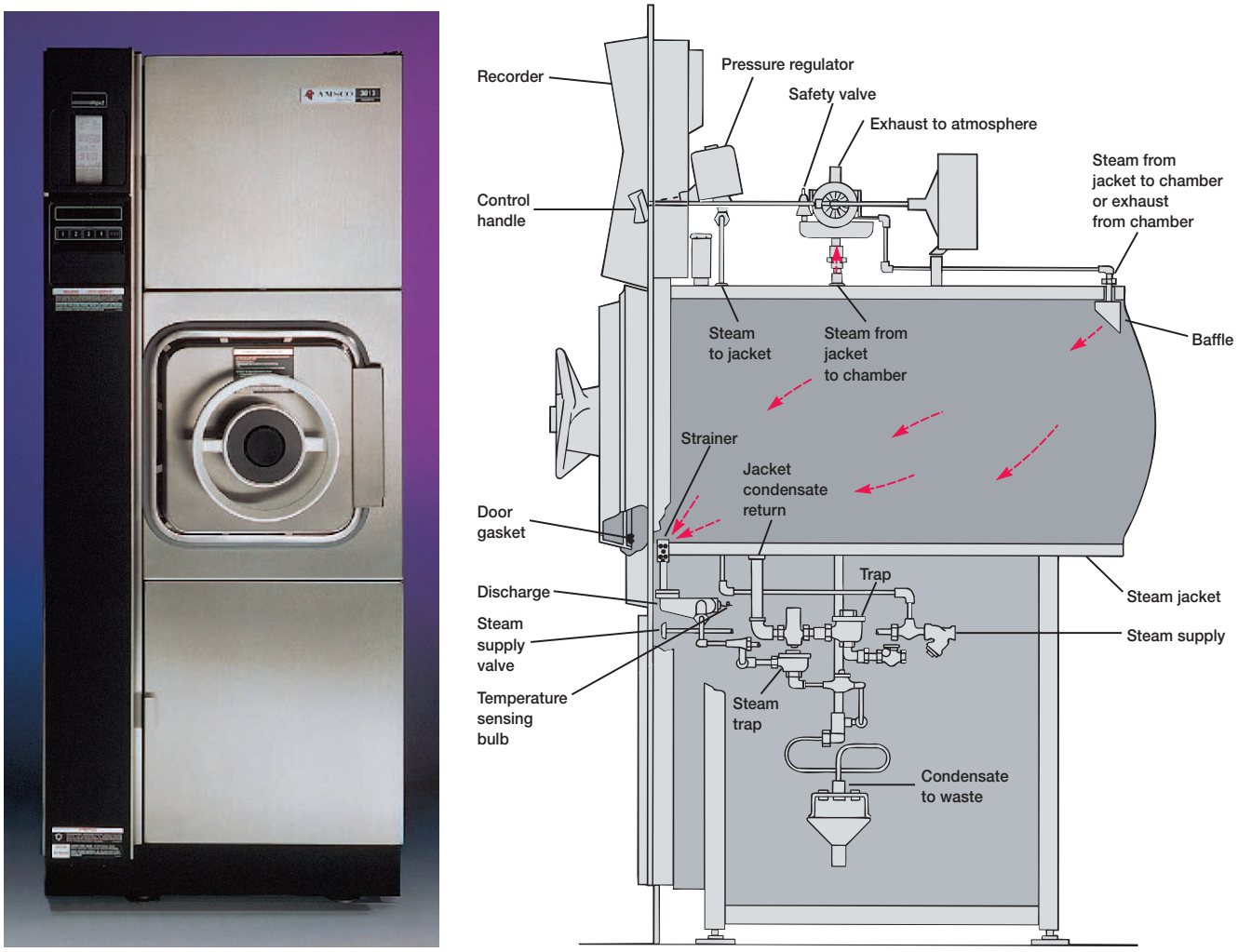



**Figure 7.3** **The Autoclave or Steam Sterilizer.** (**a**) A modern, automatically controlled autoclave or sterilizer. (**b**) Longitudinal cross section of a typical autoclave showing some of its parts and the pathway of steam. *(**b**) From John J. Perkins,* Principles and Methods of Sterilization in Health Science, *2nd edition, 1969. Courtesy of Charles C. Thomas, Publisher, Springfield, Illinois.*

pressure of 15 pounds. The chamber should not be packed too tightly because the steam needs to circulate freely and contact everything in the autoclave. Bacterial endospores will be killed only if they are kept at 121°C for 10 to 12 minutes. When a large volume of liquid must be sterilized, an extended sterilization time will be needed because it will take longer for the center of the liquid to reach 121°C; 5 liters of liquid may require about 70 minutes. In view of these potential difficulties, a biological indicator is often autoclaved along with other material. This indicator commonly consists of a culture tube containing a sterile ampule of medium and a paper strip covered with spores of *Bacillus stearothermophilus* or *Clostridium* PA3679. After autoclaving, the ampule is aseptically broken and the culture incubated for several days. If the test bacterium does not grow in the medium, the sterilization run has been successful. Sometimes either special tape that spells out the word *sterile* or a paper indicator strip that changes color upon sufficient heating is autoclaved with a load of material. If the word appears on the tape or if the color changes after autoclaving, the material is supposed to be sterile. These approaches are convenient and save time but are not as reliable as the use of bacterial endospores.

Many substances, such as milk, are treated with controlled heating at temperatures well below boiling, a process known as **pasteurization** in honor of its developer Louis Pasteur. In the 1860s the French wine industry was plagued by the problem of wine spoilage, which made wine storage and shipping difficult. Pasteur examined spoiled wine under the microscope and detected microorganisms that looked like the bacteria responsible for lactic acid and acetic acid fermentations. He then discovered that a brief heating at 55 to 60°C would destroy these microorganisms and preserve wine for long periods. In 1886 the German chemists V. H. and F. Soxhlet adapted the technique for preserving milk and reducing milk-transmissible diseases. Milk pasteurization was introduced into the United States in 1889. Milk, beer, and many other beverages are now pasteurized. Pasteurization does not sterilize a beverage, but it does kill any pathogens present and drastically slows spoilage by reducing the level of nonpathogenic spoilage microorganisms.

Milk can be pasteurized in two ways. In the older method the milk is held at 63°C for 30 minutes. Large quantities of milk are now usually subjected to **flash pasteurization** or high-temperature short-term (HTST) pasteurization, which consists of quick heating to about 72°C for 15 seconds, then rapid cooling. The dairy industry also sometimes uses **ultrahigh-temperature (UHT) sterilization.** Milk and milk products are heated at 140 to 150°C for 1 to 3 seconds. UHT-processed milk does not require refrigeration and can be stored at room temperature for about 2 months without flavor changes. The small coffee creamer portions provided by restaurants often are prepared using UHT sterilization. Pasteurization and the dairy industry (pp. 970–71)

Many objects are best sterilized in the absence of water by **dry heat sterilization.** The items to be sterilized are placed in an oven at 160 to 170°C for 2 to 3 hours. Microbial death apparently results from the oxidation of cell constituents and denaturation of proteins. Although dry air heat is less effective than moist heat—*Clostridium botulinum* spores are killed in 5 minutes at 121°C by moist heat but only after 2 hours at 160°C with dry heat—it has some definite advantages. Dry heat does not corrode glassware and metal instruments as moist heat does, and it can be used to sterilize powders, oils, and similar items. Most laboratories sterilize glass petri dishes and pipettes with dry heat. Despite these advantages, dry heat sterilization is slow and not suitable for heat-sensitive materials like many plastic and rubber items.

## Low Temperatures

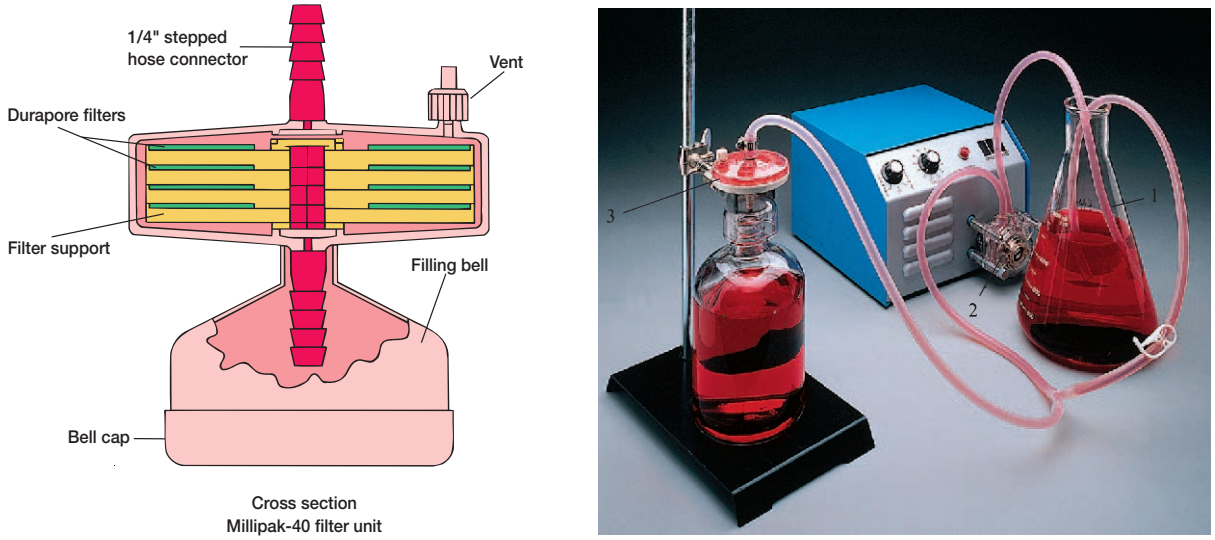
Although our emphasis is on the destruction of microorganisms, often the most convenient control technique is to inhibit their growth and reproduction by the use of either freezing or refrigeration. This approach is particularly important in food microbiology (*see p. 970*). Freezing items at −20°C or lower stops microbial growth because of the low temperature and the absence of liquid water. Some microorganisms will be killed by ice crystal disruption of cell membranes, but freezing does not destroy contaminating microbes. In fact, freezing is a very good method for long-term storage of microbial samples when carried out properly, and many laboratories have a low-temperature freezer for culture storage at −30 or −70°C. Because frozen food can contain many microorganisms, it should be prepared and consumed promptly after thawing in order to avoid spoilage and pathogen growth. Effect of temperature on microbial growth (pp. 125–27)

Refrigeration greatly slows microbial growth and reproduction, but does not halt it completely. Fortunately most pathogens are mesophilic and do not grow well at temperatures around 4°C. Refrigerated items may be ruined by growth of psychrophilic and psychrotrophic microorganisms, particularly if water is present. Thus refrigeration is a good technique only for shorter-term storage of food and other items.

## Filtration

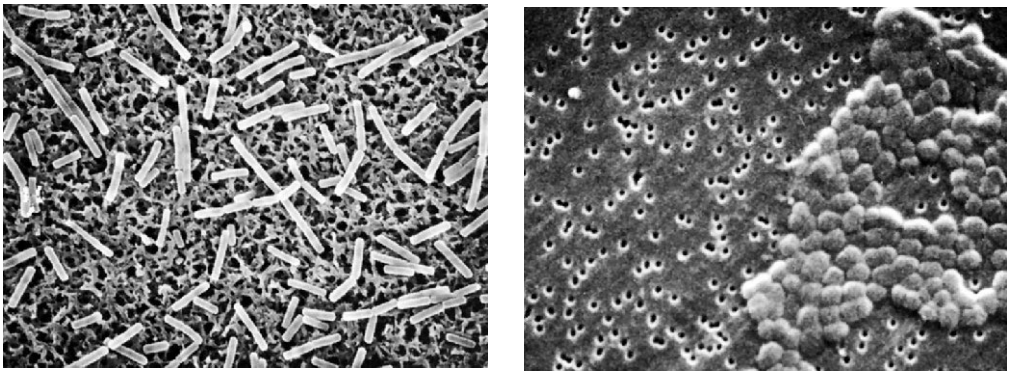
Filtration is an excellent way to reduce the microbial population in solutions of heat-sensitive material, and sometimes it can be used to sterilize solutions. Rather than directly destroying contaminating microorganisms, the filter simply removes them. There are two types of filters. **Depth filters** consist of fibrous or granular materials that have been bonded into a thick layer filled with twisting channels of small diameter. The solution containing microorganisms is sucked through this layer under vacuum, and microbial cells are removed by physical screening or entrapment and also by adsorption to the surface of the filter material. Depth filters are made of diatomaceous earth (Berkefield filters), unglazed porcelain (Chamberlain filters), asbestos, or other similar materials.

**Membrane filters** have replaced depth filters for many purposes. These circular filters are porous membranes, a little over 0.1 mm thick, made of cellulose acetate, cellulose nitrate, polycarbonate, polyvinylidene fluoride, or other synthetic materials. Although a wide variety of pore sizes are available, membranes with pores about 0.2 μm in diameter are used to remove most vegetative cells, but not viruses, from solutions ranging in volume from 1 ml to many liters. The membranes are held in special holders (**figure 7.4**) and often preceded by depth filters made of glass fibers to remove larger particles that might clog the membrane filter. The solution is pulled or forced through the filter with a vacuum




(a) (b)

**Figure 7.4** **Membrane Filter Sterilization.** A membrane filter outfit for sterilizing medium volumes of solution. (**a**) Cross section of the membrane filtering unit. Several membranes are used to increase capacity. (**b**) A complete filtering setup. The solution to be sterilized is kept in the Erlenmeyer flask, *1,* and forced through the filter by a peristaltic pump, *2.* The solution is sterilized by flowing through a membrane filter unit, *3,* and into a sterile container. A wide variety of other kinds of filtering outfits are also available.



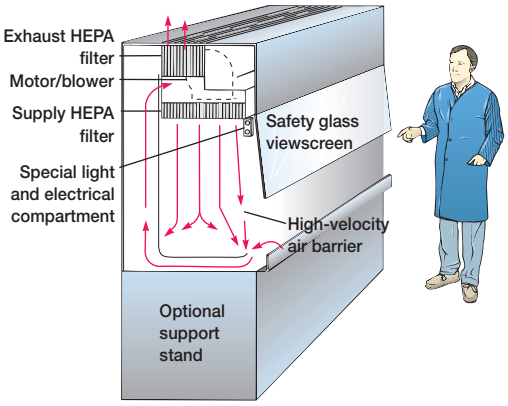
(a) (b)

**Figure 7.5** **Membrane Filter Types.** (**a**) *Bacillus megaterium* on an Ultipor nylon membrane with a bacterial removal rating of 0.2 μm (×2,000). (**b**) *Enterococcus faecalis* resting on a polycarbonate membrane filter with 0.4 μm pores (×5,900).

or with pressure from a syringe, peristaltic pump, or nitrogen gas bottle, and collected in previously sterilized containers. Membrane filters remove microorganisms by screening them out much as a sieve separates large sand particles from small ones (**figure 7.5**). These filters are used to sterilize pharmaceuticals, ophthalmic solutions, culture media, oils, antibiotics, and other heat-sensitive solutions. The use of membrane filters in microbial counting (p. 118)

Air also can be sterilized by filtration. Two common examples are surgical masks and cotton plugs on culture vessels that let air in but keep microorganisms out. **Laminar flow biological safety cabinets** employing **high-efficiency particulate air (HEPA) filters,** which remove 99.97% of 0.3 μm particles, are one of the most important air filtration systems. Laminar flow biological safety cabinets force air through HEPA filters, then project a vertical curtain of sterile air across the cabinet opening. This protects a worker from microorganisms being handled within the cabinet and prevents contamination of the room (**figure 7.6**). A person uses these cabinets when working with dangerous agents such as *Mycobacterium tuberculosis,*

(a)




(b)

**Figure 7.6 A Laminar Flow Biological Safety Cabinet.** (**a**) A technician pipetting potentially hazardous material in a safety cabinet. (**b**) A schematic diagram showing the airflow pattern.

tumor viruses, and recombinant DNA. They are also employed in research labs and industries, such as the pharmaceutical industry, when a sterile working surface is needed for conducting assays, preparing media, examining tissue cultures, and the like.

## Radiation

The types of radiation and the ways in which radiation damages or destroys microorganisms have already been discussed. The practical uses of ultraviolet and ionizing radiation in sterilizing objects are briefly described next. Radiation and its effects on microorganisms (pp. 130–31)

**Ultraviolet (UV) radiation** around 260 nm (*see figure 6.17*) is quite lethal but does not penetrate glass, dirt films, water, and other substances very effectively. Because of this disadvantage, UV radiation is used as a sterilizing agent only in a few specific situations. UV lamps are sometimes placed on the ceilings of rooms or in biological safety cabinets to sterilize the air and any exposed surfaces. Because UV radiation burns the skin and damages eyes, people working in such areas must be certain the UV lamps are off when the areas are in use. Commercial UV units are available for water treatment. Pathogens and other microorganisms are destroyed when a thin layer of water is passed under the lamps.

**Ionizing radiation** is an excellent sterilizing agent and penetrates deep into objects. It will destroy bacterial endospores and vegetative cells, both procaryotic and eucaryotic; however, ionizing radiation is not always as effective against viruses. Gamma radiation from a cobalt 60 source is used in the cold sterilization of antibiotics, hormones, sutures, and plastic disposable supplies such as syringes. Gamma radiation has also been used to sterilize and "pasteurize" meat and other food. Irradiation can eliminate the threat of such pathogens as *Escherichia coli* O157:H7, *Staphylococcus aureus,* and *Campylobacter jejuni.* Both the Food and Drug Administration and the World Health Organization have approved food irradiation and declared it safe. A commercial irradiation plant operates near Tampa, Florida. However, this process has not yet been widely employed in the United States because of the cost and concerns about the effects of gamma radiation on food. The U.S. government currently approves the use of radiation to treat poultry, beef, pork, veal, lamb, fruits, vegetables, and spices. It will probably be more extensively employed in the future.

1. Define thermal death point (TDP), thermal death time (TDT), decimal reduction time (*D*) or *D* value, *z* value, and the *F* value.
2. Describe how an autoclave works. What conditions are required for sterilization by moist heat, and what three things must one do when operating an autoclave to help ensure success?
3. How are pasteurization, flash pasteurization, ultrahigh temperature sterilization, and dry heat sterilization carried out? Give some practical applications for each of these procedures.
4. How can low temperature be used to control microorganisms?
5. What are depth filters and membrane filters, and how are they used to sterilize liquids? Describe the operation of a biological safety cabinet.
6. Give the advantages and disadvantages of ultraviolet light and ionizing radiation as sterilizing agents. Provide a few examples of how each is used for this purpose.

## Box 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 (*see Box 36.1, p. 829*) should be followed.
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.

## 7.5 The Use of Chemical Agents in Control

Although objects are sometimes disinfected with physical agents, chemicals are more often employed in disinfection and antisepsis. Many factors influence the effectiveness of chemical disinfectants and antiseptics as previously discussed. Factors such as the kinds of microorganisms potentially present, the concentration and nature of the disinfectant to be used, and the length of treatment should be considered. Dirty surfaces must be cleaned before a disinfectant or antiseptic is applied. The proper use of chemical agents is essential to laboratory and hospital safety (**Box 7.2;** *see also Box 36.1*). It should be noted that chemicals also are employed to prevent microbial growth in food. This is discussed in the chapter on food microbiology (*see pp. 971–72*).

Many different chemicals are available for use as disinfectants, and each has its own advantages and disadvantages. In selecting an agent, it is important to keep in mind the characteristics of a desirable disinfectant. Ideally the disinfectant must be effective against a wide variety of infectious agents (gram-positive and gram-negative bacteria, acid-fast bacteria, bacterial endospores, fungi, and viruses) at high dilutions and in the presence of organic matter. Although the chemical must be toxic for infectious agents, it should not be toxic to people or corrosive for common materials. In practice, this balance between effectiveness and low toxicity for animals is hard to achieve. Some chemicals are used despite their low effectiveness because they are relatively nontoxic. The disinfectant should be stable upon storage, odorless or with a pleasant odor, soluble in water and lipids for penetration into microorganisms, and have a low surface tension so that it can enter cracks in surfaces. If possible the disinfectant should be relatively inexpensive.

One potentially serious problem is the overuse of triclosan and other germicides. This antibacterial agent is now found in products such as deodorants, mouthwashes, soaps, cutting boards, and baby toys. Triclosan seems to be everywhere. Unfortunately we are already seeing the emergence of triclosan-resistant bacteria. *Pseudomonas aeruginosa* actively pumps the antiseptic out the cell. Bacteria seem to be responding to antiseptic overuse in the same way as they reacted to antibiotic overuse (*see pp. 818–20*). There is now some evidence that extensive use of triclosan also increases the frequency of antibiotic resistance in bacteria. Thus overuse of antiseptics can have unintended harmful consequences.

The properties and uses of several groups of common disinfectants and antiseptics are surveyed next. Many of their characteristics are summarized in **tables 7.4** and **7.5.** Structures of some common agents are given in **figure 7.7.**

### Phenolics

Phenol was the first widely used antiseptic and disinfectant. In 1867 Joseph Lister employed it to reduce the risk of infection during operations. Today phenol and phenolics (phenol

## 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.

## 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.

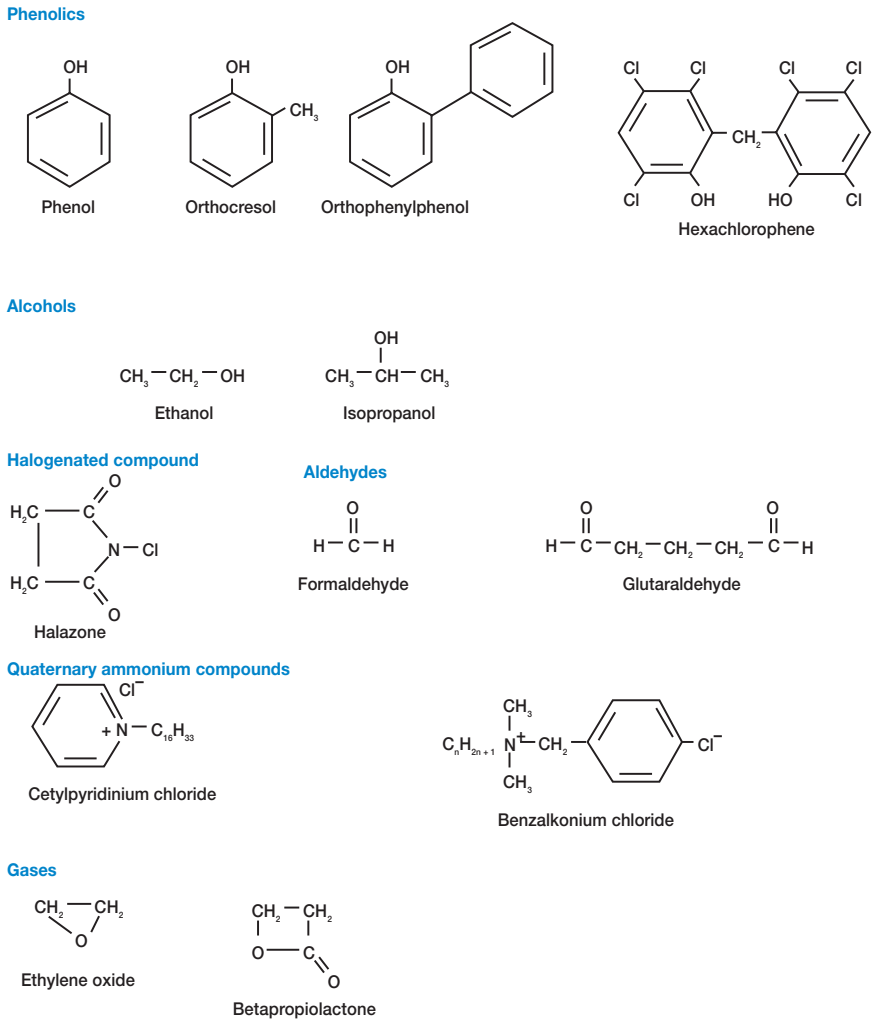



**Figure 7.7 Disinfectants and Antiseptics.** The structures of some frequently used disinfectants and antiseptics.

derivatives) such as cresols, xylenols, and orthophenylphenol are used as disinfectants in laboratories and hospitals. The commercial disinfectant Lysol is made of a mixture of phenolics. Phenolics act by denaturing proteins and disrupting cell membranes. They have some real advantages as disinfectants: phenolics are tuberculocidal, effective in the presence of organic material, and remain active on surfaces long after application. However, they do have a disagreeable odor and can cause skin irritation.

Hexachlorophene (figure 7.7) has been one of the most popular antiseptics because it persists on the skin once applied and reduces skin bacteria for long periods. However, it can cause brain damage and is now used in hospital nurseries only in response to a staphylococcal outbreak.

## Alcohols

Alcohols are among the most widely used disinfectants and antiseptics. They are bactericidal and fungicidal but not sporicidal; some lipid-containing viruses are also destroyed. The two most popular alcohol germicides are ethanol and isopropanol, usually used in 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, 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.

$$Cl_2 + H_2O \longrightarrow HCl + HClO$$

$$Ca(OCl)_2 + 2H_2O \longrightarrow Ca(OH)_2 + 2HClO$$

$$HClO \longrightarrow HCl + O$$

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 sometimes has been used successfully as an alternative to chlorination in Europe and Canada. Municipal water purification (pp. 651–53)

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 1/100 dilution of household bleach (e.g., 1.3 fl oz of Clorox or Purex bleach in 1 gal or 10 ml/liter) is combined with sufficient nonionic detergent (about 1 oz/gal or 7.8 ml/liter) 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. More recently these have been superseded by other less toxic and more effective germicides (many heavy metals are more bacteriostatic than bactericidal). There are a few exceptions. A 1% solution of silver nitrate is often added to the eyes of infants to prevent ophthalmic gonorrhea (in many hospitals, erythromycin is used instead of silver nitrate because it is effective against *Chlamydia* as well as *Neisseria*). 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

**Detergents** [Latin *detergere,* to wipe off or away] are organic molecules that serve as wetting agents and emulsifiers because they have both polar hydrophilic and nonpolar hydrophobic ends. Due to their amphipathic nature (*see section 3.2*), detergents solubilize otherwise insoluble residues and are very effective cleansing agents. They are different than soaps, which are derived from fats.

Although anionic detergents have some antimicrobial properties, only cationic detergents are effective disinfectants. The most popular of these disinfectants are quaternary ammonium compounds characterized by a positively charged quaternary nitrogen and a long hydrophobic aliphatic chain (figure 7.7). They disrupt microbial membranes and may also denature proteins.

Cationic detergents like benzalkonium chloride and cetylpyridinium chloride kill most bacteria but not *M. tuberculosis* or endospores. They do have the advantages of being stable, nontoxic, and bland 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, are highly reactive molecules that combine with nucleic acids and proteins and inactivate them, probably by cross-linking and alkylating molecules (figure 7.7). 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 now sterilized with ethylene oxide gas (figure 7.7). 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. Because pure EtO is explosive, it is usually supplied in a 10 to 20% concentration mixed with either $CO_2$ 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 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.

Recently vapor-phase hydrogen peroxide has been used to decontaminate biological safety cabinets.

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 (iodine and chlorine), heavy metals, quaternary ammonium compounds, aldehydes, and ethylene oxide.

## 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 experimental disinfectant are inoculated with the test bacteria *Salmonella typhi* and *Staphylococcus aureus,* then placed in a 20 or 37°C water bath. These inoculated disinfectant tubes are next subcultured to regular fresh medium at 5 minute intervals, and the subcultures are incubated for two or more days. The highest dilutions that kill the bacteria after a 10 minute exposure, but not after 5 minutes, are used to calculate the phenol coefficient. The reciprocal of the appropriate test disinfectant dilution is divided by that for phenol to obtain the coefficient. Suppose that the phenol dilution was 1/90 and maximum effective dilution for disinfectant X was 1/450. 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.**

**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.

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 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

1. 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.
2. Many terms are used to define how microorganisms are controlled: sterilant, disinfectant, sanitization, antisepsis, and antiseptic.
3. Antimicrobial agents that kill organisms often have the suffix -cide, whereas agents that prevent growth and reproduction have the suffix -static.
4. Microbial death is usually exponential or logarithmic (**figure 7.1**).
5. 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.
6. The efficiency of heat killing is often indicated by the thermal death time or the decimal reduction time.
7. 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**).
8. Moist heat kills by degrading nucleic acids, denaturing enzymes and other proteins, and disrupting cell membranes.
9. Heat-sensitive liquids can be pasteurized by heating at 63°C for 30 minutes or at 72°C for 15 seconds (flash pasteurization). Heating at 140 to 150°C for 1 to 3 seconds (ultrahigh-temperature sterilization) may be used.
10. Glassware and other heat-stable items may be sterilized by dry heat at 160 to 170°C for 2 to 3 hours.
11. Refrigeration and freezing can be used to control microbial growth and reproduction.
12. Microorganisms can be efficiently removed by filtration with either depth filters or membrane filters.
13. Biological safety cabinets with high-efficiency particulate filters sterilize air by filtration (**figure 7.6**).
14. Radiation of short wavelength or high-energy ultraviolet and ionizing radiation can be used to sterilize objects.
15. Chemical agents usually act as disinfectants because they cannot readily destroy bacterial endospores. Disinfectant effectiveness depends on concentration, treatment duration, temperature, and presence of organic material (**tables 7.4** and **7.5**).
16. Phenolics and alcohols are popular disinfectants that act by denaturing proteins and disrupting cell membranes (**figure 7.7**).
17. Halogens (iodine and chlorine) kill by oxidizing cellular constituents; cell proteins may also be iodinated. Iodine is applied as a tincture or iodophor. Chlorine may be added to water as a gas, hypochlorite, or an organic chlorine derivative.
18. Heavy metals tend to be bacteriostatic agents. They are employed in specialized situations such as the use of silver nitrate in the eyes of newborn infants and copper sulfate in lakes and pools.
19. Cationic detergents are often used as disinfectants and antiseptics; they disrupt membranes and denature proteins.
20. Aldehydes such as formaldehyde and glutaraldehyde can sterilize as well as disinfect because they kill spores.
21. Ethylene oxide gas penetrates plastic wrapping material and destroys all life forms by reacting with proteins. It is used to sterilize packaged, heat-sensitive materials.
22. A variety of procedures can be used to determine the effectiveness of disinfectants, among them the following: phenol coefficient test, measurement of killing rates with germicides, use dilution testing, and in-use testing.

## Key Terms

algicide *138*
antimicrobial agent *139*
antisepsis *138*
antiseptics *138*
autoclave *140*
bactericide *138*
bacteriostatic *138*
*D* value *140*
decimal reduction time (D) *140*
depth filters *142*
detergent *148*
disinfectant *138*
disinfection *138*
dry heat sterilization *142*
*F* value *140*
flash pasteurization *142*
fungicide *138*
fungistatic *138*
germicide *138*
high-efficiency particulate air (HEPA) filters *143*
iodophor *148*
ionizing radiation *144*
laminar flow biological safety cabinets *143*
membrane filters *142*
pasteurization *142*
phenol coefficient test *149*
sanitization *138*
sterilization *137*
thermal death time (TDT) *140*
ultrahigh-temperature (UHT) sterilization *142*
ultraviolet (UV) radiation *144*
use dilution test *149*
viricide *138*
*z* value *140*

## Questions for Thought and Review

1. How can the *D* value be used to estimate the time required for sterilization? Suppose that you wanted to eliminate the risk of *Salmonella* poisoning by heating your food ($D_{60}$ = 0.40 minutes, *z* value = 5.0). Calculate the 12*D* value at 60°C. How long would it take to achieve the same results by heating at 50, 55, and 65°C?
2. How can one alter treatment conditions to increase the effectiveness of a disinfectant?
3. How would the following be best sterilized: glass pipettes and petri plates, tryptic soy broth tubes, nutrient agar, antibiotic solution, interior of a biological safety cabinet, wrapped package of plastic petri plates?
4. 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.)? List all chemicals suitable for each task.
5. Until relatively recently, spoiled milk was responsible for a significant proportion of infant death.
   a. Why is untreated milk easily spoiled?
   b. Why is boiling milk over prolonged periods not desirable?
6. In table 7.3 why is the *D* value so different for the three conditions in which *S. aureus* might be found?

## Critical Thinking Questions

1. Throughout history, spices have been used as preservatives and to cover up the smell/taste of food that is slightly spoiled. The success of some spices led to a magical, ritualized use of many of them and possession of spices was often limited to priests or other powerful members of the community.
   a. Choose a spice and trace its use geographically and historically. What is its common-day use today?
   b. Spices grow and tend to be used predominantly in warmer climates. Explain.
2. Design an experiment to determine whether an antimicrobial agent is acting as a cidal or static agent. How would you determine whether an agent is suitable for use as an antiseptic rather than as a disinfectant?
3. Suppose that you are testing the effectiveness of disinfectants with the phenol coefficient test and obtained the following results.

| Dilution | *Bacterial Growth after Treatment* | | |
|---|---|---|---|
| | Disinfectant A | Disinfectant B | Disinfectant C |
| 1/20 | − | − | − |
| 1/40 | + | − | − |
| 1/80 | + | − | + |
| 1/160 | + | + | + |
| 1/320 | + | − | + |

What disinfectant can you safely say is the most effective? Can you determine its phenol coefficient from these results?

## Additional Reading


### General

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