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CHAPTER

2 LABORATORY SAFETY

Microbiology laboratory safety practices were first published in 1913 in a textbook by Eyre.[2] They included admonitions such as the necessity to (1) wear gloves, (2) wash hands after working with infectious materials, (3) disinfect all instruments immediately after use, (4) use water to moisten specimen labels rather than the tongue, (5) disinfect all contaminated waste before discarding, and (6) report to appropriate personnel all accidents or exposures to infectious agents.

These guidelines are still incorporated into safety programs in the late twentieth-century laboratory. In addition, safety programs have been expanded to include not only the proper handling of biologic hazards encountered in processing patient specimens or handling infectious microorganisms but also fire safety; electrical safety; the safe handling, storage and disposal of chemicals and radioactive substances; and techniques for the safe lifting or moving of heavy objects. In areas of the country prone to natural disasters (e.g., earthquakes, hurricanes, snowstorms), safety programs also involve disaster preparedness plans that outline steps to take in an emergency. Although all microbiologists are responsible for their own health and safety, their institution and immediate supervisors are required to provide safety training to help familiarize microbiologists with known hazards and to avoid accidental exposure. Laboratory safety is considered such an integral part of overall laboratory services that federal law in the United States mandates preemployment safety training followed by at least quarterly safety in-services. Microbiologists should find very little reason to be afraid while performing duties if the safety regulations are internalized and followed without deviation. Investigation of the causes of accidents usually shows that accidents happen when individuals become sloppy in performing their duties or when they do not believe that they will be affected by departures from safety standards.

STERILIZATION AND DISINFECTION

Sterilization is a process whereby all forms of microbial life, including bacterial spores, are killed. Sterilization may be accomplished by physical or chemical means. **Disinfection** is a process whereby pathogenic organisms, but not necessarily all microorganisms or spores, are destroyed. As with sterilization, disinfection may be accomplished by physical or chemical methods.

METHODS OF STERILIZATION

The **physical** methods of sterilization include the following:

- Incineration
- Moist heat
- Dry heat
- Filtration
- Ionizing (gamma) radiation

Incineration is the most common method of treating infectious waste. Hazardous material is literally burned to ashes at temperatures of 870° to 980° C. Toxic air emissions and the presence of heavy metals in ash have limited the use of incineration in most large U.S. cities, however.

Moist heat (steam under pressure) is used to sterilize biohazardous trash and heat-stable objects; an autoclave is used for this purpose. An autoclave is essentially a large pressure cooker. Moist heat in the form of saturated steam under 1 atmosphere (15 psi [pounds per square inch]) of pressure causes the irreversible denaturation of enzymes and structural proteins. The most common type of steam sterilizer in the microbiology laboratory is the gravity displacement type shown in Figure 2-1. Steam enters at the top of the sterilizing chamber and, because steam is lighter than air, it displaces the air in the chamber and forces it out the bottom through the drain vent. The two common sterilization temperatures are 121° C (250° F) and 132° C (270° F). Items such as media, liquids, and instruments are usually autoclaved for 15 minutes at 121° C. Infectious medical waste, on the other hand, is often sterilized at 132° C for 30 to 60 minutes to allow penetration of the steam throughout the waste and the displacement of air trapped inside

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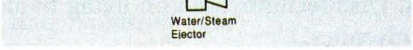

FIGURE 2-1 Gravity displacement type autoclave. **A,** Typical Eagle Century Series sterilizer for laboratory applications. **B,** Typical Eagle 3000 sterilizer piping diagram. The arrows show the entry of steam into the chamber and the displacement of air. (Courtesy AMSCO International Inc, a wholly owned subsidiary of STERIS Corp., Mentor, Ohio.)

the autoclave bag. Moist heat is the fastest and simplest physical method of sterilization.

Dry heat requires longer exposure times (1.5 to 3 hours) and higher temperatures than moist heat (160° to 180° C). Dry-heat ovens are used to sterilize items such as glassware, oil, petrolatum, or powders. Filtration is the method of choice for antibiotic solutions, toxic chemicals, radioisotopes, vaccines, and carbohydrates, which are all heat-sensitive. Filtration of liquids is accomplished by pulling the solution through a cellulose acetate or cellulose nitrate membrane with a vacuum. Filtration of air is accomplished using high-efficiency-particulate-air (HEPA) filters designed to remove organisms larger than 0.3 μm from isolation rooms, operating rooms, and biological safety cabinets (BSCs). Ionizing radiation used in microwaves and radiograph machines are short wavelength and high-energy gamma rays. Ionizing radiation is used for sterilizing disposables such as plastic syringes, catheters, or gloves before use.

The most common **chemical** sterilant is ethylene oxide (EtO), which is used in gaseous form for sterilizing heat-sensitive objects. Formaldehyde vapor and vapor-phase hydrogen peroxide (an oxidizing agent) have been used to sterilize HEPA filters in BSCs. Gluteraldehyde, which is sporocidal (kills spores) in 3 to 10 hours, is used for medical equipment such as bronchoscopes, because it does not corrode lenses, metal, or rubber. Peracetic acid, effective in the presence of organic material, has also been used for the surface sterilization of surgical instruments. The use of gluteraldehyde or peracetic acid is called **cold sterilization.**

METHODS OF DISINFECTION

PHYSICAL METHODS OF DISINFECTION

The three **physical** methods of disinfection are:

- Boiling at 100° C for 15 minutes, which kills vegetative bacteria
- Pasteurizing at 63° C for 30 minutes or 72° C for 15 seconds, which kills food pathogens
- Using nonionizing radiation such as ultraviolet (UV) light

UV rays are long wavelength and low energy. They do not penetrate well and organisms must have direct surface exposure, such as the working surface of a BSC, for this form of disinfection to work.

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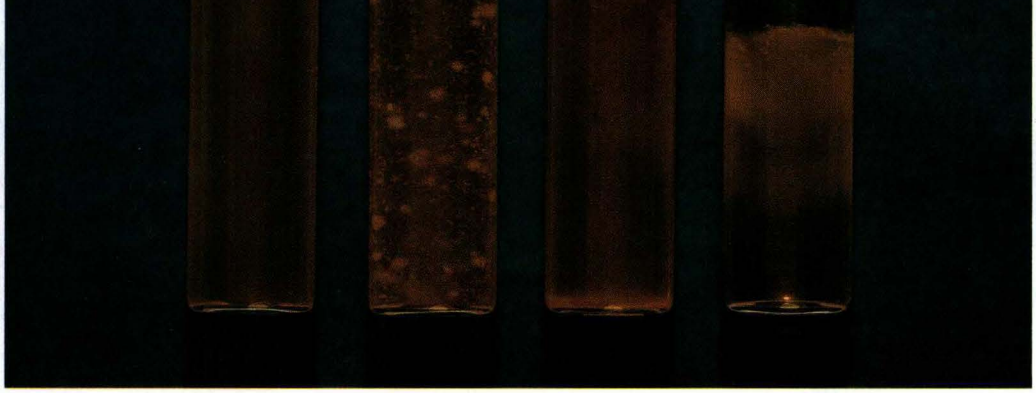

A B C D

FIGURE 12-7 Growth characteristics of various bacteria in thioglycollate broth. **A,** Facultatively anaerobic gram-negative bacilli (i.e., those that grow in the presence or absence of oxygen) grow throughout broth. **B,** Gram-positive cocci grow as "puff balls." **C,** Strictly aerobic organisms (i.e., those that require oxygen for growth), such as *Pseudomonas aeruginosa,* grow toward the top of the broth. **D,** Strictly anaerobic organisms (i.e., those that do not grow in the presence of oxygen) grow in the bottom of the broth.

many gram-negative bacilli that are not enteric pathogens and inhibits gram-positive organisms. A phenol red indicator in the medium detects increased acidity from carbohydrate (i.e., lactose, xylose, and sucrose) fermentation. Enteric pathogens, such as *Shigella* spp., do not ferment these carbohydrates so their colonies remain colorless (i.e., the same approximate pink to red color of the uninoculated medium). Colonies of *Salmonella* spp. are also colorless on XLD, because of the decarboxylation of lysine, which results in a pH increase that causes the pH indicator to turn red. These colonies often exhibit a black center that results from *Salmonella* spp. producing H_2S. Several of the nonpathogens ferment one or more of the sugars and produce yellow colonies (Figure 12-8).

Preparation of artificial media

Nearly all media are commercially available as ready-to-use agar plates or tubes of broth. If media are not purchased, laboratory personnel can prepare agars and broths using dehydrated powders that are reconstituted in water (distilled or deionized) according to manufacturer's recommendations. Generally, media are reconstituted by dissolving a specified amount of media powder, which usually contains all necessary components, in water. Boiling is often required to dissolve the powder, but specific manufacturer's instructions printed in media package inserts should be followed exactly. Most media require sterilization so that only bacteria from patient specimens will grow and not those that are contaminants from water or the powdered media. Broth media are distributed to individual tubes before sterilization. Agar media are usually sterilized in large flasks or bottles capped with either plastic screw caps or plugs before being placed in an autoclave.

MEDIA STERILIZATION The timing of **autoclave sterilization** should start from the moment the temperature reaches 121° C and usually requires a minimum of 15 minutes. Once the sterilization cycle is completed, molten agar is allowed to cool to approximately 50° C before being distributed to individual petri plates (usually 25 mL of molten agar per plate). If other

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