9

Working without Contamination

ASEPTIC (OR STERILE) technique is a way of working that maintains sterility. Before the advent of hoods, it is the way all benchwork in a lab was done. In many labs today, aseptic technique is no longer rigorously practiced; in fact, in some molecular biology labs, there is not even an attempt at maintaining sterility.

Big mistake! Although the need for aseptic practice is not as clear in a biochemistry lab as in, say, a cell biology or infectious disease lab, its use can avoid many problems that would be impossible to diagnose. Also, the demarcations between different kinds of fields of study are not so well defined, with molecular biology generally now considered to be a technique, not a field, and everyone may need to grow his own cells or freeze his own bacteria.

Aseptic technique should be to you not merely a way to open your cultures sterilely, but a way of thinking and acting *always* in the lab. There is no aspect of lab work that won't profit by extra care. Use it to prepare RNA buffers, to set up restriction enzyme digestions, to prepare membrane proteins. When you are used to working aseptically, following the rules for using radioactivity or hazardous material will seem very familiar.

All procedures are described for right-handed workers.

WHEN TO USE STERILE TECHNIQUE

When you must use sterile technique. You **must** use sterile technique whenever you are working with living organisms, or with any media, buffers, or culture containers used for living organisms. For example:

- **Setting up a culture of *E. coli* for a transformation**
- **Making LB plates**
- **Splitting cells**
- **Filtering serum for media**
- **Opening and rehydrating a vial of lyophilized bacteria**

When it helps to use sterile technique. You **should** use sterile technique whenever you don't want the contents of one container or area to enter another container or area. Yes, this is true of just about anything you do in the lab. Even when you are working with buffers with a high salt and/or detergent content, in which it is unlikely that any organism would grow, you must be careful not to introduce oils from your hands or dust from a greasy pipet.

You **should** also use sterile techniques when working with any hazardous agents, such as radioactivity or toxic chemicals. Of course, protecting yourself is of primary concern, and you must sometimes modify your procedures. Fortunately, protecting your material and protecting yourself both involve creating barriers between you and the material, and so involve the same means and the same end. For example:

- **Setting up restriction digests.** Contamination of restriction enzymes can be a huge disaster. Although most enzymes are packaged in glycerol, and glycerol concentrations over 50% are bacteriostatic, diluted enzymes and others without glycerol are susceptible to bacterial contamination. In addition, traces of common elements can inhibit enzyme reactions.

- **Setting up PCR reactions.** PCR reactions are notoriously plagued with contamination by other DNAs. By working aseptically, and setting up reactions in a room away from the PCR machine and the place where the analysis of the PCR products is done, the introduction of stray DNA can be avoided.

- **Labeling cells with [^{32}P] phosphate.** In this case, working aseptically is for your protection, and not for the protection of the cells. When you think aseptically, you will not leave open caps, generate aerosols, or accidentally reuse a radioactive pipet.

STERILE TECHNIQUE

The bottom line is this: The air is dotted and filled with dust and spores and germs, and you don't want them in your bottles or cells. Unless air currents blow them away from the working area, these potential contaminants will descend to the working surface, into open bottles, and onto pipet tips. Microorganisms will "fall" from hands and sleeves, and descend to the surface. Arm motions, rapid pipeting, and passersby will stir up unpredictable currents that can't be guarded against.

The use of sterile technique will *minimize* the exposure of your material to contaminants. It cannot completely protect in all conditions and, thus, can't completely prevent all contamination. But the better your technique is, the better your record on contamination will be.

Keep surfaces clean, bottles closed, and movements minimized.

Rules

- **The working area should be as far away from drafts and traffic as possible.** No windows should be open, and you should try not to be near an active doorway. The use of a biosafety cabinet makes aseptic technique easier to maintain, but is not at all necessary.
- **Be sure there is a clear working area.** Remove all supplies and equipment you won't be using. Old flasks and containers must not be nearby. When you are finished, remove or put away all supplies and equipment, and wipe down the area again.
- **Wipe down the working area with an antiseptic or cleaning agent before use.** 70% ethanol is fine for most labs. (But use whatever the laboratory uses, since alcohols may not be effective against a particular lab's nemesis.) Keep a squirt bottle of 70% ethanol next to every working area, and use it liberally. Wipe the work surface down at the end of the day, before the start of an experiment, and after a spill.
- **All pipets and bottles should be sterile.** If you don't KNOW something is sterile, don't use it. Reusable bottles and pipet canisters should have autoclave tape that indicates sterility, or a written note on the tape that the container has been sterilized. Disposable pipets, if opened, must be in a bag in which the sterility of the tips has clearly not been disturbed. If the bag is old, torn, or found left completely opened, do not use the pipets.
- **Set the working area up to minimize hand movements.** Have the tools you will use with the right hand placed to the right of the open space, the ones you will

use with the left hand placed to the left of an open space. For example, for the removal of a small volume from a jar by a right-hander, pipet aids, pipets, and pipettors should be to the right, the jar to the left.

- **Have ready everything you will need,** so you don't have to leave the working area to retrieve something. The more you rustle around, the more the air will be disturbed. Leaving the working area also breaks your concentration.
- **Wear latex gloves, and change them frequently.** Gloves protect the working material and the investigator. If gloves are not available, needed, or desired, wash hands before and after working. You may prefer to work without gloves, and for nontoxic material and nonpathogenic materials, that is fine. But you will need gloves for many procedures, and it is just as well to get used to working with them.

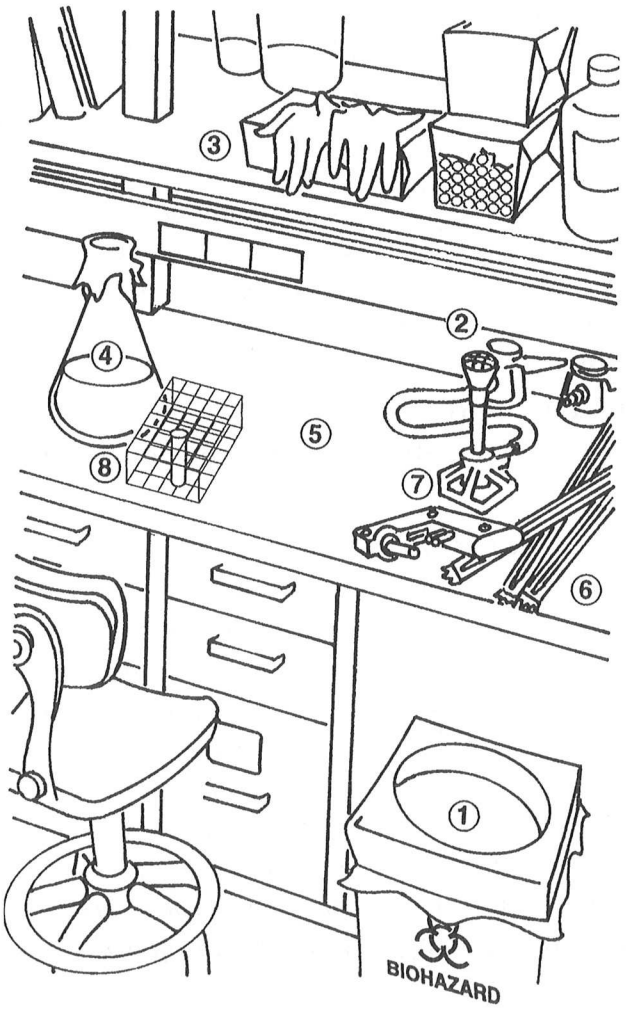


FIGURE 1.

Lab bench setup for aseptic work. **Task:** To transfer 5 ml of growing bacterial culture from a tube into 100 ml of culture medium. The supplies needed are arranged for a right-handed investigator, so that things to be manipulated with the right hand are set up to the right of the working area, and things to be manipulated with the left hand are set up to the left of the working area. **Key:** (1) *Biohazard disposal.* (2) *Bunsen burner.* (3) *Box of gloves.* (4) *Flask of culture medium.* (5) *Clear area.* (6) *Individually wrapped pipets.* (7) *Pipet aid.* (8) *Test tube with bacterial culture, in a test tube rack.*

Technique Tips

Every task you will do will be a bit different. This is why it is necessary to think carefully about your actions: it is not possible to describe every action one will perform.

Generally, what you will be trying to do is to minimize the encounter of your material with potential contaminants. To do this, minimize all actions.

- **Minimize:**

  Distance. The closer all supplies are to you and to each other, the less movement you will make.

  Exposure. The more you move something through the air, the more airborne particles it will encounter. The longer a bottle is left open, the more airborne particles can enter the jar. Flaming a bottle or pipet fixes particles to the surface and creates an upward flow that minimizes exposure to airborne particles.

  Flaming is not done to sterilize. 1–3 seconds through a flame is sufficient.

  Motion. Motions create air currents. Faster motions create faster air currents. Make all movements necessary, and make them gentle. Don't wave pipets in the air, or cross your hands over your open working area.

  Pouring. Pouring creates aerosols, which disturb the air and can carry contamination to unwanted areas. Also, the fluid left on the lip of a bottle after pouring is one of the greatest sources of contamination to the contents of the bottle, as it creates a bridge from the outside to the inside (Freshney, p 56). Use a pipet to transfer liquids whenever possible.

  Pipets are available that can pipet up to 50 ml. If you use one, be sure the pipet is set firmly into the pipet aid. 50-ml pipets are larger, and if they are not set in squarely during use, the vacuum will be lost and the fluid will drip out.

- **Open all bottles with the bottle pointed at approximately a 45° angle.** This minimizes airborne contamination and the creation of aerosols when pipeting.
- **If you must put a cap or lid down, place it face down on a clean surface.** Try not to do this. With a face-up lid, there is more chance of contamination by hands and bottles moving above it.

  There are two schools of thought on the placement of the cap, face up, or face down. Both are right: Choose one method, and stick to it. If you leave the caps up, avoid moving your hands over it. If you leave the caps down, be sure the surface below has been well disinfected.

- **Flame glass pipets and open bottles before manipulation.** Position the Bunsen burner between your working hand and the bottles, etc. you will be working on.
- **Do not flame plastic bottles or pipets!** Work quickly, minimize open bottles, and tilt all bottles at a 45° angle when pipeting, whether you flame the opening or not.

- **Pipet gently and don't swirl bottles.** This minimizes aerosols. Also, be careful when opening centrifuge bottles.
- **Don't leave any bottle open.** When you place an open bottle down, cover it immediately with the cap or lid. Do not leave bottles with pipets in, and the cap off.
- **Stop what you are doing.** There is a great deal of overkill in working aseptically, so things usually work out fine. Relax and stay aware. But there are a few *common mistakes* that should result in immediate termination of whatever action you are performing.

Mistakes that break sterility

1. Pipeting up too far in the pipet. Discard the pipet and check the pipettor: You may need to change filters.
2. Touching the tip of the pipet against a bottle, the ground, the outside of the pipet container, or anywhere solid. Discard the pipet.
3. Dropping an opened container or tube to the ground. Discard it.
4. Touching anything, including a gloved hand, to either the HEPA filter that prevents the suction of particulates through the vacuum system, or to the filter used in a flask vacuum system. This is a major source of contamination in hoods. Discard whatever touches the filter.
5. Reusing pipets while working. Once a pipet has been wetted, it is much more likely to pick up airborne contaminants.

Pipeting

Pipeting with reusable glass pipets. Reusable pipets are stored usually in metal canisters, or cans. For work with cells or bacteria, the pipet should be plugged with cotton.

A small canister top could be held in the last two fingers of the left hand.

1. Loosen the top of the canister.
2. Hold the canister in the left hand, and remove the top of the canister with the right hand.
3. Flame the top and the open end of the canister. Place the top down, on its side.
4. Hold the canister horizontally in your left hand and gently shake and tip the canister so the tops of one or two pipets stick out of the top of the canister about an inch and can be easily grasped.

5. Lay down the canister top on its side, or hold it in your left hand, and remove a pipet. Slide it out, holding it with your thumb and index finger about 2 inches from the top, without touching the pipet to the side of the canister.
6. Pass the bottom third of the pipet through the flame for 1–3 seconds. Rotate the pipet 180° as you pass it through the flame.
7. Insert the pipet into the pipet aid.
8. Hold the bottle from which you will pipet at a 45° angle with your left hand. While holding the pipet aid in the right hand, open the donor bottle with the last two fingers of the right hand. Retain the cap in those fingers.
9. With the left hand, pass the opening of the bottle through the flame.
10. Pass the pipet through the flame again.
11. Place the tip of the pipet into the container from which you are removing liquid. Don't touch the tip to the inside at all, but place it straight into the liquid. Pipet the volume you require and carefully withdraw the pipet.

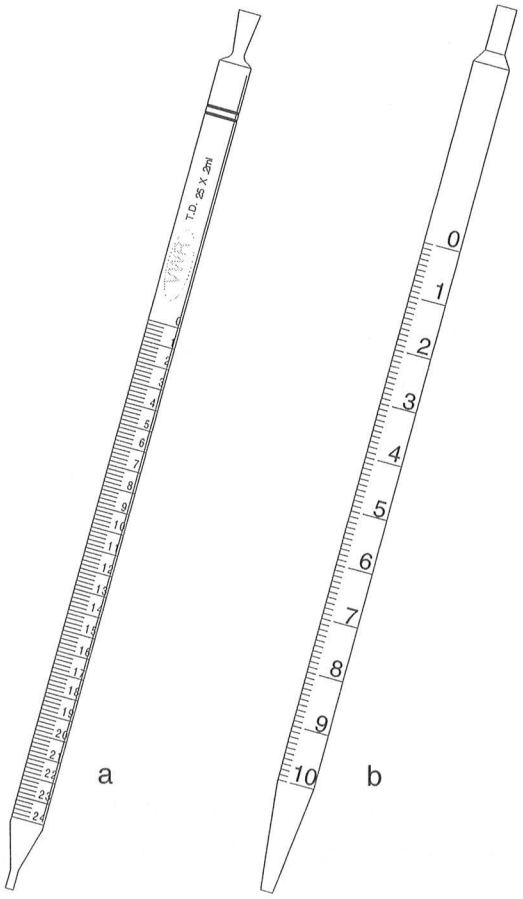


FIGURE 2.

Note carefully the type of measurement on the pipet. Most pipets are made to deliver (marked T.D., to deliver, on the top of the pipet) the chosen volume when the fluid is released entirely from the pipet (*a*). Other pipets (marked T.C., to contain) are made to release the chosen volume only when the fluid is released to a measured point (*b*).

12. Flame the donor bottle and recap it. Place it to the side.
13. While holding the pipet as still as possible, open the receptacle bottle as in steps 7–9.
14. Place the pipet into the receptacle bottle carefully, as in step 10, and dispense the liquid.
15. Withdraw the pipet and place in the appropriate place to the side. This may be a beaker for temporary storage. Afterward, all pipets will generally go into a soaking basin.
16. Flame the receptacle bottle, recap, and put down.

All other pipeting will be similar. In fact, all other manipulations will be variations of the ones described above.

Pipeting with disposable pipets

1. Be sure the top of the plastic package has been opened (or, if taped, the tape has been removed) so it will be easier to quickly take a pipet.
2. Hold the pipet package in the left hand while you remove a pipet with the thumb and index finger of the right hand. Try not to touch the inside of the bag or the other pipets with the tip of the chosen pipet. Gently squeezing the bag to make a tube helps to create an open area in the bag through which you can withdraw the pipet.
3. Lay the package of pipets to the left.
4. Do all steps except flaming, as described for reusable pipets.

Contamination tends to occur when withdrawing the final inch of pipet from the package. Fingers may touch the tops of the pipets in the package, and a touch of the tip of the pipet against the top of the packaged pipets can contaminate the pipet and anything else it touches.

How to open an individually wrapped pipet

1. Hold the pipet in your left hand, about 3/4 to the top. Point the tip toward the left.
2. Tighten your left hand, so that the plastic wrapping cannot slip.
3. Grab the top of the plastic wrapping with your right hand.
4. While holding firmly to the pipet with the left hand, use the right hand to pull the wrapping against the top of the pipet, puncturing the top.
5. Continue pulling the wrapper down, folding 2 inches or so over the outside of the wrapper and pipet. Now you have a sterile tunnel through which the pipet can be withdrawn.
6. Withdraw the pipet from the wrapper, being careful not to touch the pipet to any surface. Put the wrapper to the left side, and dispose of it when you are through pipeting.

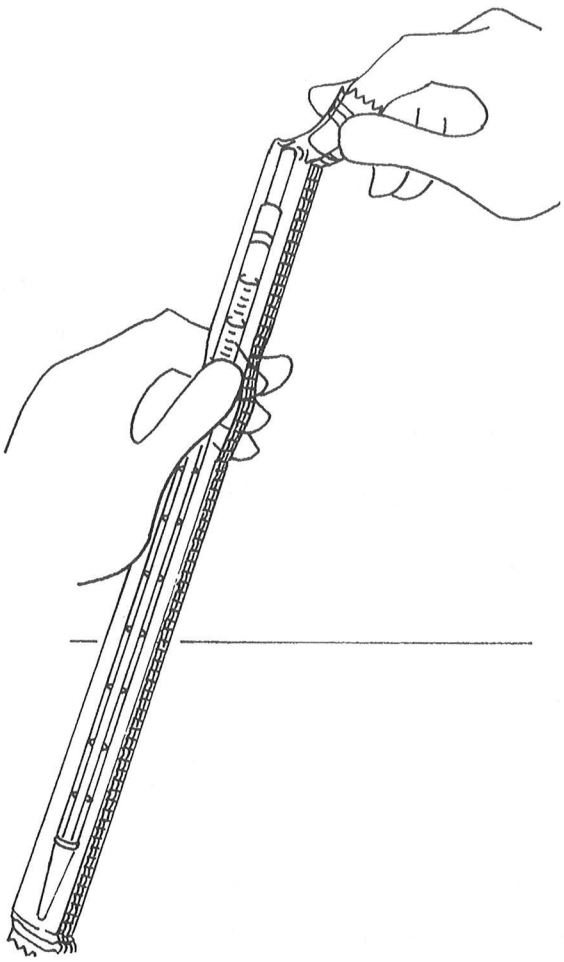


FIGURE 3.
Opening a disposable pipet.

Pouring

Hold the recipient container in the left hand, the donor in the right.

1. Flame the recipient container, with the flask pointed at an angle. Move in toward the left, keeping the container angled.
2. Flame the donor container. Wait 1–2 seconds (so the fluid in the container doesn't get scorched).
3. Maintain the recipient container at an angle, and hold it steady. Put the donor container, angled toward the recipient, approximately 2 inches above the recipient.
4. Tilt the donor and pour.
5. Flame the open mouth of both containers.

Since the chance of contamination is much greater when pouring than when pipeting, pour only when the volume is too large to be easily pipeted.

Filter Sterilizing

Filtration of a small volume with a syringe filter

Materials

- A disposable 0.2 µm syringe filter
- A syringe of the volume of the solution. (1, 2, 5, 10, or 20 ml. 50-ml syringes are awkward to handle.) Use a sterile, disposable syringe to ensure cleanliness.
- A sterile tube as a receptacle
- Tube holder

1. Unwrap only the top of the syringe filter. This is the wider end, which will be twisted onto the end of the syringe.
2. Remove the wrapping from the syringe, and take out the plunger. Place the plunger on a clean surface.
3. Remove the protective cap from the tip of the syringe, and immediately twist the filter onto the syringe. Keep the wrapping on the exit tip of the filter, and lay the setup down.
4. Remove the cap from the sterile tube and place the tube in a holder.
5. Remove the wrapping from the exit end of the filter. Place the exit end in the tube, and hold the syringe upright.
6. Pour the solution into the syringe, replace the plunger, and push gently and firmly until the entire solution has passed through the filter into the tube.
7. Cap the tube, label it, and dispose of the syringe in biohazard/sharps waste.

You can directly draw up into the syringe the solution to be sterilized, before putting on the filter. But the syringe often won't fit the tube the solution is in.

Filtration of a large volume with a disposable cup filter

Materials

- A disposable 0.2 µm filter
- A holder or stand for filter
- A sterile bottle as a receptacle

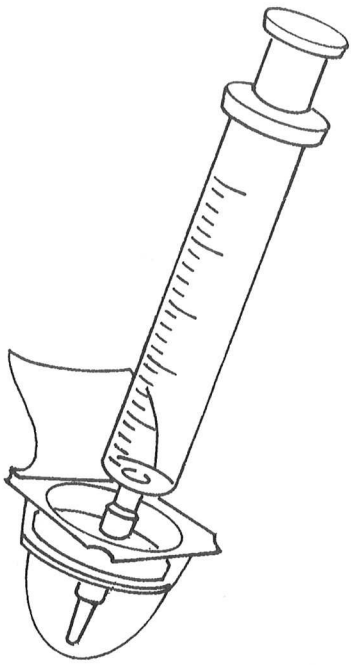


FIGURE 4.
Keep the filter in the wrapper until you are ready to dispense the contents.

- Vacuum source with attached tubing. House vacuum, found on lab benches, is the best and most common. The vacuum can also be water driven, or provided by a gentle pump.

1. Attach the vacuum hose to the filter. Place the filter in a stand where it will remain stable during manipulations.
2. Pour the solution into the filter. Don't worry if you have a bit more solution than will fit in the filter; you can add more after some of the solution has been filtered.
3. Put the lid on the filter and slowly turn on the vacuum. The solution will be pulled through the filter into a sterile receptacle. For some filters, this receptacle is actually a sterile bottle that can be used to store the buffer.
4. Turn off the vacuum and wait a minute.
5. Twist off the vacuum hose. Immediately pour the filtered solution into a sterile bottle and cap it. If the sterile bottle is part of the filter apparatus, just cap it.
6. Label the bottle. Be sure to note that the contents have been filter-sterilized (FS). Discard the filter.

There are also reusable filter apparatuses, with disposable filters, which can be used to sterilize large volumes of solutions. Attach the filter setup with a 0.2-μm filter to a sterile sidearm flask (to which the vacuum hose is attached). Turn on the vacuum, pour in the solution, and continue to pour until the entire solution has passed through the filter into the flask. Remove the vacuum hose, flame the opening, and pour the solution into a sterile bottle.

Aspirating

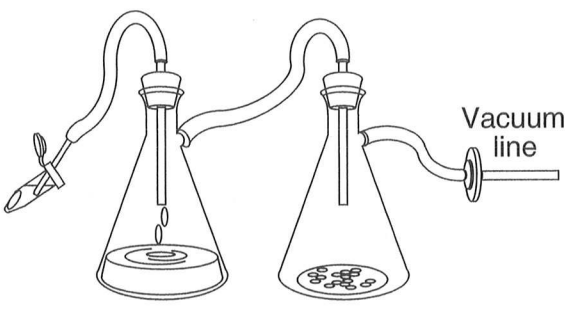
The motions involved in removing fluid with an aspirator are very similar to those used in transferring liquid with a pipet. Set your working area up as if you were pipeting from one container to another. Just add a test tube or beaker that can be used to place the used pipet and tubing temporarily.

A pipettor tip can be inserted on the end of a pasteur pipet, making flaming unnecessary. Gently stab the pipet into the tip in the tip box.

1. Open a package of pipets or pasteur pipets. One will be attached to the aspirator.
2. Holding the pipet in the left hand, insert it into the tubing that is held in the right hand.
3. Hold the pipet near the place it enters the tubing.
4. If you are using a glass pipet, flame it lightly.
5. Turn on the vacuum.
6. Open the container or tube from which you will be removing fluid. Hold the cap in the last two fingers of the right hand. Maintain the tube at a 45° angle.

The tilt of the tube not only helps prevent airborne contamination, but also protects the integrity of pellets while removing supernatants.

7. Flame the open container.
8. Insert the tip of the pipet just below the surface of the liquid. As the level of the liquid goes down, follow it with the pipet tip.
9. As you near the bottom, gently lift and move the pipet around the surface of the pellet, gently testing the strength of the pellet without touching it.
10. Remove the pipet and insert it into the tube or beaker until you have a chance to deal with it.
11. Flame the container, cap it, and put it down.
12. Hold the tubing and pipet pointing upward, to be sure all the fluid is drained from it.
13. Turn off the vacuum. Remove the pipet and discard it.



An aspirator used for potentially infectious material must be equipped with a hydrophobic filter between the flasks and the vacuum supply. This prevents the uptake of material into the vacuum system. If the vacuum suddenly is reduced, it is usually because the filter has become wetted and must be replaced. Be sure you know where the replacements are.

PROTECTING THE INVESTIGATOR

Biomedical investigators work with a host of potentially infectious elements. These include virus-infected cells, human blood and waste products, and pathogenic bacteria. Organisms are classified according to the risk of transmitting disease. Several health organizations have established guidelines to be followed when working with different kinds of biohazard (potentially infectious) material: Your own institution may have additional rules. *Following the guidelines protects not only the immediate investigator, but also the other people in the laboratory.*

> *"However, he would then review the protocol... It was in this manner that I was introduced to Avery's extraordinary rigorous bacteriological technique ...he... had agreed that they would treat all bacterial cultures as if they contained the plague bacillus. They realized that it was a common failing to become sloppy in handling nonpathogenic organisms which in turn led to some relaxation of acceptable techniques when dealing with more infectious agents." McCarty, p 125.*

TABLE 1. Systems for Classifying Microorganisms on the Basis of Hazards to Laboratory Workers and the Community

	Hazard			
USPHS (1974)	Class 1 none or minimal	Class 2 ordinary potential	Class 3 special, to individual	Class 4 high, to individual
WHO (1979)	Risk Group I low individual low community	Risk Group II moderate individual low community	Risk Group III high individual low community	Risk Group IV high, to individual and community
ACDP (1990)	Hazard Group 1 unlikely to cause human disease	Hazard Group 2 possibly to laboratory workers, unlikely to community	Hazard Group 3 some hazard to laboratory workers, may spread to community	Hazard Group 4 serious hazard to laboratory workers, high risk to community

Several organizations have established guidelines to follow when working with each level of biohazard. The system of the U.S. Department of Health and Human Services (USPHS), a branch of the NIH, is used in the majority of descriptions in the United States.

Biosafety Level Requirements

In the USA, containment facilities are referred to as BL1, BL2, BL3, and BL4, BL standing for *B*iosafety *L*evel protection. They are sometimes also called P1, P2, P3, and P4, the "P" being an abbreviation for protection. The USPHS hazard classification for microorganisms corresponds to the Biosafety 1 containment level require-

ments. In other words, the Class III organism would require BL3 containment.

Most of the guidelines concern the containment of the hazard. Containment is effected by the use of different levels of biosafety cabinets, which provide a closed environment with control of airflow and exhaust.

The procedure performed can also influence the actual hazard of the organism. Disruptive actions, such as sonication, that can cause aerosols increase the potential hazard and the protection needed is greater.

TABLE 2. Summary of Biosafety/Containment Level Requirements

Level	Facilities	Laboratory practice	Safety equipment
1	Basic	GMT[a]	None. Work on open bench
2	Basic	GMT plus protective clothing, biohazard signs	Open bench plus safety cabinet for aerosol potential
3	Containment	Level 2 plus special clothing, controlled access	Safety cabinet for all activities
4	Maximum containment	Level 3 plus air lock entry, shower exit, special waste disposal	Class III safety cabinet, pressure gradient, double-ended autoclave

[a]GMT = Good microbiological technique.

Biosafety Cabinets

Biological safety cabinets are divided into three classes, I, II, and III, based on the amount of protection provided (see Table 3).

STERILE TECHNIQUE IN THE CLASS II BIOSAFETY CABINET

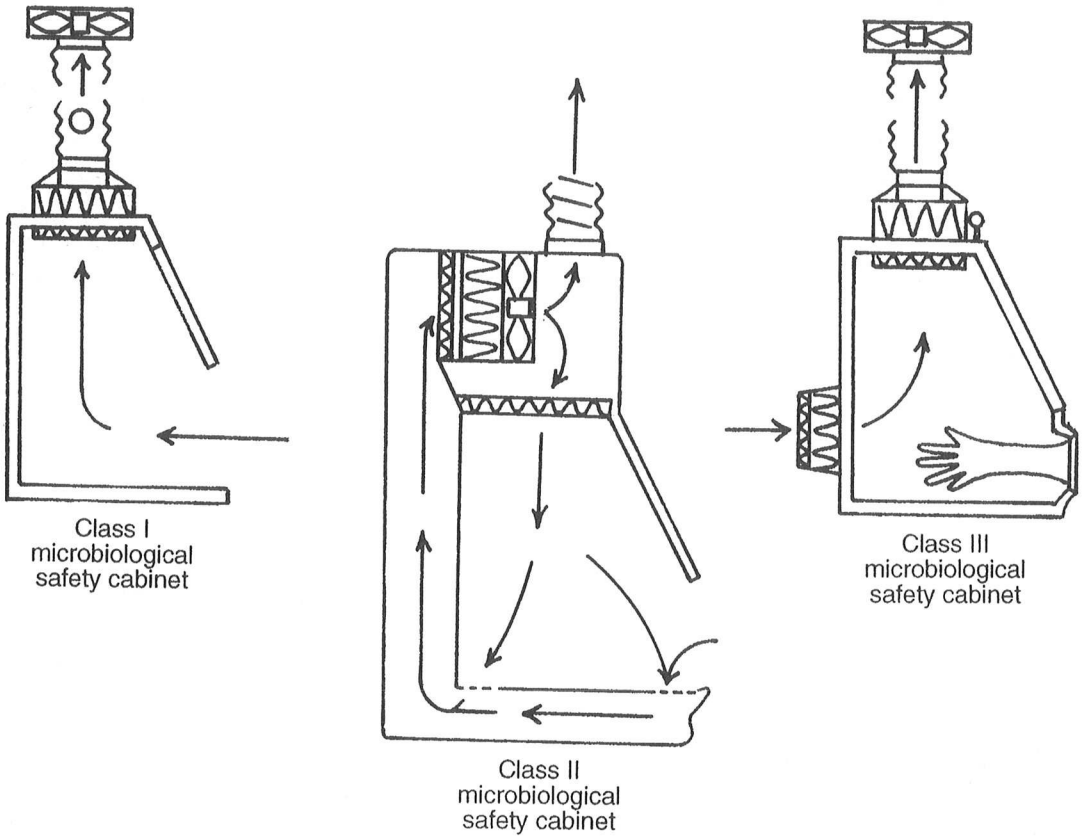
The highest level of biohazard most investigators routinely deal with is with class 2. The class II vertical flow cabinet is sufficient to deal with this.

Infected human blood and pathogenic organisms require BL3 (and sometimes BL4) containment, which is provided by a negative pressure room, a facility not found in many institutions.

The *biosafety hood*, also known as the *laminar flow hood* or *class II vertical flow cabinet* provides filtered and recirculated airflow within the workspace. The airflow makes a curtain that stops passage of the outside air into the hood, and the air into the room. This protects the working investigator from the contents of the hood and makes the hood a good place to deal with potentially dangerous organisms. The exhaust air is filtered through a HEPA (high efficiency particle air) filter, so that biohazard material isn't released into the room or building.

However, most hoods are used to *protect the experiment*, not the experimenter. Their most common use in labs is with routine tissue culture, to prevent contamina-

TABLE 3. The Use of Biological Safety Cabinets



Class	Investigator protected?	Experimental materials protected?	Suitable for
I	Partially Circulating air barrier; HEPA-filtered exhaust	No No sterile work surface; unfiltered room air is drawn across the work area.	Low-risk oncogenic viruses Routine lab operations such as sonicating. Provides personnel protection for work with chemical carcinogens, low-level radioactive materials, volatile solvents. Similar to fume hood, filtration not as effective.
II	Partially Circulating air barrier; HEPA-filtered exhaust	Yes All intake air is filtered.	Low- to moderate-risk oncogenic viruses CDC class 1–3 agents, anything requiring BL2 containment. Cell culture *or* bacterial culture.
III	Yes Physical barrier (glove box); HEPA-filtered exhaust	Yes All intake air is filtered. Poorer air circulation than in class II; material not as protected	Used for CDC class 4 agents requiring BL4 containment. Highly toxic chemicals and carcinogens (provided that effluents are treated)

The Biosafety 1 containment levels of the hazard classification for microorganisms do not correspond to the classes of biosafety cabinets.

tion of the cells during splitting and experimental manipulations. Because of the design of the laminar flow hood, there will be fewer particles or microorganisms floating in the air, waiting to leap into your opened bottles. Thus, you can relax *somewhat* on your benchtop aseptic technique.

People who have first learned aseptic technique in a biosafety cabinet tend to be sloppier about their technique than a bench-trained person. They assume the hood will take care of all lapses. This isn't true! Vigilance is still necessary to prevent contamination. *The major cause of contamination is movement of the arms in and out of the cabinet, which breaks the air curtain and disturbs the flow.*

*Work with **radiolabeled iodine**, often done sterilely, must **not** be done in a class II biosafety cabinet. Iodine is volatile, and must be used in a fume hood equipped with a TEDA charcoal filter. Be sure your fume hood is certified to do ^{125}I work before you think about an experiment.*

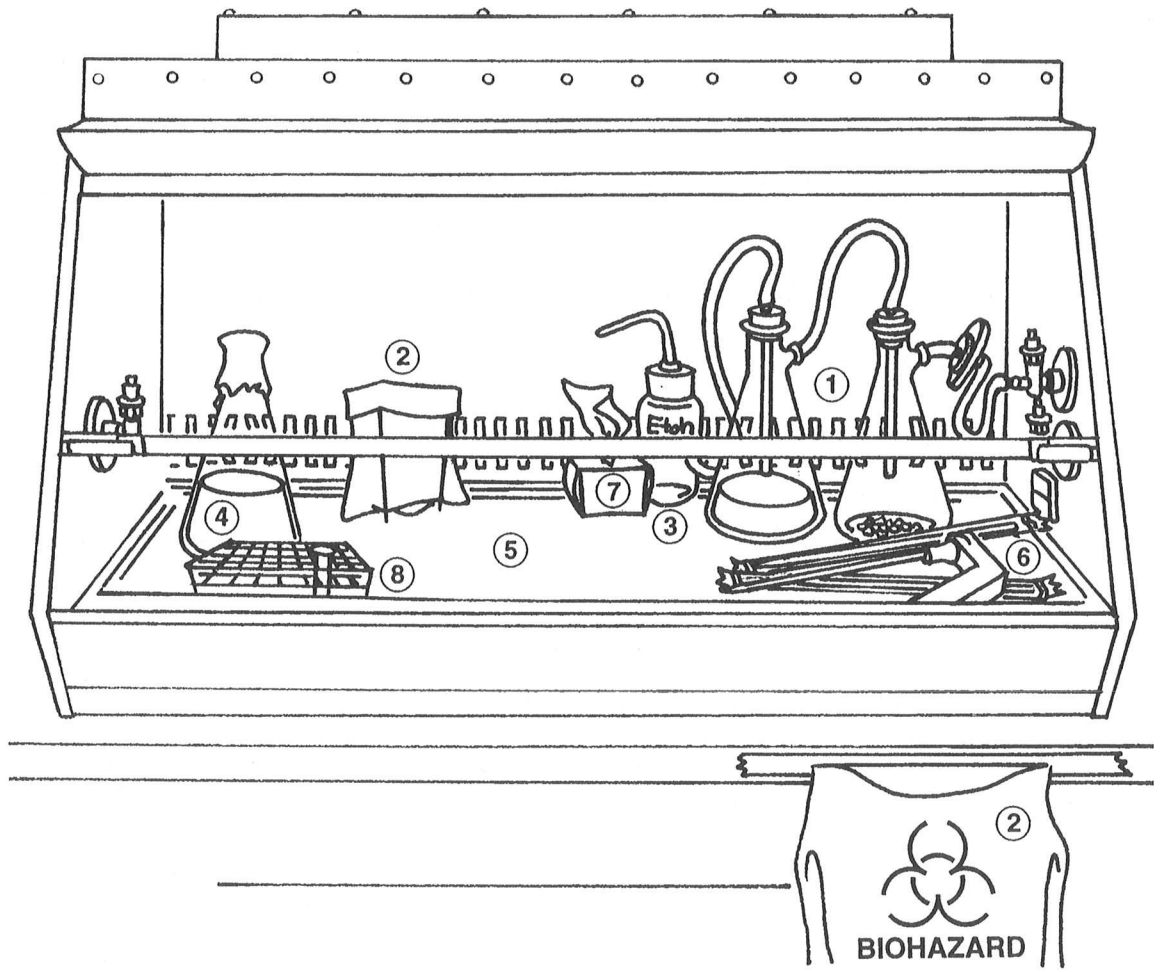


FIGURE 5.

Biosafety cabinet set up for aseptic work. **Task:** To transfer 5 ml of cell culture from a tube into 40 ml of culture medium. The supplies needed are arranged for a right-handed investigator, so that things to be manipulated with the right hand are set up to the right of the working area, and things to be manipulated with the left hand are set up to the left of the working area. **Key:** (1) *Aspirator with filter.* (2) *Biohazard disposal.* (3) *70% ethanol in squirt bottle.* (4) *Flask of culture medium.* (5) *Clear area.* (6) *Individually wrapped pipets and pipet aid.* (7) *Kimwipes.* (8) *Test tube with cell culture, in a test tube rack.*

Working sterilely in a biosafety cabinet

1. **Verify that the hood is on and air is circulating (on/off switch, sound, and dials).** The hood should be left on continuously, 24 hours a day. It sometimes is not, because of noise and heating issues: In this case, turn it on 5 minutes before use to allow the airflow to establish itself.
2. **Lower the sash to the calibration mark.** If there is no mark, lower sash either to the 100 ± 10-ft/min level (the readout is on the front of the hood) or to 12–14 inches. The sash must be below chin level. If you are creating aerosols, lower sash as far down as you can.
3. **Do not block airflow.**
   a. Don't cover the space between tapered metal front lip and the work surface, e.g., with spill paper.
   b. Do not block the rear exhaust slot. Place bulky items to rear and sides on a supporting mesh; elevate at least 2 inches.
   c. Don't block the face of the hood with shielding or large equipment.
   d. Locate work at least 6 inches inside the hood.
   e. Don't sit with your body flush against the cabinet.
4. **Secure papers and other lightweight material to prevent their entrapment in the exhaust line.** Don't write yourself notes on stray pieces of paper.
5. **Wipe down the surface with 70% ethanol or isopropanol or another disinfectant before each use of the hood.**
6. **If you must put a sterile cap down, put it face down on a clean surface.**
7. **Minimize hand movement in and out of the hood.** Bringing arms in and out of the hood disturbs the airflow. Before you start work, put everything you need inside the hood, including a receptacle to hold trash.
8. **Do NOT use a flame in a biosafety cabinet.** It interferes with the control of the airflow patterns.

Although it is no longer recommended that biosafety cabinets have UV lights installed (because they have been deemed to be ineffective against contaminants, and, therefore, to give a false sense of security), older hoods do have them and labs do use them. Just be sure the UV light is off before you work in the hood.

Maintaining hood function

Get into the habit of checking the magnehelic dial on the outside of the hood whenever you sit down to work. If the reading changes, it indicates a problem with the airflow, and the cleanliness and protection afforded by the hood will be compromised.

The door must be closed to a level marked on the hood, in order to be effective. Lifting the hood sounds an alarm, indicating that the airflow has been perturbed. Every now and again, lift the door and be sure the alarm sounds. If it doesn't, notify EHS.

Biosafety cabinets are inspected regularly and certified for safety and integrity of airflow. The date of the previous and next inspection should be posted on the cabinet. Be sure the cabinet is inspected on time by the EHS department. At this time, the HEPA filters (which trap the particles in the hood) are usually changed, and the cabinet may need to be decontaminated with formaldehyde or another agent to permit the EHS representative to work safely. This takes at least a day, and the recertification of hoods in the lab should be coordinated so everyone always has access to at least one hood.

Working courteously in a biosafety cabinet. There are seldom enough biosafety cabinets in a laboratory to satisfy all the investigators that require one. To ensure the safety of all investigators who use the hood, the rules of courtesy must be followed meticulously.

- Use and obey any sign-up sheet.
- Get in and out of the hood as quickly as you can. Be well organized before you start.
- Replenish supplies such as bleach and 70% ethanol as soon as they run out.
- Empty sharps containers and biohazard disposal when they near being full. Don't wait until they spill.
- Remove all of your supplies when you are finished. Clean the hood well.

RESOURCES

Collins C.H., Lyne P.M., and Grange J.M. 1991. *Microbiological methods,* 6th edition. Butterworth-Heinemann, Oxford, England.

Freshney R.I. 1994. *Culture of animal cells. A manual of basic technique,* 3rd edition, pp. 51–59. Wiley-Liss, New York.

Gershey E.L., Party E., and Wilkerson A. *1991. Laboratory safety in practice: A comprehensive compliance program and safety manual.* Van Nostrand Reinhold, New York.

Heidcamp W.H. 1995. *Cell biology laboratory manual.* Gustavus Adolphus College, St. Peter, Minnesota.
http://www.gac.edu/cgi-bin/user/~cellab/phpl?index-1.html

McCarty M. 1985. *The transforming principle. Discovering that genes are made of DNA.* W.W. Norton & Company, New York.

U.S. Department of Health and Human Services. 1988. *Biosafety in microbiological and biomedical laboratories.* Public Health Services. Centers for Disease Control. HHS Publication No. (NIH) 88-88395. 2nd edition.