8

Storage and Disposal

AFTER YOU MAKE IT, after you use it, when you are done with it, you have to put it somewhere! *Everything in a lab must be accounted for and placed in an appropriate place at all times.* For the storage of experimental materials, this is obvious. Incorrect storage can ruin reagents and organisms. But it is less obvious for trash. Here the issues are of safety and expediency, and not necessarily your own safety and expediency, so people are more casual. Improper disposal of material can be a health hazard to those responsible for removing it. It can be harmful to the environment. It is illegal in many places. And it can be grounds to have the entire lab shut down.

EMERGENCY STORAGE

When in doubt, the general rule for emergency short-term storage is: COLD IS BEST.

Where should I put it??? It is 1 A.M., your experiment took 3 hours longer than you expected, everyone else has gone home, and you forgot to ask where to put your tubes. There are certain assumptions you can make about where things should go. In the morning, find out about long-term storage for the material and move it.

- **Acids or bases.** Leave on your bench until the morning. Store acids and bases separately, in polyethylene trays that could contain the spill if a bottle breaks.
- **Antibodies, monoclonal and polyclonal.** 4°C. Most purified antibodies are stored long term at 4°C, but some are stored at –20°C.

- **Assay tubes.** You can try 4°C, but assay stability is extremely variable. Most colorimetric assays will not be readable the next day.
- **Bacteria.**
  Plate or stab cultures, 4°C.
  Liquid cultures, 4°C.
  Lyophilized cultures, 4°C.
  Frozen cultures, –70°C.
- **Buffers.** 4°C.
- **Cells.** Back where they came from, or discard: *Never* put living cells where you don't KNOW they belong.
- **Detergents.** Room temperature.
- **DNA.** 4°C.
- **Enzymes.** Most restriction enzymes are stored at –20°C, but read the tube to check, as some can't be frozen. Most other enzymes should also be stored at –20°C.
- **Ethanol.** Room temperature.
- **Growth factors and cytokines.** –20°C.
- **Hazardous chemicals.** You cannot compromise with even the overnight storage of hazardous chemicals. Before you begin work with such a substance, you must find out all relevant storage and disposal information.
- **Lipids.** –20°C. Many lipids are unstable and are sensitive to oxygen or light, or to changes in temperature.
- **Media.** 4°C.
- **PCR reactions.** 4°C. You may find the previous user's tubes in the cycler.
- **Radioisotopes.**
  ^{32}P: nucleotides at –20°C, phosphate at 4°C.
  ^{33}P: 4°C.
  ^{35}S: methionine at –70°C.
  ^{125}I: protein A at 4°C, iodine in a fume hood at room temperature.
  ^{3}H: 4°C.
  ^{14}C: 4°C.

Ethanol is a fire and explosion hazard. Large volumes are stored at room temperature, in a high-density polyethylene or steel (terne plate, galvanized, or type 316 stainless) safety can. However, some protocols call for cold ethanol: Two common uses of cold ethanol are cell and tissue fixation and nucleic acid precipitations. Investigators often try to keep a small bottle of 100% ethanol in the refrigerator, but EHS teams always remove it during inspections. If it is stored cold, it should be kept in an explosion-proof refrigerator. Otherwise, remove some and keep it on ice before the experiment to cool it.

- **RNA.** –20°C with ethanol, –70°C in water.
- **Serum.** 4°C.

STORING REAGENTS

For long-term storage, you want a situation for each chemical in which the biological and chemical activity of the material is preserved as best as it can be, in as safe a location as possible for all lab personnel. These requirements are specific to *each* chemical and reagent, and you must check the situation for the individual reagent, and not merely for the class or type of reagent.

What you need to know to store material

- **Temperature requirement.** Must it be stored in an oven, at room temperature, refrigerated, frozen, deeply frozen, or in liquid nitrogen?
- **Gas requirements.** Is the material sensitive to oxygen? Does it require another gas?
- **Moisture sensitivity.** If water vapor would harm the material, it needs to be desiccated. Must it also be under vacuum?
- **Associated hazards.** Is the material radioactive, flammable, extremely toxic, or volatile? If it is a solution, is the solvent organic?

Reagents must be stored with all storage requirements satisfied. For example, if a substance is radioactive and oxygen-sensitive and requires cold storage, it must be stored under nitrogen and placed in an area of a freezer in which radioactive material is permitted to be stored.

How to find storage and disposal information

- **The Materials Safety Data Sheet (MSDS),** which comes with every chemical, describes the composition and properties, toxicology, and instructions for handling, spill control, and waste disposal. If you cannot locate one, call the manufacturer and ask them to fax one to you.
- **The Merck Index.**
- **EHS department.** The EHS (Environmental Health and Safety) department probably keeps the data sheet on file for every chemical used by the department. Even if you have this information, you should be in frequent

To open a vacuum-sealed desiccator

1. Slowly open the stopcock to release the vacuum.
2. Try to slide the lid off.
3. If it won't slide, release the vacuum by slowly prying and wiggling a flat-edged weighing spatula between the top and bottom. A razor will also work, but you must be careful.
4. Once you hear the hiss of the releasing vacuum, slide the top off while holding the bottom firmly. Never try to lift the top off.

To open a desiccator that is kept in the cold

The standard wisdom says that the desiccator must be brought to room temperature before it is opened, in order to avoid condensation inside the container. The problem is that the reagents inside react adversely not only to water, but also to warmth.

For non-vacuum desiccators and grease-free desiccators, it is fine to open the jar immediately, remove the reagent you need, and quickly replace the lid and the jar in the refrigerator. If it makes you feel better, quickly wipe the inside of the container with a Kimwipe before you put the lid on, but it probably won't matter.

Let vacuum desiccators come to room temperature before you open them. Cold grease won't seal well, so you can't return greased desiccators to the cold immediately, anyway. You might as well avoid any chance of condensation and open the desiccator only when it is warmed.

How to store reagents that are light-sensitive

If something arrives from the manufacturer in a brown bottle, assume that it is light-sensitive. If possible, store the reagent in this container. When you make a solution of the material, you will have to store that solution away from light, also. Either obtain a brown or amber reagent bottle, or cover a clear bottle with aluminum foil and tape. Once you have capped the bottle, cover the cap and the top of the bottle with a square of foil.

Small tubes of the reagent can be kept in a box. A freezer box is good for this, or the shipping box. Label the box with the usual information, as well as with a label saying "Light-sensitive material inside!" But the light-sensitive material should be the only material in the box: You don't want to be rifling through the box for tubes and bottles.

Straight-edge razors are a hazard in the lab. Either discard razors and scalpels immediately after use, or keep only one active at a time. Store it by inserting the sharp edge into a piece of styrofoam or into a styrofoam tube rack, so you can't inadvertently grab the sharp edge. Dispose of razors in a sharps container.

Some common light-sensitive reagents are actinomycin D, mitomycin C, nitroblue tetrazolium, phenol, Rifampicin, and tetracycline.

Don't rely on the fact that the freezer or refrigerator door is usually closed to store your reagent in a clear bottle.

How to store reagents that are sensitive to oxygen

Gaseous nitrogen is usually used to drive off the oxygen in oxygen-sensitive reagents: This is what the phrase "under nitrogen" means.

1. A cylinder of nitrogen must be set up next to a fume hood, as it is usually solutions with organic solvents that require nitrogen. Turn the hood on.
2. Be sure the cylinder is strapped into place. (See Chapter 10 for the use of gas cylinders and pressure regulators.)
3. Insert a pasteur pipet into the tubing connected to the regulator.
4. Turn on the nitrogen. Adjust the flow until you can barely feel it when applied closely to the back of your hand.
5. Open your tube or bottle in the hood. It should be held firmly in a rack.
6. Lower the pipet tip into the mouth of the container until you can just see the surface liquid ripple. Leave it there for 5–10 seconds for tubes, longer for containers with a higher liquid-to-air ratio.

The use of aseptic technique (Chapter 9) will prevent the introduction of contaminants into the tube while you gas the tube.

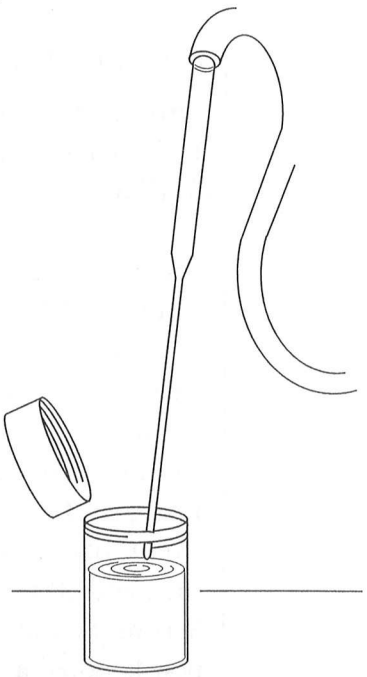

FIGURE 2.

Control the flow of the nitrogen so the surface of the fluid ripples gently.

7. Cap the container quickly.
8. Turn off the nitrogen.
9. Store the vial. It usually must be at –20°C, and may also be put under vacuum.

ALIQUOTING

Many substances in the lab are stored in small volumes.

Why aliquot?

The stock solution is the concentrated solution that will be aliquoted. The working solution is the final concentration of the material after the aliquot has been diluted at the time of use.

- **To prevent breakdown of the stock solution by repeated freeze-thawing.** Many materials are unstable to freeze-thawing, and small aliquots can be used once or twice and discarded, or held for a short time at 4°C. Examples of this are *serum* and *antibodies.*

- **To prevent contamination by multiple users.** The more people that use a stock solution, the greater the chance of introducing a contaminant. This is true even if the stock is used at the same temperature at which it is stored. Examples are *enzymes* and *media.*

Commonly aliquoted material

Antibiotics. 1:100 or 1:50 is a typical and useful dilution. See Chapters 10 and 11 for concentrations of tissue culture and bacterial culture antibiotics.
Antibodies. Most antibodies react unfavorably to repeated freezing and thawing.
Bacteria. The most common use for aliquoted bacteria is for transformations. Competent bacteria are stored at a concentration that enables the investigator to remove a tube and immediately perform the transformation.
Cells. Cells must be stored, in liquid nitrogen or in deep freeze, at a concentration that buffers the inevitable cell death but doesn't allow a freshly started culture to overgrow.
Enzymes. Generally, it is best to buy in small volumes, even if it is more expensive. Only a very large lab, with a dedicated person in charge, can spare the time and effort to aliquot enzymes without costly mistakes.
Serum. Serum is cheaper if purchased in large volumes, but it retains activity best if stored at –20°C, without being frozen and thawed.

- **For physical convenience.** It is much easier to manipulate a 10-ml tube than a 500-ml bottle!
- **To save time.** Instead of weighing out a powder and dissolving it every time, this can be done once and an aliquot merely tipped into place.

Don't aliquot when

- The diluted substance has little stability.
- You will only use a substance infrequently.
- You will be using varying and wide-ranging concentrations of the substance.

Stock and working solutions are given either in w/v or molarity. Keep your stock and working solutions in the same units.

How to aliquot

1. To make a stock solution, you need to know:
   - The working concentration of the substance. Working concentration is also given as the final concentration. It may be given as w/ml or in molar concentration.

     For example, the working concentration of chloramphenicol to use for amplification of plasmids is 170 µg/ml, and for selection of resistant bacteria, 10 to 30 µg/ml.

   - *What to dissolve the substance in.* Not everything can be dissolved in water. Some substances must be dissolved in another solvent at the stock solution concentration, but can then be dissolved in water at the working concentration.

     You will find the working concentration of a substance in a protocol, a manual, or a paper, or from a colleague. Be sure you get the working concentration for your particular application.

     Chloramphenicol can be dissolved in methanol or absolute alcohol, or in warm water at low concentrations. Since methanol is more poisonous to cells and bacteria than ethanol, the concentrated stock solution is made in ethanol.

- *The volumes you will be dissolving the aliquots in.* The volume of the aliquot should be appropriate to the volume of the final diluent.

To find the solubility of a substance in water or other solvent, check the substance container or its data sheet, the catalog, or call the manufacturer. Manuals and other sources of protocols often describe how to make a stock solution of the substance.

2. Decide how many aliquots to make. This is a compromise between *convenience and need.* It is difficult to weigh out very small amounts, so you may have to make more aliquots than you will ever need. 10–100 is usually a good amount.

Make aliquots so they must be used at convenient ratios such as 1:1000 or 1:100 to arrive at the desired final concentration.

3. Set up sterile tubes. Label well. Keep the tubes on ice if the aliquots must be frozen or refrigerated (as they usually must be). Loosen the caps.

You want a 20 mg/ml stock solution of chloramphenicol, to make a final 1:1000 dilution for 20 µg/ml.
If you will be making 1 liter (1000 ml) of medium, you need 1-ml aliquots.
If you will be making 100 ml of medium, you need 100-µl aliquots.

4. Make the stock solution and filter it, if necessary. Work in a laminar flow hood or a place without drafts.
5. Dispense the sterile solution into the tubes. Place in a freezer box or rack at the appropriate temperature.
6. Record the aliquot information in your lab book.

Label each tube. The label should include the name and concentration of the substance, the date the solution was made, and your initials. Your name and the contents must be clearly identifiable by anybody in the lab or by safety personnel. If you will have a rack or a box of only one type of aliquot, you can label the rack or box and put only an identifying mark on the lid of each tube.

REFRIGERATORS AND FREEZERS

- *Use the appropriate refrigerator or freezer.* If radioactive or biohazard materials are stored within, no food is permitted, and there will be a sign on the door to this effect. Flammable materials (such as ethanol) must be stored in an explosion-proof refrigerator, which has an enclosed motor to eliminate sparking.

- *Only open the door when you need to.* If you must manipulate, remove the box or rack and put it on ice while you search for a certain tube.
- *All containers in the refrigerator or freezer must be completely labeled and securely capped.* No loose tubes in styrofoam cups, or tucked into the egg rack! Every tube must be able to withstand shaking and moving (such as you would find if a frantic investigator were pawing wildly through a refrigerator to search for a lost tube) without falling from a rack.
- *Periodically discard material you no longer need.* Bottles with only 10 ml of medium left, petri dishes of bacterial medium, and duplicate sample retained "just in case" can take up a lot of room and make it difficult to find anything.
- *Keep a record of the location of all of your reagents.* It is very easy to quickly slip a tube in a box, with the intention of moving it later to a labeled box. Have a system set up so it is effortless to record the placement of any reagent.
- *Respect private space.* Space in refrigerators is frequently allocated to investigators, with everyone given a shelf or rack. Don't spill over onto someone else's space. If, after throwing away or rearranging everything you can, you still need space, speak with the czar of the freezers and refrigerators and request more space.

If the stock solution needs to be filter-sterilized, make more (10%) than you need. Check the dry material, for it may be sterile: If you use the entire amount and dissolve it in sterile water or ethanol, you won't need to filter-sterilize it.

Frost-free freezers. *It is often recommended that enzymes and growth factors, which are sensitive to changes in temperature, not be kept in a frost-free freezer. This is because the frost-free freezer cycles slightly in temperature to prevent frost buildup.*

Frost-free refrigerators. *Refrigerators should be frost-free to prevent water damage.*

How to defrost a freezer

Defrosting a laboratory freezer is a laboratory job, and requires the cooperation and assistance of all lab members. It is a 1- to 3-day job: The contents must be moved, the defrosting actually done, the freezer washed and brought back to temperature, and the contents repacked.

- *The defrosting must be planned a week in advance.* All workers should be notified of the

Finding a place for the contents of a low-temperature freezer is a formidable task, because that freezer and every other one is usually completely filled. EHS departments can sometimes arrange a "loaner" low-temperature freezer for emergencies and for short-term storage. You should know where to find another freezer before an emergency occurs.

date and time by which they must remove all of their reagents from the freezer. Ideally, an empty freezer is available for temporary storage; otherwise, everyone must find a bit of cold space somewhere.

- *The pre-defrosting cleanup is a good time to unload unnecessary reagents.* Each investigator should discard expired reagents and ones that will never be used. Go through the tubes, and update your records.
- *Pack all tubes carefully for the move, no matter how short a distance you are going.* There should be no microfuge racks or any holders from which a tube can spill. Every box and container must be labeled with the contents and your name. Do not rely on location to recognize your reagents!
- *Start the defrosting as early as possible in the morning.* Do not do it overnight—huge amounts of water can be generated, and someone must be there at all times. Pull the plug, and open the door.
- *Wear gloves for all fiddling and removal of the ice.* Medium-weight rubber gloves (such as used for washing dishes) are excellent for protecting your hands against the cold and whatever nasty material might have spilled into the ice. Beware of broken tubes embedded in the ice.
- *Have ready:* Mop and bucket, newspaper, paper towels, bench diapers, anything that will absorb the water. Also useful are basins into which the sodden material can be thrown.
- *Help the defrosting along,* but be careful. Everyone says not to chip at the ice, but everyone does: If you do, be sure the ice is partially melted, and that it is thick enough that you are in no danger of puncturing the freezer. Try to pry, rather than chop.

Ice buildup on inner doors and gaskets of –70°C freezers is a chronic problem and may prevent the doors from closing tightly. This, in turn, leads to condensation and more ice accumulation. Once a week, scrape the ice free with a plastic windshield ice scraper.

- *Hairdryers are used for small freezers,* but the danger of electrocution is high, and this should not be done. Buckets filled with hot water are a good compromise. Fill a bucket with as hot water as you can, and place the bucket inside the freezer and shut the door. Replace the bucket in 20 minutes. You can also spill almost-boiling water inside the freezer; this will speed up the defrosting but add to the amount of water you must clean up.
- *Remove all water as you go along.* This is relatively easy but tedious for upright freezers, as most of the water will go on the floor or in the bottom of the freezer, where it can be collected and mopped. In horizontal freezers, the water is difficult to reach on the bottom. The water can be

siphoned or pumped out, or mopped. There are also inexpensive, disposable plastic pumps that can be used for this.

- *Keep the area surrounded by dry material, so the floor stays dry.* A wet lab floor is a death trap.
- *Clean the defrosted freezer.* Once the freezer has been thoroughly defrosted, wipe it down with a mild disinfectant.
- *Only when the freezer is completely dry can you plug it back in.* It will take hours, certainly overnight, before the freezer will be back down to temperature. Make sure the temperature is stable before you reintroduce tubes.
- *Record the placement of all material.* Space may be allocated by one worker as a laboratory job, but each investigator should know and record the contents of his or her own boxes.

DISCARDING LAB WASTE

Every paper, cell, chemical, pipet, or tube has its own place to be discarded—nothing can be casually thrown away. Consult your institution's safety office for the specifics of trash disposal at your institution. Be very careful, and not only for safety reasons: *Throwing garbage in the wrong place is an easy way to tick off everyone in the lab.*

What You Need to Know to Dispose of Material as Waste

You must check with the EHS department of your own institution for the rules regarding storage and disposal.

- **The chemical composition.**
- **If it is hazardous or nonhazardous.**
- **If it is radioactive.**
- **If it is a biohazard.**
- **If it can be recycled.** Newspaper and other paper can be recycled. So can some reagent containers, dry ice shipping containers, and shielded casings for radioactive material. Each is disposed of in a separate place.

NEVER

- put radioactive waste anywhere but in the designated radioactive waste area, not even for a minute.
- put sharp items, such as needles, pasteur pipets, or scalpels, in regular or regular biohazard trash. They must be placed in a special sharps disposal box and are usually treated as biohazard material.
- put broken glass anywhere but in a box or container dedicated to that purpose. If the glass contained biohazard material, it must be autoclaved first.

Acids. Small amounts (<100 ml) may be neutralized (check with pH paper) and slowly poured down the drain with large amounts of water. Larger amounts are handled as hazardous chemical waste.

Acids and bases should not be mixed.

Aluminum foil. Recycle: look for a bin in the hall or department.

Antibodies. Biohazard waste.

Bacteria. Biohazard waste. Plates and slants go into solid biohazard waste. Reusable flasks and bottles are autoclaved or rinsed with 10% Clorox. Liquid cultures are either brought to 10% bleach by the addition of Clorox, or autoclaved, before being poured down the sink. Supernatants from bacteria should be treated as a liquid culture (yes, even for regular old *E. coli*) and should be autoclaved or bleached before disposal down the sink.

Do not autoclave solutions containing Clorox!

Bases. Small amounts (<100 ml) may be neutralized (check with pH paper) and slowly poured down the drain with large amounts of water. Larger amounts are handled as hazardous chemical waste.

Biohazard waste is anything derived from a living thing or that comes in contact with living things. Both liquid and dry waste will be generated.

- Liquid (over 1 ml) biohazard waste should have Clorox added to 10%, be allowed to sit for 30 minutes, and be poured down the sink.
- Dry biohazard waste is discarded in bins lined with biohazard bags.

Don't put acidic or basic waste (pH less than 3 or greater than 9) in metal cans, which can corrode.

Buffers. Most buffers can be poured down the sink. See "Chemical waste–hazardous" for exceptions.

Cells. Biohazard waste. Liquid cultures are either brought to 10% bleach by the addition of Clorox, or autoclaved, before being poured down the sink. Aspirate liquid from disposable plates, dishes, tubes, bottles, and flasks before throwing the containers into solid biohazard waste. Reusable flasks and bottles (unusual for cells) are either rinsed with 10% bleach or are autoclaved.

Keep organic waste separate from aqueous waste.

Chemical waste–hazardous. Generally, you should not mix chemicals. When mixing is done, it is for small volumes of solvents from the same category; for example, all halogenated solvents. Check first with EHS. Of course, it is also done if mixtures are part of a process, such as from a DNA synthesis machine, or from extractions (phenol-chloroform).

All bottles must be labeled and/or tagged. The full chemical name, the percentage of the mixture, if any, the waste volume, the location, and your name must be on the tag or label, which is often provided by EHS.

Use the correct bottles. You cannot just pick up any spare bottle in the lab and pour hazardous waste into it: There may be the danger of an explosion or fire or a leak. Ask EHS for the correct bottle and cap to use for disposal for every single chemical you will dispose of.

Do not flush flammable, water immiscible, water reactive, or highly toxic materials down the sink.

Hold the waste bottles in the appropriate holding area until pickup (or dropoff) by EHS. Some waste must be neutralized before it is picked up. It is a good idea to check the pH of all waste—now you get to use all that pH paper! Record the pH on the label. Check with EHS about safe neutralization instructions.

- Examples of hazardous chemical waste

Acetonitrile
Acrylamide
Benzene
Chloroform
Chromic acid
Cyanogen bromide
Diisopropyl fluorophosphate (DFP)
Hydrogen peroxide
Diethyl ether
Dimethyl formamide (DMF)
Dimethyl sulfoxide (DMSO)
Ethidium bromide
Formaldehyde
Hydrazine
Hydrofluoric acid
Hydrogen cyanide
Mercury
Methylene chloride
Methylmercuric hydroxide
Osmium tetroxide
Peracetic acid
Perchloric acid
Phenol and phenol solutions
Picric acid
Pyridine
Trichloroethylene
Xylene

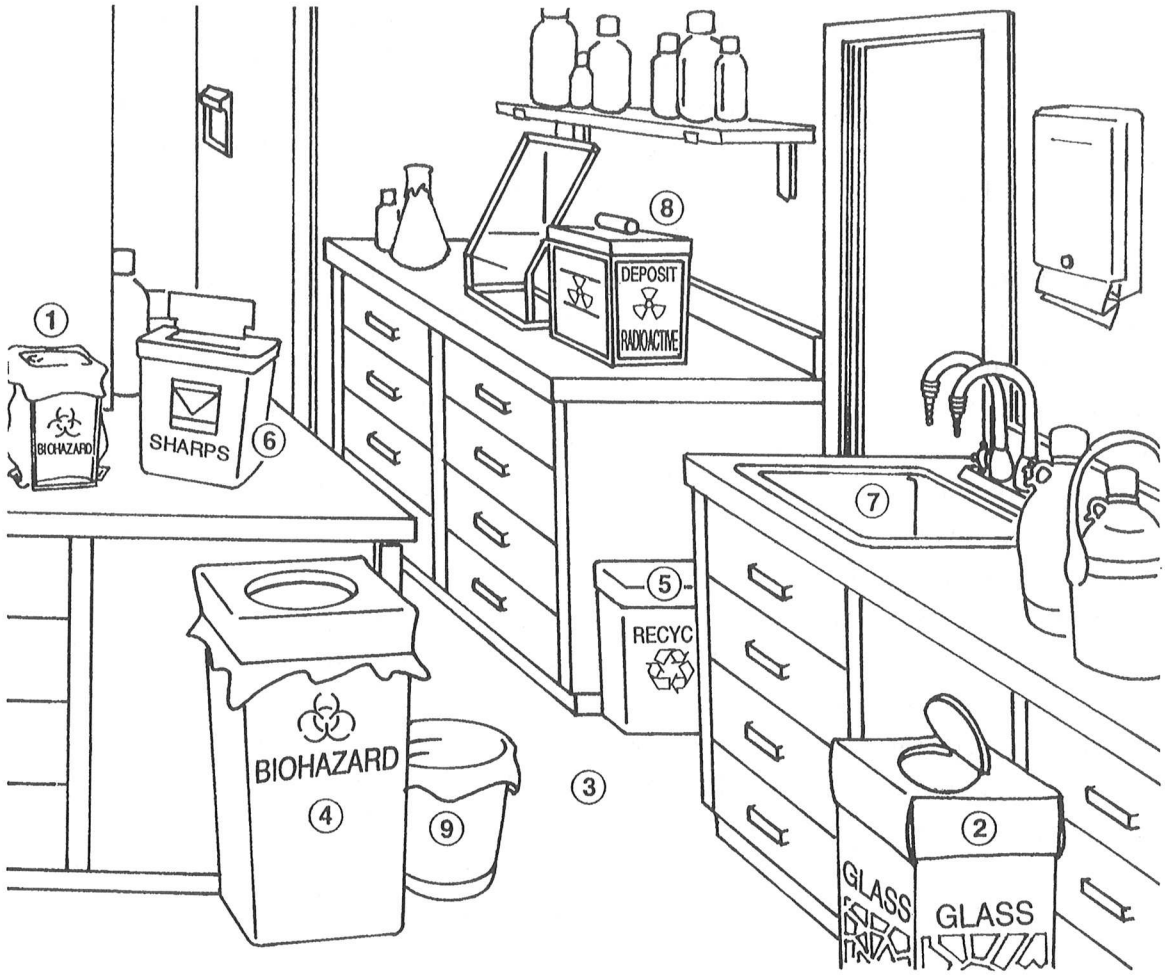

FIGURE 3.

Waste disposal areas are found throughout the laboratory. **Key:** (1) *Benchtop biohazard bag.* (2) *Glass disposal box.* (3) *Hazardous waste pick-up area.* (4) *Large biohazard trash bag.* (5) *Paper to be recycled.* (6) *Sharps disposal.* (7) *Sink.* (8) *Radioactive waste.* (9) *Trash.*

Chemical waste–nonhazardous. Dispose of as trash if solid. Liquids should be flushed down the drain in the laboratory sink, followed by large amounts of water.

- Organic chemicals
  - Acetates: Ca, Na, NH_4, and K
  - Amino acids and their salts
  - Citric acid and salts of Na, K, Mg, Ca, and NH_4
  - Lactic acid and salts of Na, K, Mg, Ca, and NH_4
- Inorganic chemicals
  - Bicarbonates: Na, K
  - Borates: Na, K, Mg, Ca
  - Bromides: Na, K

Carbonates: Na, K, Mg, Ca
Chlorides: Na, K, Mg, Ca
Fluorides: Ca
Iodides: Na, K
Oxides: B, Mg, Ca, Al, Si, Fe
Phosphates: Na, K, Mg, Ca, NH_4
Silicates: Na, K, Mg, Ca
Sulfates: Na, K, Mg, Ca, NH_4

Nonflammable, noncorrosive, nonmetallic, nontoxic, odorless, water-soluble substances may be discarded down the sink. Most buffers can be discarded in this way.

DNA. Biohazard waste.

Dry ice. Let the dry ice evaporate in the ice bucket or container. Do not dispose of it down the sink, even while running the water, because the pipes can freeze and crack.

Gels. Biohazard waste.

Glass. Dispose of in a sealed and clearly labeled box. Autoclave. Many labs have a dedicated glass disposal box.

Gloves. If gloves are used for work with biohazard material, dispose of them in the solid biohazard waste. Otherwise, gloves can go in regular trash (but check, because some institutions require that anything that has the appearance of biohazard material should go into biohazard trash).

Needles. Sharps disposal box.

Never remove a needle from a syringe. Never recap a needle—this is where most needle accidents happen! Throw the syringe with attached needle directly into the sharps disposal box!

Paper. Recyclable paper is put in a dedicated lab area, bin or box. Non-recyclable paper, such as paper towels, goes into trash.

Phenol and phenol-containing solutions. Dispose of as hazardous chemical waste. Keep a working bottle at your bench, and dispose of appropriately when it is filled, noting on the label the concentration of phenol and other solvents such as chloroform and isoamyl alcohol. Obtain a waste bottle with advice from EHS: Don't use ordinary lab bottles.

Photographic fixer, developer, stop bath. Diluted material may be discarded down the sink, or they may be collected by EHS. If they are, keep fixer, developer, and stop bath separate from each other.

Pipets. Disposable pipets are usually discarded directly into biohazard trash. However, pipets can pierce the biohazard bag, so double-bag to prevent leaks or

injury to personnel. Your lab may use a biohazard bag-lined box, dedicated to pipets. Reusable pipets are usually placed in a pipet bucket that can be put into a pipet washer. Since this causes an aerosol, it is preferable to use cotton-plugged pipets and place them in a horizontal container.

Protein, cell extracts. Biohazard waste.

Radioactive waste. Radioactive waste includes all paper towels, absorbent paper, pipet tips, and everything used during the experiment.

Do not dispose of volatile chemical waste, such as chloroform or ether, by allowing it to evaporate in a fume hood or on the bench. Treat volatile material as hazardous chemical waste.

RNA. Biohazard waste.

Serum. Biohazard.

Sharps. (Pasteur pipets, needles, syringes with needles attached, automatic pipet tips, glass slides and coverslips, razor blades and scalpels) in a sharps disposal box. Depending on the kind of lab, there may be separate "regular" and biohazard sharps disposal boxes, or a biohazard sharps box into which all sharps, biohazard or not, are placed. Radioactively contaminated sharps must be collected in a separate sharps disposal box.

Solvents. Hazardous chemical waste—do not pour down the sink! Use a waste container with a volume as close to that of the waste as possible. Do not combine different solvents: Of course, some waste is already a mixture and should be labeled accordingly.

For disposal of the products of manual synthesis or of other processes generating complex wastes, separate the solvents into:

- Halogenated (e.g., dichloromethane, dichloroethane, chloroform)
- Flammable (e.g., toluene, xylene, benzene)
- Aqueous (e.g., HPLC waste, amino acid analysis waste)
- Phenol-chloroform

Supernatants. Supernatants from centrifugation spins of cells, viruses, and bacteria are liquid biohazard waste. Autoclave or bring to 10% Clorox before pouring it down the sink. While centrifuging, keep a bottle for pouring or aspiration of supernatants: Do not pour supernatants down the sink directly.

Syringes. With attached needle, in biohazard sharps disposal. Syringe alone, in solid biohazard waste (for appearance sake) or sharps container. Check with EHS for local regulations.

Never remove a needle from a syringe. Throw the syringe with attached needle directly into the sharps disposal box!

Thermometers. Mercury thermometers must *not* be discarded with glass waste. Most mercury thermometers are encased with plastic or resin to contain the mercury in case of breakage: If the integrity of the seal has not been broken, pick up the thermometer (with gloves), place in a sealed box or beaker, and contact EHS for pickup. If mercury has been spilled you should call EHS immediately. Thermometers filled with alcohol or mineral spirits can be discarded with glass waste.

Don't throw pipet tips in the solid biohazard disposal or in the trash. Pipet tips are considered sharps because they can pierce through plastic biohazard bags, exposing lab and custodial personnel to the contents of the bag.

Tips. Sharps disposal box.

Trash. Nonrecyclable, nonradioactive, nonbiohazard, nonsharp, nonhazardous: There won't be very much of this! This will contain mostly paper towels. Usually disposed of in a bin, either unlined or lined with a plain (not biohazard) black or green bag. Remember—you aren't supposed to be eating in the lab, so there shouldn't be food items in the trash! This is a red flag to EHS personnel, so get rid of soda cans and sandwich wrappings outside of the laboratory.

WATCH FOR YOUR TURN IN REMOVING THE WASTE. Every lab has its own policy on removal of waste from the lab, and custodians are seldom responsible for anything other than "trash." It will fall to the lab members to do everything else (and new lab members are probably not exempted!). This may be done by a rotating task list, by honor, or by assignment: Take this very seriously, and don't miss your turn.

Volatile chemicals. Dispose of volatile materials according to their chemical composition (hazardous, organic, etc.). Do not dispose of volatile chemicals by allowing them to evaporate in a fume hood.

Order of Priority for Disposal

Much of the waste in the lab falls under several categories at once. It may be only biohazard, or it may be biohazard and radioactive, or it may be biohazard and radioactive and an organic solvent. Dispose of the waste according to its highest numbered priority:

1. Radioactive, solid.
2. Radioactive, liquid.
3. Hazardous chemical.
4. Biohazard.
5. Sharp.
6. Nonhazardous chemical.

RESOURCES

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