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Safe Laboratory Techniques

Research with disease-producing microorganisms has always presented hazards for the unwary. In the late nineteenth century, as the relationship between microorganisms and human diseases began to be understood, the scientists who were studying microorganisms that had been isolated from disease victims sometimes also became victims of the same disease. For example, in 1884, typhoid bacilli were first isolated and less than a year later the first case of laboratory-acquired typhoid fever occurred. 1884 was also the year that the famous German bacteriologist, Robert Koch, first isolated the etiologic agent of cholera, and in 1886 a laboratory-acquired cholera infection was reported, presumably the result of a pipetting accident, probably aspiration. Three years later, in 1889, the tetanus bacillus was isolated and four years after that, a syringe-and-needle accident resulted in a tetanus infection in a laboratory worker. Other laboratory-acquired infections involving pipets and syringes and needles occurred with the agents of diphtheria, glanders, and brucellosis during 1897 and 1898. In fact, the history of microbiology is replete with stories of medical martyrs who, in the absence of knowledge of the extreme hazards of the organisms they studied, both deliberately or accidentally became infected and died of laboratory-acquired disease. Working with agents of such deadly diseases eventually taught research workers the proper techniques necessary for handling hazardous agents, and these techniques are now among the fundamental skills that every worker in a biomedical laboratory must learn.

The most basic safety principles deal with proper handling of laboratory equipment, which is the topic of this chapter. The pioneers of microbiology learned that even the most common of the laboratory techniques, those that are repeated constantly, such as pipetting and using syringes and needles, have associated hazards. These hazards can be grouped into three general categories: inoculation of agents through puncture wounds or cuts, or splashes onto mucous membranes; ingestion of agents by, for example, mouth contact with contaminated fingers or aspiration of liquids during mouth pipetting; and inhalation of agents that have become airborne as aerosols.

Many of the precautions discussed in this chapter are concerned with avoiding aerosol production, which is the single greatest hazard associated with such operations as pipetting, centrifuging, lyophilizing, blending, sonicating, and similar techniques in which mechanical energy is imparted to fluids. However, hazards from self-inoculation (cuts or puncture wounds caused by pipets or syringes and needles), ingestion, or even mechanical injury resulting from the improper use of such equipment as centrifuges, freezers, or shakers must not be ignored. This chapter discusses common biomedical laboratory operations, with particular attention given to identifying sources of hazards and listing suggestions for safe practices to avoid injury.

S. R. Rayburn, *The Foundations of Laboratory Safety*

1.1 Pipets*

Pipets (351, 373) are one of the basic tools of biomedical research. They are used for volumetric measurement of fluids and for the transfer of these fluids from one container to another. The fluids that are handled are frequently hazardous in nature, containing infectious, toxic, corrosive, volatile, or radioactive agents. A pipet can become a hazardous piece of equipment if improperly used (209, 350, 499). Safety pipetting techniques are required to reduce the potential for exposure to hazardous materials. The most common hazard associated with pipetting procedures involves the application of mouth suction (209, 350, 499). More than 13 percent of all known laboratory accidents that resulted in infection were caused by oral aspiration through a pipet. Also, contaminants can be transferred to the mouth if a contaminated finger is placed on the suction end of the pipet. There is also the danger of aspirating aerosols created by pipetting liquids when using unplugged pipets, even if no liquid is drawn into the mouth.

Additional hazards of exposure to aerosols are created when liquid drops from a pipet to a work surface (474), when cultures are mixed by alternate suction and expulsion (209, 350, 499), when an inoculum is forcefully ejected onto a culture dish, or when the last drop is blown out (113, 209). It has been demonstrated by high-speed photography that an aerosol of approximately 15,000 droplets, most under ten micrometers in diameter, is produced when the last drop of fluid in the tip of a pipet is blown out with moderate force (Figure 1.1). While the aerosol hazard associated with pipetting procedures can only be reduced by use of safe techniques and use of biological safety cabinets (240), the potential hazards associated with oral ingestion can be eliminated by use of mechanical pipetting aids (426, 428).

A list of safe pipetting techniques is shown in Table 1.1. Figures 1.2, 1.3, and 1.4 illustrate various pipetting aids. Many more varieties of mechanical pipetting aids are available from scientific supply vendors or safety equipment suppliers. The careless use of pipetting aids has its own associated hazards. One of these hazards arises when the pipet is inserted into the device. The pipet can break if force is improperly applied, possibly injuring the worker. (See Section 1.11 and Figure 1.8.)

FIGURE 1.1 Aerosol from a pipet. This aerosol was created by blowing out the last drop from a pipet. (Source: National Institutes of Health.)

It is also necessary to use a safe method for storing contaminated pipets until they can be washed. They should be placed horizontally in a pan containing enough suitable decontaminant solution to allow their complete immersion (209), rather than being dropped vertically into a cylinder. There are several reasons for avoiding the use of vertical cylinders. First, hydraulic pressure will force an aerosol of residual liquids and contaminants from the bore of the pipet out through its upper end as it is dropped into the cylinder. Second, flat trays can be placed directly in the autoclave, thus

*Excerpted from (477).

TABLE 1.1 Safe pipetting practices

1. Never pipet by mouth. Always use some type of pipetting aid.
2. If working with infectious or toxic fluid, pipetting operations should be confined to a safety cabinet or hood.
3. Pipets used for the pipetting of infectious or toxic materials always should be plugged with cotton. This avoids collecting aerosols of the pipetted solution within the mechanical pipetting device.
4. No infectious material should be prepared by bubbling expiratory air through a liquid with a pipet.
5. Infectious material should not be mixed by alternate suction and expulsion through a pipet.
6. No infectious material should be forcibly expelled from a pipet.
7. When pipets are used, avoid accidentally dropping infectious cultures from the pipet. Place a disinfectant-soaked towel on the working surface and autoclave the towel after use.
8. Mark-to-mark pipets are preferable to other types, since they do not require expulsion of the last drop.
9. Discharge from pipets should be as close as possible to the fluid or agar level, or the contents should be allowed to run down the wall of the tube or bottle whenever possible, not dropped from a height.
10. Contaminated pipets should be placed horizontally in a pan containing enough suitable decontaminant to allow complete immersion. They should not be placed vertically in a cylinder.
11. Discard pans for used pipets are to be housed within the biological safety cabinet.
12. The pan and the pipets should be autoclaved as a unit and replaced by a clean pan with fresh disinfectant.

From (477).

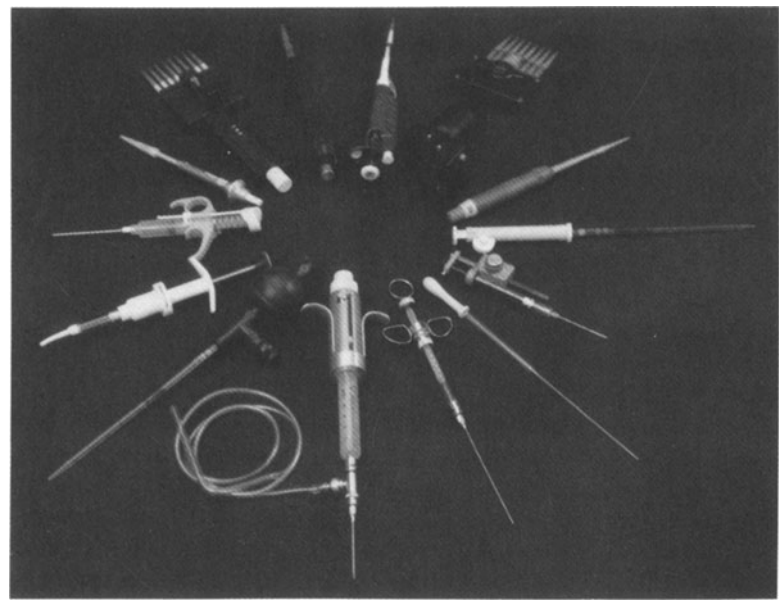

FIGURE 1.2 Mechanical pipetting devices used to avoid the hazards of mouth pipetting.

minimizing handling of contaminated pipets and disinfectant solution. Finally, vertical cylinders will not fit well in biological safety cabinets and hand motions back and forth into and out of the work chamber must be minimized (see Chapter 9).

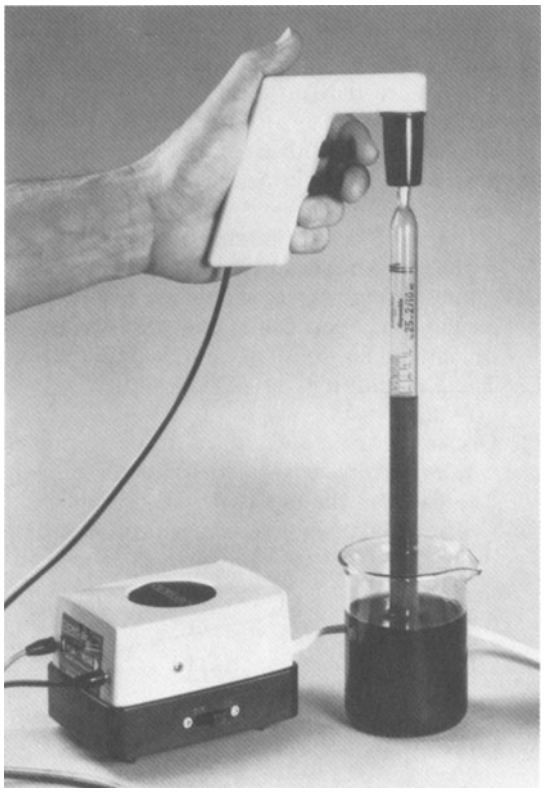

FIGURE 1.3 Mechanical pipettor operated by an air pump. (Courtesy of Drummond Scientific Co.)

Since no independent studies have been performed on the safety characteristics of the various pipets available, it is advisable that each research group evaluate pipet performance according to the following criteria.

- No uncontrollable discharge (leakage from the pipet or disposable tip).
- No contamination of the suction end of the pipet and, of course, of the pipetting aid, operator, and/or vacuum lines.
- Will not produce aerosols or splash to create surface contamination.
- Can be cleaned and/or sterilized in the case of accidental contamination or during routine maintenance.
- Is reasonably easy and convenient to use without requiring undue manipulation or effort.
- Will function effectively and easily for all pipet volumes in its design range.

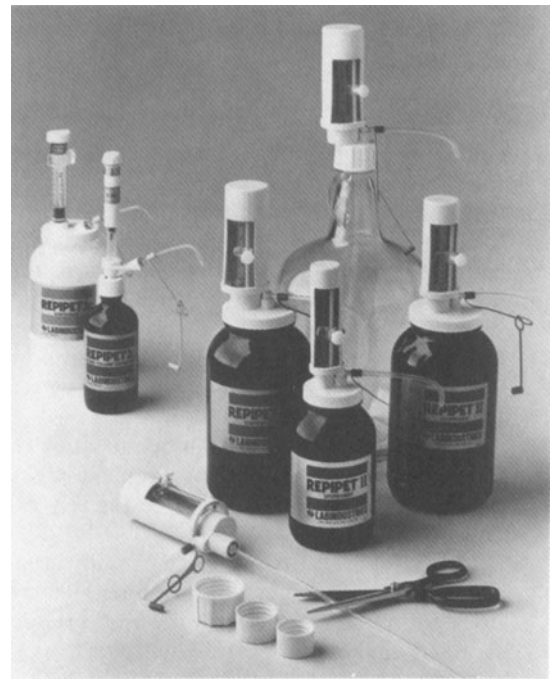

FIGURE 1.4 Bottle dispensers. These devices allow the safe dispensing of liquids from large containers. (Courtesy of Labindustries, Inc.)

The Pasteur pipet requires special mention. Because of its sharp tip, it requires handling and disposal with great care to prevent injury. Many laboratory workers as well as custodial and service workers have been jabbed by the extremely sharp tip. Such injuries have occurred to workers' hands and arms, and even to legs (from carrying waste bags). See Chapter 7 for a discussion of disposal of such "sharps."

1.2 Syringes and Needles*

The hypodermic needle is a potentially dangerous instrument (192, 474). A syringe and needle should never be used as a substitute for a pipet. This will avoid two common sources of accidents: needle punctures, and exposure to aerosols of hazardous materials. The following procedures should be used when inoculating animals with a syringe and needle.

*Excerpted from (477).

- Use a regular beveled needle for parenteral injections and a blunt needle or a cannula on a syringe for oral or intranasal inoculations.
- For inoculation of pathogens or other hazardous materials, use the syringe and needle in a biological safety cabinet (see Chapter 9) only and avoid quick and unnecessary movements of the hand that holds the syringe.
- After washing and prior to sterilization, examine glass syringes for chips and cracks and reuseable needles for barbs and plugs.
- Use needle-locking (e.g., Luer-Lok™ type) syringes only (209), and be sure that the needle is locked securely into the barrel. A disposable syringe-needle unit (where the needle is an integral part of the unit) is preferred. Figure 1.5 illustrates a wide variety of available syringe and needle units.
- Wear surgical synthetic rubber gloves for all manipulations with needles and syringes.
- Fill the syringe carefully to minimize air bubbles and frothing of the inoculum.
- Hold the syringe vertically and expel excess air, liquid, and bubbles into a cotton pledget (swab) moistened with the proper disinfectant, or into a small bottle of sterile cotton (209).
- Do not use the syringe to expel fluid forcefully into an open vial or tube for the purpose of mixing. This practice can create considerable aerosols. Mixing with a syringe should be performed only with the tip of the needle held below the surface of the fluid in the tube.
- If syringes are filled from test tubes, take care not to contaminate the hub of the needle, as this may result in transfer of hazardous material to the fingers.
- When removing a syringe and needle from a rubber-stoppered bottle, wrap the needle and stopper in a cotton pledget moistened with the proper disinfectant (209). If there is danger of the disinfectant contaminating sensitive experiments, a sterile dry pledget may be used and discarded immediately into disinfectant solution.
- Inoculate animals with the hand "behind" the needle to avoid punctures.
- Be sure the animal is properly restrained prior to the inoculation, and be on the alert for any unexpected movements of the animal.
- Before and after injection of an animal with a biohazardous agent, swab the site of injection with a disinfectant (209).
- Discard syringes into a pan of disinfectant without removing the needle. The syringe may first be filled with disinfectant by immersing the needle and slowly withdrawing the plunger, and finally removing the plunger and placing it separately into the disinfectant. This filling action clears the needle and

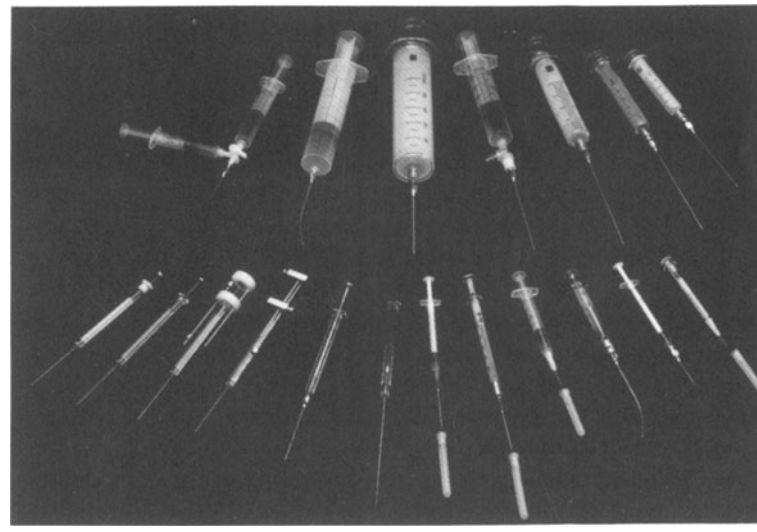

FIGURE 1.5 Syringe and needle units. Needles should only be used for inoculation of animals, for withdrawing blood, other body fluids, or tissues, and for piercing seals of bottles for the transfer of liquids under sterile conditions. Use blunt needles or cannulas for other operations.

dilutes the contents of the syringe. *Autoclave syringes and needles in the pan of disinfectant.*

- Use separate pans of disinfectant for disposable and nondisposable syringes and needles to eliminate a sorting problem in the service area.
- Do not place used syringes on the work surface or on gauze or tissue to be discarded. Place used syringes into a holder or into a container used only for this purpose. This avoids injury from needle punctures from overlooked needles when the gauze or tissue is gathered up for disposal.
- Do not discard syringes and needles into pans containing pipets or other glassware that must be sorted out from the syringes and needles.
- After decontamination, destroy disposable needles and syringes, taking care to label the discards to prevent accidental injury to the custodial staff. See Chapter 7 for further details.

1.3 Centrifuging*

Centrifuging (26, 374) presents the possibility of two serious hazards: mechanical failure and creation of aerosols. A mechanical failure, such as a broken drive shaft, a faulty bearing, or a disintegrated rotor, can produce not only aerosols but also hazardous fragments moving at great velocity. If these fragments escape the protective chamber of the centrifuge they could cause great injury. A well-functioning centrifuge, however, may still produce hazardous aerosols of biological materials or chemicals if improperly or carelessly used. Observe the manufacturer's instructions carefully to minimize the risk of mechanical failure; follow sound laboratory practices and use appropriate centrifuge safety equipment (188) or biological safety cabinets (244, 375, 415) to eliminate production of aerosols. Be certain that all users of the centrifuge are properly trained, and prominently post on the device operating instructions that include safety precautions.

*Excerpted from (477).

Although improper use of centrifuges and associated equipment leads to fewer accidents than improper use of pipets and syringes and needles, when such accidents do occur they usually result in the formation of aerosols. Because of the size of the equipment, an aerosol produced by a centrifuge accident can potentially affect more people than pipet- or syringe-produced aerosols (502).

Practices such as filling centrifuge tubes, removing cotton plugs or caps from tubes after centrifuging, removing the supernatant, and resuspending the sedimented pellet may also release aerosols into the environment. The greatest hazard associated with centrifuging biohazardous materials results if a centrifuge tube breaks. When tubes break or crack and the contents are retained in the rotor cavity or bucket under centrifugal force, relatively little aerosol is produced compared to breakage that releases the fluid into the centrifuge chamber.

Safety procedures for centrifuges

When used with biohazardous materials, centrifuge tubes, rotors, and accessories should be filled and opened in a biological safety cabinet (215). If centrifuging of biohazardous substances is to be performed outside a special containment cabinet, a sealed safety bucket or tube should be used (Figure 1.6). After the safety bucket or tube is filled and sealed, it should be considered potentially contaminated and should be wiped with a cloth soaked in disinfectant. Since some disinfectants are corrosive to centrifuge rotors and buckets, rinsing the rotor or bucket with clean water is desirable after an appropriate contact time in disinfectant has elapsed.

If the potential for a leak exists, and safety buckets that seal with "O" rings are not available, tubes can be enclosed in sealed plastic bags before centrifuging (309). Should the tube break, however, the plastic bag is likely to rupture. Therefore, this technique normally only prevents the escape of materials that otherwise may contaminate the outside of the bucket.

Before centrifuging, check and discard tubes with cracks and chipped rims, inspect the inside of each tube cavity or bucket, correct

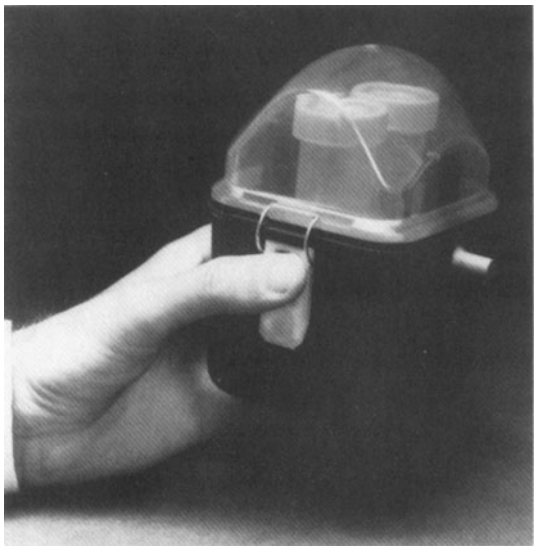

FIGURE 1.6 Safety bucket. This container is designed to prevent the release of aerosols should a tube break or leak. (Courtesy of Beckman Instruments, Inc., Spinco Division.)

rough walls caused by erosion or adhering matter, and carefully remove bits of glass and other debris from the rubber cushion (209, 479).

A disinfectant should be added between the tube and tube cavity or bucket to disinfect the culture in case of accidental breakage. This practice also provides an excellent cushion against shocks that might otherwise break the tube (209, 479). Care must be taken, however, not to contaminate the culture material with the disinfectant. Be aware that if the tube breaks, this relatively small amount of disinfectant may not completely inactivate the infectious material because of the dilution of the disinfectant and the high concentration and packing of the sedimented materials.

It is best not to decant, or pour off, supernatant material from centrifuge tubes. Decanting will always result in some liquid spilling down the outside of the tube or becoming trapped between the rim of the tube and the closure (cap or plug), and will remain to contaminate the hands or, in a subsequent centrifugation step, biohazardous fluid may be spun off as droplets that form an aerosol (209, 479). If tubes must be decanted, wipe off the outer rim with a disinfectant afterwards. Using a vacuum system with appropriate in-line safety reservoirs and filters (Figure 1.7) is preferable to decanting from centrifuge tubes or bottles.

Minimize the amount of aerosol created by using a swirling, rotary motion rather than shaking to resuspend a sediment after centrifuging, especially if the sediment is composed of packed infectious microorganisms or other hazardous material. Table 1.2 gives the spray factors for some laboratory practices. If vigorous shaking is essential to suspend the material or achieve homogeneity, allow a few minutes to elapse before opening the container to permit the aerosol to settle. Shaking always contaminates the closures; thus there is the added hazard of liquids dripping from the closure or running down the outside of the container. A biological safety cabinet may be required to ensure the safety of the laboratory worker when performing some of these operations (see Chapter 9).

It is important to avoid filling the centrifuge tube to the point where the rim, cap, or cotton plug becomes wet with culture (209, 479). Screw caps or caps that fit over the rim outside the centrifuge tube are safer than plug-in closures. Some fluid usually collects between a plug-in closure and the rim of the tube. Even screw-capped bottles are not without risk: if the rim is soiled and sealed imperfectly, some fluid will escape (102). Aluminum foil should not be used to cap centrifuge tubes containing toxic or infectious materials because these lightweight caps often become detached or rupture during handling and centrifuging (102).

Buckets and tube cups are often improperly balanced. Take care to ensure that matched sets of buckets, adapters, and plastic inserts do not become mixed. If these components are not inscribed with their weights by the manufacturer, application of a drop of colored paint will help to identify matched sets. In balancing rotors, consider the tubes, buckets, adapters, and inserts, and any disinfectant solution or water added for balancing. Account for solution densities in the balancing proce-

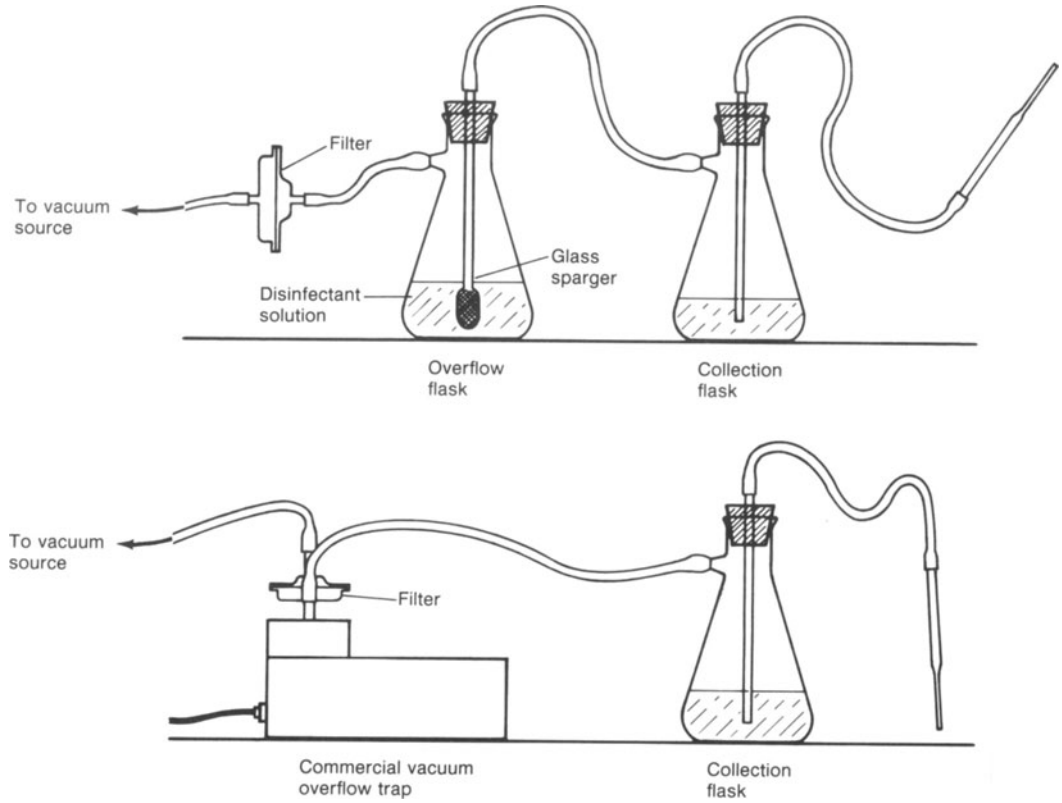

FIGURE 1.7 Two safety vacuum systems. The upper arrangement uses parts available in the laboratory. The lower system employs a commercial vacuum overflow trap, in which a float detects the rise of liquid in the trap and activates a solenoid valve, disconnecting the vacuum source.

dure. The basic concern is that the centers of gravity of the tubes be equidistant from the axis of rotation. To illustrate the importance of this, two identical tubes containing 20 grams of mercury and 20 grams of water, respectively, will balance perfectly on the scales; however, their performance in motion is totally different, leading to violent vibration with the potential for damage and injury (113).

Low-speed and small portable centrifuges

Centrifuges that do not have aerosol-tight chambers have been shown to allow the escape of aerosol created from various sources:

- Biohazardous fluid remaining on the lip of the tube after decanting the supernatant fluid.
- Leakage from a tube in an angle-head centrifuge rotor resulting from overfilling a tube and placing aslant in the rotor.
- Leakage from nonrigid tubes that distort under centrifugal forces.
- Fluid trapped in the threads of screw caps.

Safety buckets should be used to prevent escape of aerosol in the event the primary culture container held in the bucket should break or in any other manner allow the release of agent into the bucket. Handling of the cultures, filling of centrifuge tubes, and placing them in the buckets should be performed in a biological safety cabinet. The outside of the bucket should be decontaminated before the bucket is removed for centrifuging. Subsequently, the bucket should be returned to and opened in the biological safety cabinet (209).

TABLE 1.2 Spray factors for various operations

Operation	Spray factors*	
	Minimum	Maximum
Sonic oscillation	5×10^{-7}	9×10^{-5}
Blender (opened)	1×10^{-6} (tight cover on, open 1 min later)	2×10^{-3} (open)
Blender (closed)	9×10^{-9} (screw cap on)	2×10^{-8} (plastacap, loose)
Dropping liquids, 90 cm		2×10^{-6}
Mixing with pipet	2×10^{-6} (not blowout type)	1×10^{-4}
Centrifuge "spill" (drop of culture on rotor)		2×10^{-6}
Vortex mixer (capped tube)		0
Shaking dilution bottle	7×10^{-9}	2×10^{-7}
Lyophile tube breakage:		
Milk and broth		3×10^{-9}
Nutrient broth		2×10^{-9}
With cotton pledget (wet)		0
With cotton pledget (dry)		6×10^{-11}
Transfer of lyophilized material (shaking tube)		2×10^{-10}
Removing cotton plugs from test tubes	6×10^{-8}	2×10^{-7}
Flaming loop	3×10^{-9}	3×10^{-7}

*When the spray factor is multiplied by the number of bacteria or virus per milliliter of solution the expected number of airborne particles released by the operation can be calculated.
From (125).

In fact, a small centrifuge could be placed in a biological safety cabinet to reduce the escape of any aerosol produced during the run.

Some of the older clinical centrifuges have been shown to be hazardous (390). The microhematocrit centrifuge, in particular, has been shown to produce aerosols. One frequently sees blood samples centrifuged in tubes without closures or with cotton plugs secured in the tubes by means of tape or other unsecure means. Remember that some tissue specimens contain viable infectious microorganisms, particularly hepatitis virus, and that open tubes, contaminated closures, and concomitant release of aerosols from blood samples and tissue suspensions can be hazardous to laboratory personnel.

High-speed centrifuges

Centrifuging at high speeds poses additional hazards because of the higher stresses and forces applied to the rotor and tubes. In addition to the recommended practices listed above, precautions should be taken to filter the air exhausted from the vacuum lines, to avoid metal fatigue resulting in disintegration of rotors, and to use proper techniques for cleaning, handling, and using centrifuge components. Some of these precautions are discussed briefly below.

In high-speed centrifuges, the chamber is connected to a vacuum pump. If infectious materials are released into the chamber, the pump and the oil in it will become contaminated. For operations requiring a higher degree of containment, a filter should be placed between the centrifuge chamber and the pump (102).

High-speed rotors are prone to metal fatigue. To keep track of their usage, a record for each rotor separate from the instrument log must be kept, especially when rotors are used in more than one machine. Each rotor must be accompanied by its own record card or book indicating the number of hours run at normal or derated speeds. Failure to observe this precaution can result in dangerous and expensive rotor failures. Frequent inspection, proper cleaning, and timely drying of rotors are important to prevent corrosion or other damage that may lead to the development of pitting or cracks. If the rotor is treated with a disinfectant, it should be rinsed with clean water and dried as soon as the disinfectant has adequately decontaminated the rotor (see Chapters 5 and 6). Rubber "O" rings and tube closures must be examined for deterioration and must be coated with the lubricant recommended by the manufacturer. If tubes of different materials are used (e.g., celluloid, polypropylene, stainless steel), care must be taken that the tube closures designed specifically for the type of tube in use are employed. These caps are often similar in appearance,

but are prone to leakage if applied to tubes of the wrong material. Properly designed tubes and rotors that are well-maintained and properly handled will not leak (113).

Cleaning and disinfection of tubes, rotors, and other components requires considerable care. Although it is unfortunate that no single process is suitable for all items, the various manufacturers' recommendations must be followed meticulously in order to avoid metal fatigue, distortion, and corrosion. One hazard that can easily be avoided is the use of celluloid tubes. Celluloid (cellulose nitrate) centrifuge tubes are not only highly flammable and prone to shrinkage with age and distortion on boiling, but also can be highly explosive in an autoclave (113). Use a chemical disinfectant appropriate to the biohazardous agent to decontaminate celluloid tubes.

Continuous-flow centrifuges

Modern biotechnology research and production techniques have led to the increasing use of continuous-flow centrifuges, zonal rotors, or both, to collect biological products from fermentors or other process vessels used in large-scale experiments or pilot plant operations. Continuous-flow centrifuges, as the name implies, allow the continuous harvesting of the product while the centrifuge operates at full speed. The *zonal* centrifuge or rotor, another type of continuous flow device, separates the product according to its density or buoyancy under centrifugal force. These devices allow the collection and concentration of large amounts of biological products in a short time period compared with the "batch" method used in ordinary centrifuging. This very ability to collect and concentrate large volumes of product makes it essential that the centrifuging process not create a biological hazard.

The most common of the continuous-flow centrifuges are the industrial or pilot plant open bowl units. Some of these centrifuges are over twenty years old but are still quite serviceable. Their drives operate on air or steam turbines, or by electric motors. Many units have refrigeration coils in the bowl to keep the product cool during centrifuging. The process culture to be harvested is added through a feed line and the supernatant is withdrawn through an effluent line; these lines are connected to the rotor through rotating seals at the drive shaft.

Continuous-flow centrifuges, particularly the older ones, produce a massive aerosol that is almost impossible to contain, even with sealed centrifuge bowls. This problem is best solved by enclosing the centrifuge in a specially designed ventilated safety cabinet (Figure 1.8). The aerosol problem limits the use of the continuous-flow centrifuge to minimal risk agents or to those projects having sufficient resources to provide the necessary containment equipment.

Zonal centrifuges and rotors provide a higher degree of containment than do the industrial centrifuges, but these units also require special handling to avoid producing an aerosol hazard. Although many zonal centrifuges and zonal rotors used in laboratory centrifuges are designed to minimize aerosol production, much of the success of avoiding aerosol production is a direct result of operator care in handling the cultures, setting up the run, and disassembly and decontamination of the apparatus after the run is completed.

Some of the conditions that can lead to production of aerosols during zonal centrifuging include the following.

- *Leaky rotor seals.* The rotating rotor seals are very susceptible to leaks. Some of the major causes of seal leaks are nicks or other damage to the seals, improper assembly, and overpressurization. If the culture materials are pumped into the rotor using a positive displacement pump, a blockage (caused by an air bubble, for example) can overpressurize the seal causing it to leak or even rupture.
- *Drops of culture in chamber or on rotor.* Drops of culture materials can fall into the chamber or on the rotor during centrifuge set up. Such stray droplets can result in the production of considerable quantities of aerosol.
- *Snagging tubing and tubing connections.* Connecting zonal rotors to the culture sys-

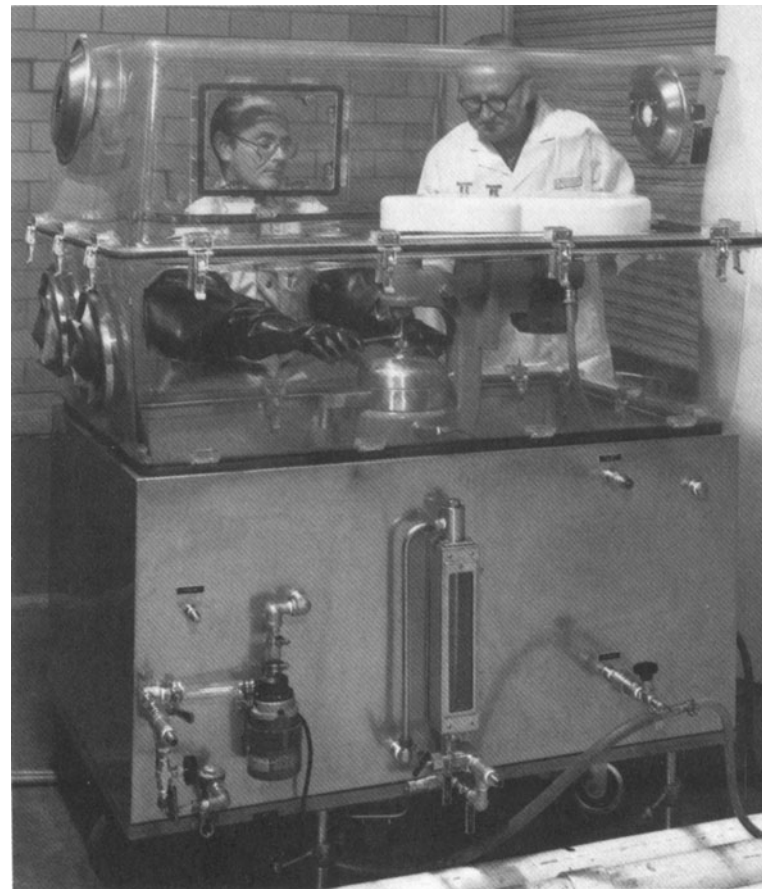

FIGURE 1.8 Continuous-flow Z41 CEPA centrifuge installed in a glove box for the separation of Namalva cells from supernatant in the mass production of lymphoblastoid interferon. (Courtesy of New Brunswick Scientific Co., Inc.)

tem generally involves long lengths of tubing with, perhaps, valves, tees, connectors, and other plumbing fittings. Each of these connections is a potential source of leaks, and the tubing itself can be snagged by the operator, moving parts, or passers-by if not properly clamped and secured.

- *Disassembly and decontamination.* When the run is over, the rotor, chamber, and all associated tubing that was in contact with the agent must be decontaminated. Do not neglect the underside of the chamber lid. If the centrifuge is equipped with a vacuum system, any aerosol of agent may have contaminated the vacuum oil, pump, or piping. The chamber drain in those instruments that have one may also require decontamination.

Further information on large-scale centrifugation can be found in (26, 27, 477).

Chapter 6 contains suggestions for operating a centralized common centrifuge facility in an efficient and safe fashion.

1.4 Blenders, Ultrasonic Disruptors, Grinders, and Lyophilizers*

All these devices release considerable amounts of aerosols during their operation. The safety practices described below will provide maximum protection to the operator during the blending of hazardous materials (278). In addition, some operations may produce consid-

*Excerpted from (477)

erable noise and require the use of hearing protection.

Operate all blending, cell-disrupting (460), and grinding equipment in a biological safety cabinet (474). Use safety blenders designed to prevent leakage (217) from the rotor bearing at the bottom of the bowl (474). If the rotor is not leak proof, inspect the rotor bearing at the bottom of the blender bowl for leakage prior to each operation. Test it in a preliminary run with sterile saline or methylene blue solution prior to use with the hazardous material (474). If the blender is used with biohazardous materials, use a towel moistened with disinfectant over the top of the blender (474). Disinfect the device and residual contents promptly after use.

Avoid using glass blender bowls with hazardous materials because of potential breakage. If they must be used, glass bowls should be covered with a polypropylene jar to prevent spraying of glass and contents in the event the bowl breaks (102). Blender bowls sometimes require supplemental cooling to prevent damage to the bearings and to minimize thermal effects on the product (415). Before opening the safety blender bowl, permit the blender to rest for at least one minute to allow settling of the aerosol cloud.

Lyophilizing has certain hazards associated with aerosol production (371,372). Depending on the design of the lyophilizer, aerosol production can occur when material is loaded, or when sample containers are removed from the unit. For work with hazardous agents, load the samples in a biological safety cabinet. The exhaust from the vacuum pump should be filtered to remove any hazardous agents (see Chapter 9), or the pump can be vented into a safety cabinet.

After completion of the run, wipe with a disinfectant all surfaces that have been exposed to the agent. If the lyophilizer is equipped with a removable sample chamber, close off the chamber and move it to a biological safety cabinet for unloading and decontamination. Try to minimize handling of cultures, and utilize vapor traps wherever possible.

1.5 Water Baths

Water baths may become contaminated by the cultures that are incubated in them (201); therefore precautions are needed to prevent growth of organisms that can present a hazard or cause cross-contamination problems. Water baths used to inactivate or incubate hazardous substances should contain a disinfectant, such as chlorine bleach (0.8 ml per liter of water) or a phenolic detergent (8 ml per liter). The phenolic detergent is preferred as it is less corrosive and more stable than chlorine (see Chapter 5). For chilled water baths, 70 percent propylene glycol is recommended (209, 479). Sodium azide should not be used as a bacteriostatic agent, as it creates a serious explosion hazard.

1.6 Cold Storage

Deep freezers, liquid nitrogen freezers, and refrigerators should be checked and cleaned out periodically to remove any broken ampoules, tubes, etc., containing hazardous material, and then disinfected. Use rubber gloves and, if necessary, respiratory protection during this cleaning. All infectious or toxic material stored in refrigerators or freezers should be properly packaged and labeled. Safety measures should be commensurate with the hazards present (209, 479).

The degree of hazard represented by contaminated liquid nitrogen reservoirs will be largely dependent upon the infectious potential of the stored microorganisms (403), their stability in liquid nitrogen, and their ability to survive in the airborne state. Investigations suggest that storing tissue culture cell lines in containers other than sealed glass ampoules might result in potential intercontamination among cell lines stored in a common liquid nitrogen repository. When preparing vials (especially glass) for freezing in liquid nitrogen freezers, be especially careful to prevent the possibility of leakage of liquid nitrogen into the vial. If liquid nitrogen is trapped in the vial, it will vaporize when the vial is removed

from the freezer resulting in a powerful explosion, and possibly cause splashing of the face and eyes with liquid nitrogen, broken glass, and culture material. Testing for a complete seal may be done before freezing by immersing the sealed vial in a solution of cold (4°C) methylene blue* and observing for leakage (491).

To avoid the hazards of a vial rupturing upon removal from the liquid nitrogen, glass vials on canes should always be protected by covers of cardboard safety tubes. Plastic vials may be pulled partially out of the freezer and held in the neck of the freezer for one minute to allow equilibration before complete withdrawal. Draping a towel or a piece of bench liner around the cane as it is removed is also recommended (491).

Always wear gloves and safety glasses or a face shield when removing or inserting racks, checking liquid level, or filling the freezers with liquid nitrogen.

There are special hazards involved in the use of **liquid nitrogen**. In liquid form, nitrogen has a boiling point of −196°C and a volume expansion ratio of 696.5 from a liquid at one atmosphere to a gas at 21°C and one atmosphere. This means that one liter of liquid nitrogen, upon boiling, occupies 696.5 liters of volume. If liquid nitrogen is allowed to boil in a confined, poorly ventilated area, an asphyxiation hazard caused by displacement of oxygen-laden air can result. Exposure to an atmosphere containing less than 15 percent by volume of oxygen will cause symptoms of hypoxia in individuals exposed to this depleted atmosphere; concentrations of less than 12 percent will cause unconsciousness without warning. Therefore, liquid nitrogen freezers must be located in well-ventilated areas to minimize the hazard from oxygen displacement.

Another hazard resulting from liquid nitrogen storage is the potential for concentrating oxygen in or near the cryogenic freezer. Oxygen boils at −183°C, whereas nitrogen has a boiling point of −196°C. The temperature of the liquid nitrogen can actually condense the oxygen out of the air coming into contact with poorly insulated containers; liquified oxygen can become absorbed into liquid nitrogen which has been standing around for a period of time. This can present a considerable fire hazard. Therefore, precautions must be taken to minimize the prolonged storage of liquid nitrogen and to keep oxidizable materials away from cryogenic storage containers. Also, because of this potential for the fractional distillation of oxygen from air by liquid nitrogen, avoid the use of formed plastic containers. The localized oxygen enrichment can cause autooxidation of the plastic, resulting in a fire hazard.

*A recommended solution is 0.03 percent methylene blue in phosphate buffer (PBS) 20 percent, in methanol 80 percent.

1.7 Ampoules

When a sealed ampoule containing a lyophilized or liquid culture is opened, an aerosol may be created (177). To prevent or minimize creation of aerosols, ampoules should be opened in safety cabinets. When recovering the contents of an ampoule, workers must try not to cut gloves or hands, or spray broken glass into eyes, face, or the laboratory environment. In addition, the biological product itself should be protected from contamination with foreign organisms or with disinfectants. To accomplish this, work in a safety cabinet and wear gloves. Nick the ampoule with a file near the neck, then wrap it in disinfectant-wetted cotton. Snap the ampoule open at the nick, being sure to hold the ampoule upright. Alternatively, apply a hot wire or rod to develop a crack at the file mark on the neck of the ampoule. Then, wrap the ampoule in disinfectant-wetted cotton, and snap it open. Discard the cotton and ampoule tip into disinfectant. The contents of the ampoule are reconstituted by slowly adding fluid to avoid aerosolizing the dried material. Mix contents without bubbling, and withdraw it into a fresh container (177). Ampoules of liquid cultures are opened in a similar fashion. Some researchers may desire to use commercially available ampoules prescored for easy open-

ing. However, consider the possibility that prescored ampoules may be particularly fragile and may break during handling and storage.

1.8 Shaking Machines

These items of equipment should be examined carefully for potential causes of breakage of flasks or other containers being shaken. Screw-capped, durable plastic or heavy-walled glass flasks should be used. These should be securely fastened to the shaker platform. Check to be certain that all clamp connections are secure and that the load is balanced on the platform. This is especially important if large flasks are used. Unbalanced shakers can oscillate with enough force to shift the center of balance and "walk" across the room. Never grab flasks while the platform is moving because this may result in a broken flask and probable injury.

For additional protection against the escape of cultures, enclose the flask in a plastic bag to which an absorbent material can be added as an additional safety measure. Infrequent users of this equipment may not be familiar with some of these hazards and the appropriate precautions. Post operating instructions prominently for users to see and follow.

1.9 Laboratory Sterilizers

Autoclaves are pressure vessels which, if improperly or carelessly operated, can result in damage to the apparatus or injury to personnel. The chief hazards in the use of the autoclave are the high pressures and temperatures which are developed during operation. The hazards of live steam and boiling liquids are always present, as well as the mechanical hazards of the large, heavy door, loading carriage (if so equipped), and presence of glass. All personnel must be fully trained in the operation of the autoclave so that they are familiar with its proper operation.

To ensure that infrequent users do not neglect proper operating techniques, post instrument operating instructions in close proximity to the autoclave and remind all users to follow these instructions closely. Be sure that the instructions adequately cover the precautions to be followed in opening the autoclave after use. See Section 5.2 for a discussion of other autoclave hazards.

Be aware that evacuating the air from the chamber of a vacuum steam sterilizer prior to sterilization of contaminated material can create a potential hazard by releasing infectious material to the atmosphere (30). Such sterilizers, which operate on the vacuum principle, should not be used for disinfection of potentially infectious waste unless the vacuum cycle can be disabled or turned off.

A discussion of the use of the ethylene oxide sterilizer is beyond the scope of this text. However, the use of ethylene oxide is covered in Section 5.8.

1.10 Harvesting Cultures from Laboratory Animals or Eggs

In vivo sources of cells to be cultured, regardless of whether or not infectious agents are present, require precautions in handling. Harvesting cultures from laboratory animals or embryonated eggs is a hazardous procedure and leads to heavy surface contamination of the surgical or preparation tray, surgical tools, nearby surfaces, the environment, and the hands of the operator. Even if no infectious agent is present, the worker is exposed to foreign proteins, which may cause an allergic reaction (see Chapter 20). It is essential that operations of this type be conducted in a biological safety cabinet. A suitable disinfectant should be at hand and used frequently.

1.11 Laboratory Vacuum

When laboratory vacuum is used to manipulate biohazardous materials, a suitable trap must be employed to insure that the laboratory vacuum lines, water aspirator, or vacuum pump do not become contaminated (209). A

heavy-walled vacuum flask must be used and should be protected or enclosed to minimize flying glass in the event of an implosion. Figure 1.7 illustrates two versions of a filtration apparatus that avoids this problem.

A glass vacuum desiccator must also be enclosed in a box or shielding device, or should be securely taped to minimize flying glass in the event of implosion. Be sure that atmospheric pressure is restored before attempting to open the desiccator. Frozen lids may be freed by wedging a single-edged razor blade in the frozen joint and tapping the blade gently with a wood block.

Dewar (vacuum) flasks also pose a hazard of implosion if they are subjected to mechanical shock. These containers should be securely wrapped with tape to prevent injury from flying glass.

1.12 Glass and Glassware

Glass items and apparatus are widely utilized in laboratory research and are responsible for many laboratory accidents. There are many varieties of glass that have application in research. Most glasses used for laboratory applications fall into one of these major categories: borosilicate (e.g., PYREX™, KIMAX™), used for many types of labware; fused silica, used for optics; soda-lime, used for lamp bulbs, glass sheets, and other common applications; lead-alkali, shielding and electronics; aluminosilicate, for high temperature applications, fiber glass, and similar glasses; and non-silicate glass for optics and other specialized areas. In fact, there are approximately 700 different glass compositions in commercial use (424).

Glass is an extremely strong material. Its intrinsic strength is such that it can withstand pressures as high as 210,000 to 280,000 kilograms per square centimeter (three to four million pounds per square inch, psi). However, it can also be extremely fragile, especially if it has surface flaws, which may fail at stresses as low as 140 kg/cm^2 (2000 psi). In fact, any flaw, whether created in manufacturing or in careless handling, may cause the glass to fail. Therefore, it is important to use care in handling glassware or glass apparatus. Sharp and localized impacts, scratches on the surface of a container, and localized and intense heating are to be avoided as much as possible (424).

In order to avoid damage to glassware during storage, be especially careful to avoid excessive bumping of the glassware (jostling and rolling into each other) on shelves and in drawers. Keep heavier items in low cabinets, drawers, or shelves, while more delicate items should be kept in protective containers in drawers or cabinets. To avoid damage to glass tubing or rods, store them in a horizontal position. Use heating mantles to heat flasks and reactor vessels to achieve an even heat distribution.

Avoid the hazard of cuts when handling glass tubing and inserting or removing glass tubing from rubber stoppers by exercising care in following appropriate procedures to minimize breakage. Inserting glass pipets into mechanical pipettors, if performed carelessly, can also be hazardous. Hold the pipet close to the suction ends* when inserting the suction end into the pipettor rather than grasping the pipet barrel in the middle (Figure 1.9). This avoids placing stress on the pipet.

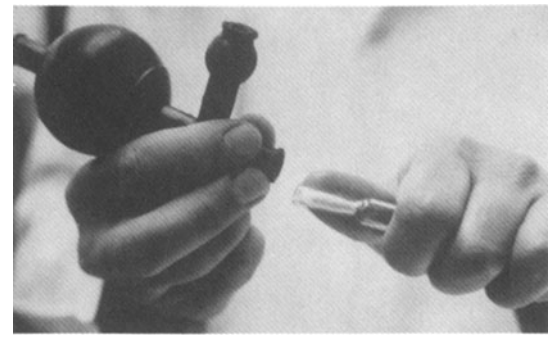

FIGURE 1.9 Handling glassware. The proper way to insert a pipet into pipetting aid: Keep the hand close to the upper end of the pipet, and use a twisting motion of the pipet aid to insert the pipet. Do not force the pipet, or apply any amount of lateral pressure. (Source: National Institutes of Health.)

*Sometimes called the "mouthpiece," an unfortunate term that should be banned.

When cutting tubing, use leather gloves or a towel placed over the scored spot on the tubing when breaking; fire polish the cut ends of the tubing; select stoppers with the proper bore size; and use leather gloves for insertion of tubing into the stopper (Figure 1.10) after lubricating the tubing with water or glycerine. Another technique that can be used to insert tubing into a stopper is to insert a stopper borer into the hole in the stopper, insert the tubing from the other end, and withdraw the borer. Wear gloves for this operation also.

A technique that is sometimes effective for removing tubing frozen into stoppers is to place the stopper on a hard surface and roll the stopper with a wooden or metal block. This causes the rubber to flex and breaks the bond between the rubber and glass. Or, one can run a stopper borer over the frozen tube into the stopper to free the tubing, or, finally, one can cut the stopper to free the tubing. Wear gloves. Don't take chances—if the assembly won't come apart, it is better to scrap the item than risk injury.

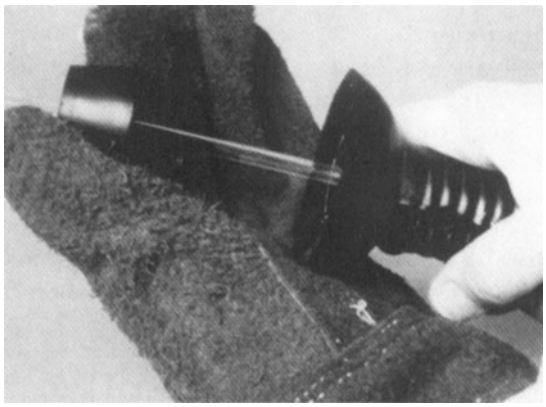

FIGURE 1.10 Working with glass tubing. The worker is using a tubing guard and wearing leather gloves. (Source: National Institutes of Health.)

1.13 Controlling Aerosols

As discussed earlier, aerosols of potentially hazardous agents can be produced by many laboratory practices. Aerosols produced by larger laboratory and process equipment, such as fermentors, can be controlled by filter entrainment of exhaust air, or by passing the exhaust air through an air incinerator. Each such installation must be separately evaluated for the appropriate hazard control measures to be taken. Wherever possible, aerosol-producing operations should be performed in a biological safety cabinet (see Chapter 9) if the aerosol may present a hazard to the laboratory worker (103, 349). Table 1.2 summarizes certain aerosol-producing operations and gives the ranges of resulting airborne particle output from each process.