14

Centrifugation

HOW FAST, WHICH ONE, what speed, what temperature? The main use of the centrifuge is to separate biologically important substances, and very few experiments can be done without at least one spin in a centrifuge. Centrifuges are used to concentrate purified proteins, wash DNA, and pellet cells; there are specialized tubes, rotors, and centrifuges for just about any job. The centrifuge supplies the driving force, and the rotor dictates the functional specialization of centrifugation. You will probably use whatever centrifuge someone points you to, but know that, by choosing your centrifuge, rotor, and tubes carefully, you can get that sample just where you want it.

The ubiquitousness of the centrifuge in the laboratory should not cause you to become casual with it. It is an important and complicated instrument that can ruin samples and cause personal injury if used carelessly.

BACKGROUND

A centrifuge is a device for separating particles from a solution. In the biological research lab, these particles are usually cells, organelles, or large molecules, such as DNA.

Centrifugation

There are two main kinds of centrifugation procedures: **Preparative**, the isolation of specific particles; and **analytical**, the measuring of the physical properties of a sedimenting particle.

Most of the centrifugation done in a molecular or cell biology lab is preparative centrifugation, and most of the routine preparative centrifugation done is differential centrifugation.

The *g*-force and revolutions per minute (rpm) listed are approximate: They are dependent on the centrifuge model and rotor used.

Differential centrifugation (pelleting)

Theory: Samples are spun at a given speed, resulting in a supernatant and a pellet fraction. The sample is isolated by sedimentation velocity that, at constant centrifugal force, is proportional to the size of the particle and the difference between the density of the particle and the liquid.
Disadvantages: The pellet is a mixture of all the sedimented components, not all of which are desired.
Rotor used: Fixed, swinging bucket.
Examples: Pelleting bacteria or cells from growth medium, collecting precipitated DNA.

Density gradient centrifugation

- **Rate-zonal centrifugation**

Theory: Separates particles having a similar buoyant density but differing in shape or particle size. Sample is layered on top of a gradient of sucrose

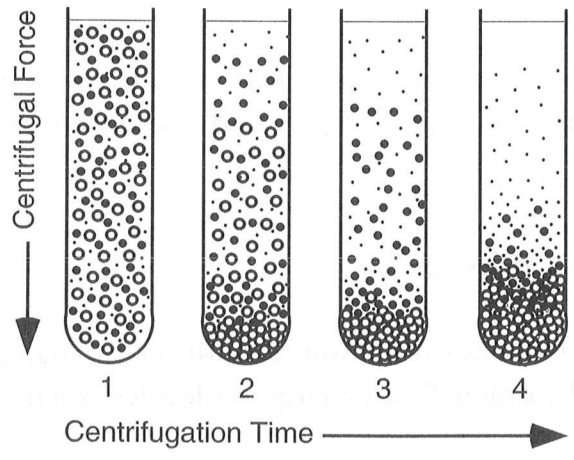

FIGURE 1.
Differential centrifugation (pelleting). (Redrawn, with permission, from Griffith 1986; Beckman Instruments.)

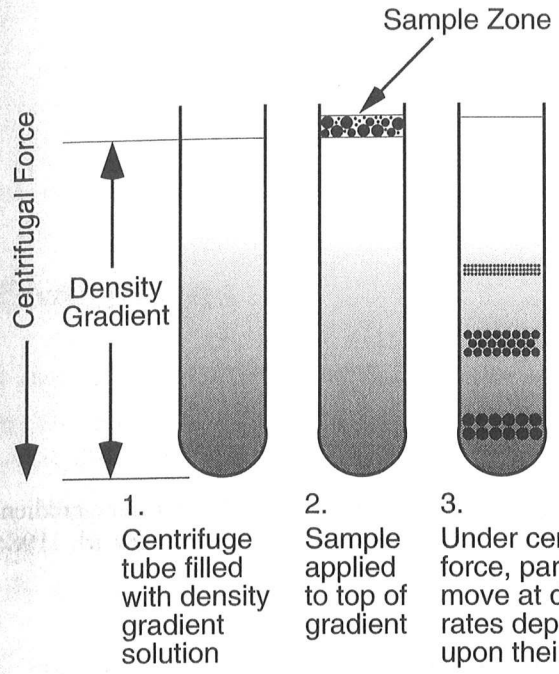

FIGURE 2.
Rate zonal separation in a swinging-bucket rotor. (Redrawn, with permission, from Griffith 1986; Beckman Instruments.)

or other viscous medium: The particle density is higher than the liquid density, so the particle will ultimately pellet. Centrifugation must be stopped when the particle(s) has been separated, but before all particles have reached the bottom of the tube.
Rotor used: Swinging bucket or specially designed zonal rotor/centrifuge.
Examples: Isolation of ribosomal subunits on a 15–40% (w/v) sucrose gradient.

- **Isopycnic (Isodensity) density gradient centrifugation**

  Theory: Like equilibrium density gradient centrifugation, used to separate particles on the basis of buoyant density. Sample is mixed with gradient material such as cesium chloride to provide a density equal to the average density of the particle. This homogeneous suspension is spun and a gradient formed during the spin. (Cesium chloride has a low viscosity, and it is difficult to make preformed gradients with it.) Particles cease sedimenting when they reach their buoyant density.
  Rotor used: Swinging bucket, vertical, fixed angle. Fixed angle and vertical are preferable, since the shorter path length allows a shorter spin. For subcellular particles, 18–72 hours at 100,000–200,000*g* are needed.
  Examples: Isolation of plasmid DNA in a cesium chloride gradient.

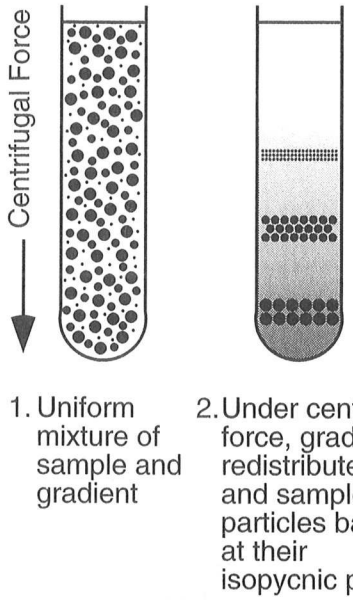

FIGURE 3.

Isopycnic separation with a self-generating gradient. (Redrawn, with permission, from Griffith 1986; Beckman Instruments.)

- **Equilibrium density gradient centrifugation**

Theory: Used to separate particles on the basis of buoyant density instead of sedimentation velocity, equilibrium density gradient centrifugation is actually a variant of isopycnic centrifugation, done with a preformed gradient instead of a self-generated one. The sample is centrifuged in a density gradient of a medium of density higher than the density of the cells or particles until an equilibrium is reached, at which each particle has

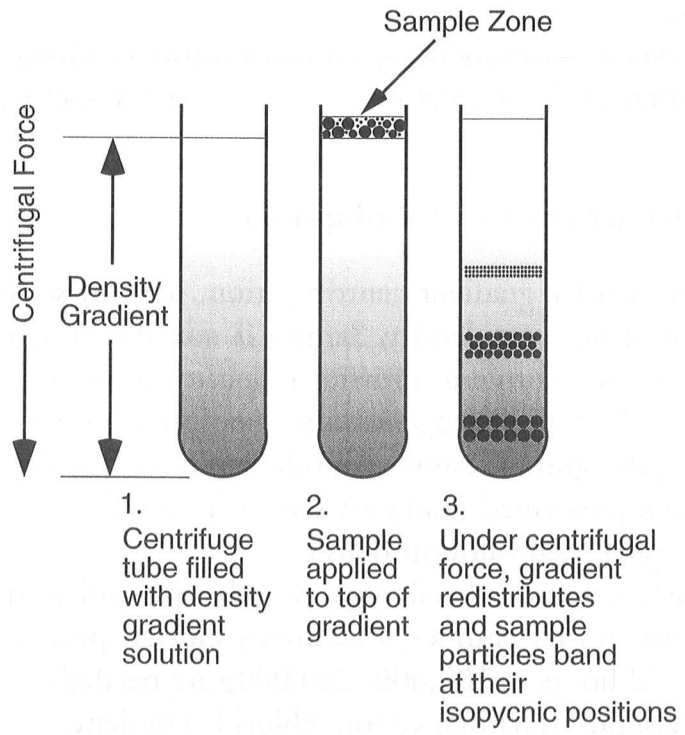

FIGURE 4.

Equilibrium density gradient centrifugation. (Redrawn, with permission, from Griffith 1986; Beckman Instruments.)

migrated to a point in the gradient where it has the same density as the surrounding solution.
Rotor used: Swinging bucket, fixed angle, vertical.
Examples: Isolation of lymphocytes on a Ficoll gradient.

Centrifuges

There are descriptive terms for types of centrifuges, but these are not strict definitions, and one centrifuge may fit into several categories. In the lab, centrifuges are generally called by the manufacturer's name.

High speed and ultracentrifuges are built with refrigeration units, needed because of the heat generated by high-speed spins. The other centrifuges are available in both refrigerated and non-refrigerated models.

- **Benchtop centrifuge.** Also known as a multipurpose centrifuge. It is not necessarily on a benchtop, and is often found under a bench.

  Uses: Pellet cells and bacteria, phenol extractions.
  g-Force and rpm: Fixed angle 17,000*g*/14,000 rpm. Swinging bucket 3,800*g*/4800 rpm.
  Rotors: Fixed angle, swinging bucket, microplate.
  Tube volumes: 2.0 ml–180 ml.

- **Clinical centrifuge**

  Uses: Serum, urine, cell, and blood sedimentation.
  g-Force and rpm: 4600*g*/6000 rpm.
  Rotors: Fixed and swinging bucket.
  Tubes: A variety of usually glass tubes, hematocrit capillaries to 75 ml.

- **Microfuge**

  Uses: Mini-phenol extractions and ethanol precipitations. Cell, at low speed.
  g-Force and rpm: Fixed angle 16,000*g*/13,000 rpm. Horizontal angle 13,000*g*.
  Rotors: Fixed, some rare swinging bucket.
  Tubes: Eppendorfs, 0.5 ml–2.0 ml.

- **High-speed centrifuge.** Also known as a high-performance centrifuge.

  Uses: Large-volume ethanol precipitations, pelleting bacteria, spin columns, protein precipitations.
  g-Force and rpm: Newer models can achieve 75,000*g*.
  Rotors: Fixed, swinging bucket.
  Tubes: Polyallomer, Pyrex.

- **Ultracentrifuge**

  Uses: Virus concentration, membrane and subcellular fraction isolation, DNA and RNA isolation.
  g-Force and rpm: 800,000*g*/100,000 rpm
  Rotors: Fixed angle, swinging bucket.
  Tubes: Nitrocellulose, polyallomer.

- **Benchtop ultracentrifuge**

  Uses: Membrane preps, virus isolation, subcellular fractionation. CsCl DNA plasmid isolations in 30 minutes.
  g-Force and rpm: 625,000*g*/120,000 rpm
  Rotors: Fixed angle, swinging bucket.
  Tubes: Nitrocellulose, polyallomer.

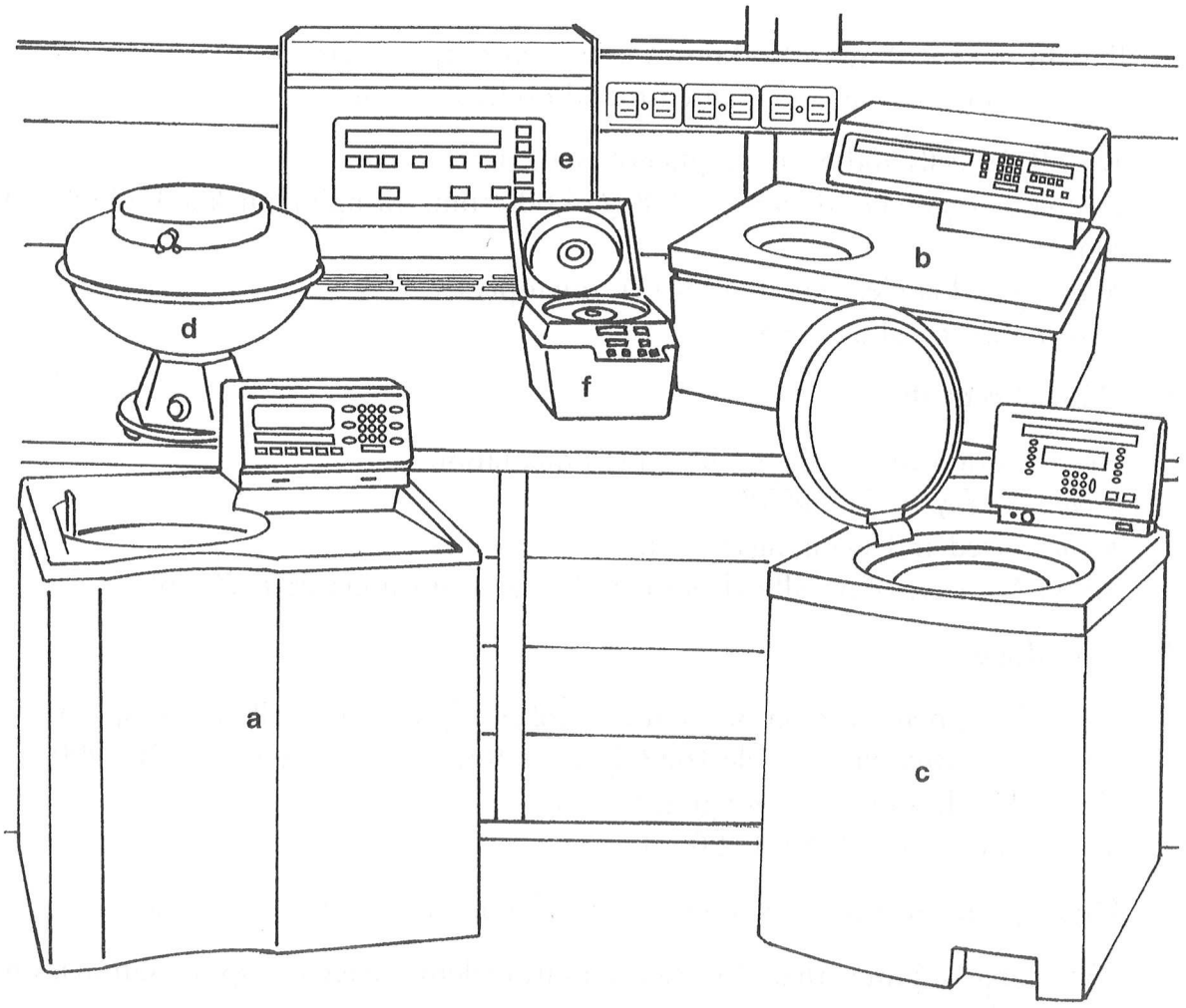

FIGURE 5.

Commonplace centrifuges are the ultracentrifuge, floor (*a*) and bench (*b*) models; the high-speed (*c*); clinical (*d*); general purpose (*e*); and microfuge (*f*).

Rotors

There are four main types of rotors: the fixed angle, swinging bucket, continuous flow, or zonal. Only the fixed-angle and swinging-bucket rotors are standard laboratory equipment: the other two are for very specialized use.

Fixed angle

Uses. Sample concentration. This is the workhorse of the lab.
Description. Sample is held at a given angle to the rotation plane.
Advantages. Works the fastest. Substances have an increased relative centrifugal force and are sedimented faster than in a swinging-bucket rotor. Few moving parts, so few mechanical failures.
Disadvantages. Materials are forced against the side of the centrifuge tube, and then slide down the wall of the tube, leading to abrasion of the particles along the tube wall.
Examples. Sorvall SS-34, or the Beckman JA-20, near vertical rotor (NVR) (Beckman), vertical rotor for high-speed spins.

Swinging bucket (also known as horizontal rotor)

Uses. Material separation. Used often in clinical work to gently separate cells.
Description. Sample is allowed to swing out on a pivot onto the plane of rotation.
Advantages. Materials must travel down the entire length of the centrifuge tube and through the media (often viscous) within the tube: This is gentler to the sample, and allows the formation of gradients and layers. Buckets can be exchanged in the rotor, so different sizes and shapes of tubes can be used. Less likely to cause aerosols.
Disadvantages. Longer apparent centrifugal force, takes longer time to precipitate than for fixed angle. There are many moving parts, which are prone to failure with extended use.
Examples. SW 55 Ti (Beckman).

Continuous flow

Uses. Separation of particles or cells from large volumes of fluid: For example, pelleting liters of bacteria, monoclonal antibody production.
Description. Designed with inlet and outlet ports for separation of large vol-

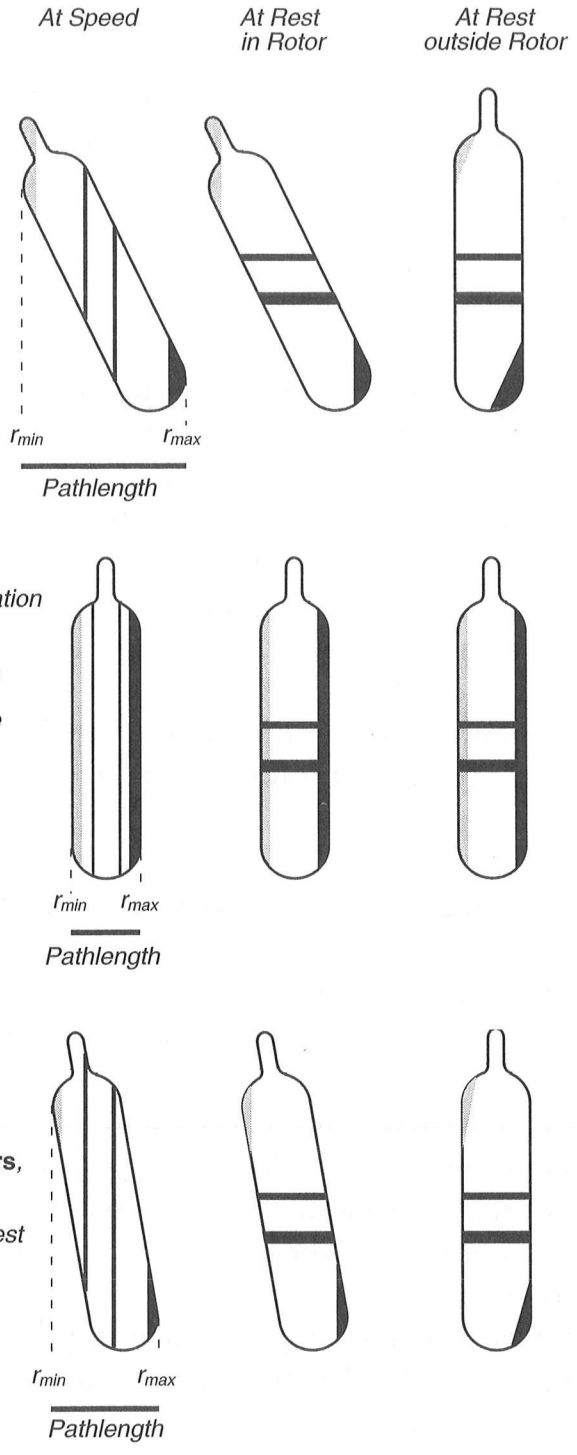

FIGURE 6.

Relative positions of the components of a plasmid prep after centrifugation through CsCl in three rotor types. The Near Vertical Tube Rotor (*C*) is the best choice for gradient centrifugation. Black areas represent pelleted material, gray shaded areas are floating components, and black lines indicate bands. (Redrawn, with permission, from Beckman Instruments.)

umes. As volume is slowly and continuously added from outside the centrifuge, the pellets in the tubes grow larger and larger. Different systems can handle 10 to 100 liters an hour.
Advantages. Large volumes can be used.
Disadvantages. Centrifuge must be adapted with inlet and outlet ports. Cleaning and maintenance take time. Many parts which can be lost or broken. Generates aerosols.
Examples. Sharples™ (for cream separation), Z-60 (Beckman).

Zonal

Uses. Large-scale separation of particles on density gradients; can separate liters of solution and pounds of cells or tissue samples.
Description. Cell suspensions and density media can be pumped into the rotor through specialized ports, and the speed can be altered to selectively disgorge cells of different densities.
Advantages. Large volume handling.
Disadvantages. Fairly complicated for unskilled worker.
Examples. Centrifugal elutriator, CF-32 Ti (Beckman).

WORKING RULES

- Don't spin radioactive, biohazard, or infectious material in any centrifuge until you have checked that you are *using the appropriate and designated centrifuge* for those materials.

Not everyone may be careful when working with biohazard material in the centrifuge, and the rotor and the inside of the centrifuge may be contaminated. Use gloves when you are loading or cleaning a centrifuge.

- *Balance all tubes and tube holders, caps and tops, shields and trunnions.* Don't merely balance the tubes—balance the entire assemblance of removable parts. Be careful that matched sets of trunnions, shields, and adapters are not mixed: Trunnions and shields usually have the weight stamped on them. If you use a balance tube, fill it with material similar to that of the material you are spinning: Water is fine for spinning bacteria out of media, for example, and for most spins, but not to balance cesium chloride.

If trunnions, shields, and tubes are not inscribed with their weights by the manufacturer, mark matching sets with paint or colored Sharpies.

- If there is a *sign-up sheet,* be meticulous about recording the necessary information. This information is needed to keep track of rotor usage, since many high speed and ultracentrifuge rotors will be down-rated (judged incapable of performing safely at the highest speeds) after a certain number of usage hours have been reached.

- *Clean up every time* you use any centrifuge, including microfuges. Wipe down the inside of the centrifuge. Remove buckets from swinging-bucket rotors, rinse with distilled water, and dry, inverted. Rinse out the entire fixed-angle rotor.

- *Know the speed limitations of each centrifuge and rotor and do not pass them.* Remember that swinging-bucket rotors cannot go as fast as fixed-angle rotors, so don't make presumptions. If the centrifuge sounds labored when running, you have probably surpassed the recommended speed.

- *Use appropriate tubes and tube holders.* This is particularly important during high-speed spins, when more stress is put on the tube. Tubes should be neither loose nor tight in the holders or rotor. Many tubes and tube holders require adapters.

- *Cover tubes.* Use closures made for the tubes, or wrap the top with parafilm. Do not use aluminum foil, which detaches or ruptures and doesn't contain aerosols.

- *Fill tubes 1–2 cm from the top.* If you fill too much, you will get leaks even from screw-top tubes. If you fill too little, the tube may collapse.

- *Do not use a tube if it is cracked* or compromised in any way. Dispose of it immediately, don't try to save it for slow spins, etc. Check even wrapped disposable tubes for cracks. A tube with a minuscule crack may hold liquid without leaking before a spin, and will only break with the force of the centrifugation.

- *Always run all buckets of a swing-out rotor.* Open and inspect all buckets before and after use—one tube left in an apparently unused bucket can unbalance the centrifuge. Be sure the individual buckets are seated properly and swing freely.

- *Spin infectious material only in enclosed tubes, with covered containers.* Centrifuges generate aerosols, and infectious particles can be dangerously dispersed, even without an overt spill. Heat-sealed tubes should be used for highly toxic or pathogenic materials.

- *Don't forget the cover of the rotor!* Most fixed-angle, and some swinging-bucket, rotors have a lid that fits over the top of the rotor. If you find out you have left it off after your run has started, stop the centrifuge and put on the cover.

- *Close the lid on refrigerated centrifuges between runs to avoid condensation.*

- *Remove samples from the centrifuge immediately.* Never let samples sit after a run. The pellet could become dispersed, and the tubes might be moved by someone who needs the centrifuge. Also, it is extremely seedy to allow an ultracentrifuge run to go extra time because you don't want to come in when the run is ready.

HOW TO SPIN

No matter what the sample or the centrifuge, the basic steps of centrifugation are the same.

1. Choose tubes appropriate to the volume and the nature of what you will spin. Use as few tubes as possible, so find tubes as close to your volume as possible. If you are spinning a liter of bacteria, don't choose 20 x 50-ml tubes if you can choose 4 x 250-ml bottles. Choose tubes of a composition appropriate to your sample. Know the speed at which you will spin (step 2). Most tubes are suitable for low-speed spins, but you must be more particular about tubes for high-speed spins.

Fill tubes to within 2 cm of the top.

2. Choose a centrifuge and rotor appropriate to your sample and what you want done with it. Steps 1 and 2 actually must be decided at the same time. Know how fast the sample must be spun. Should the sample be refrigerated? Most should. Heat is generated during a centrifuge spin, and this can damage biological samples.

Rotors are often kept in a cold room, the better to keep the samples cold after they are loaded into the rotor.

3. Balance the tubes. Each tube must be spun with a tube of exact weight across from it. This is true for every rotor and every centrifuge, including microfuges, and for low-speed spins.

How to balance tubes

Only tubes across the rotor from each other need to be of the same weight.

Microfuge tubes. Adjust by volume, not weight: Add the same volume to each pair of tubes.

Ultracentrifuge tubes. Weigh tubes individually on a mettler balance. Tare the balance with a beaker, and weigh one tube at a time.

Benchtop and high-speed centrifuges. It is most convenient to use a pan balance, and balance tubes against each other.

If the sample is sterile, balance by eye as well as you can, and adjust the pairs by adding 70% alcohol or water to the space between the tube and bucket or adapter.

Use the same medium as your sample to balance lone tubes.

4. Put the tubes in the centrifuge, always in the same orientation. If the tube has an asymmetry, such as a lip on the closure, always put every tube in the holder in the same way, lip facing in or out. This way, you will always know where to look for the pellet.

FIGURE 7.
Maintain the tube at the angle of the spin, to avoid perturbing the pellet.

5. Check and recheck that every tube has a balance placed correctly across from it. Especially when you have a lot of tubes, it is easy to forget a balance when loading the rotor. Check when you add the tubes to the rotor, and check again before you screw on the lid.

Missing balance tubes and incorrectly balanced tubes cause most centrifuge mishaps.

6. Put the cover on the rotor. Although samples will usually be safe, you must use the lid every time. Keep it near the centrifuge so you can't forget to put it on. Screw it finger tight.

7. Close the lid of the centrifuge. You will hear a click with most centrifuges. And, unless the lid is properly closed, most centrifuges will not start.

8. Adjust the settings of the centrifuge. All settings must be checked, every time.

   Speed: Turn to 0. If it is a high-speed spin, start at 1000 and turn slowly up to the desired rpm once the centrifuge has been turned on. Some new centrifuges will calculate rpm if the *g* force is entered.
   Temperature: Cold for cells and bacteria, room temperature for phenol spins.
   Brake: Generally, on for pelleting, off for gradients. Check the protocol.
   Timer: Set for the amount of time you need to spin. The actual centrifugation time will be slightly longer because the rotor, even with a brake, will not stop immediately.

Do not stop a rotor manually! Not only can this cause injury to yourself, but it can be damaging to the motor shaft and to the brakes. Wait for the rotor to come to a complete stop by itself.

9. Always wait for the centrifuge to come to full speed before you walk away. If there are any problems, such as unbalanced tubes, the problem usually

announces itself before full speed is reached. Don't be alarmed by a slight and momentary shudder as the centrifuge picks up speed, and the motor reaches its vibration point, as this is normal. But turn off the centrifuge immediately if you hear loud thunks or if a vibration continues.

10. Remove the tubes very slowly and carefully, so you don't disturb the pellet or band. Maintain the angle of the tube as you take the tube from the rotor. Note the position of the pellet, and mark the position of the pellet on the tube with a sharpie, if you are worried about finding the pellet. Have a bucket of ice or tube holder ready to hold the tubes.
11. Remove the supernatant. This can be done by decanting or aspiration.
12. Wash the pellets. (This is not always necessary. It usually improves the purity of the pellet, but do it only if specified.) To the pellet, add approximately half a tube of wash liquid. Cover and vortex until the pellet is resuspended. If the pellet remains fixed, use a sterile pipet to dislodge it from the side of the tube, and vortex again. Fill the tube, balance, and vortex again before spinning.
13. Remove the pellets. After removing the last wash liquid, resuspend the pellet in a small (2–5x the size of the pellet) volume of resuspension or wash fluid, and remove to a smaller tube.
14. Clean up. Wash tube holders, the rotor, and the inside of the centrifuge. Dispose of disposable centrifuge tubes in the appropriate trash (usually biohazard) and put glassware to soak (after rinsing with 10% bleach for biohazard material) or wash.

Although ultracentrifuge use basically requires the same steps, there are many other steps that are integral to safe use. For example, you might have to choose acceleration and deceleration times, or pump a vacuum to a certain level before achieving speed. Have someone demonstrate the ultracentrifuge before you use it!

How to Determine Centrifuge Speed

When choosing a centrifuge for your samples, the speed you require is the main consideration. The volume of your sample is the second consideration.

Speed will be given as either gravitational force (*g*) or revolutions per minute (rpm). *g*-Force is also called RCF, or relative centrifugal force. The gravitational force is the force exerted during centrifugation. Protocols usually give centrifuge speed in *g*, which is a constant. Typical centrifuge speeds are 500*g* for mammalian cells, 3000*g* for bacteria, but this, of course, will vary.

For the most part, the kind of centrifuge you need is the one that is available. Labs tend to adapt protocols to centrifuges that are accessible: Centrifuges are expensive, and are generally used until they die.

The rpm is dependent not only on the force exerted during centrifugation, but also on the type and size of the rotor and the centrifuge model.

Calculating *g*. You can calculate the *g* force from the rpm, and vice versa, by using this formula:

$$g = 1.12 \times 10^{-5} \times \text{radius (cm)} \times \text{rpm}^2$$

Measure the radius from the center of the rotor to the tip of the tube. For a fixed-angle rotor, how to do this is obvious and simple—just measure to the middle of the cavity. Most manufacturers give three radius measurements: the maximum, minimum, and average radius, or the distances from the center of rotation to the bottom, top, and middle of the sample tube. For most uses, the radius measurement won't matter, and you can use either the tip or middle of the tube measurement.

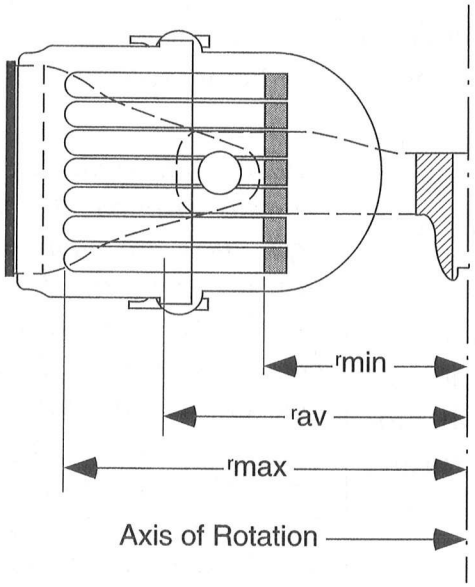

FIGURE 8.
To measure the radius for a swinging-bucket rotor, hold the tube out to approximate its position during centrifugation and measure from the tube to the rotor center. (Redrawn, with permission, from Beckman Instruments.)

The radius measurement can also be used to calculate the rpm or *g* from a nomogram (see Fig. 9). Use a ruler to draw a line from the radial distance to either the RCF or rpm, to find the unknown RCF or rpm.

Centrifuge Tubes

Considerations for choosing tubes

- Volumes can range from microliters to liters. The object is to try to use as few containers as you can. Fewer bottles means fewer manipulations, and this usually means a bigger yield.

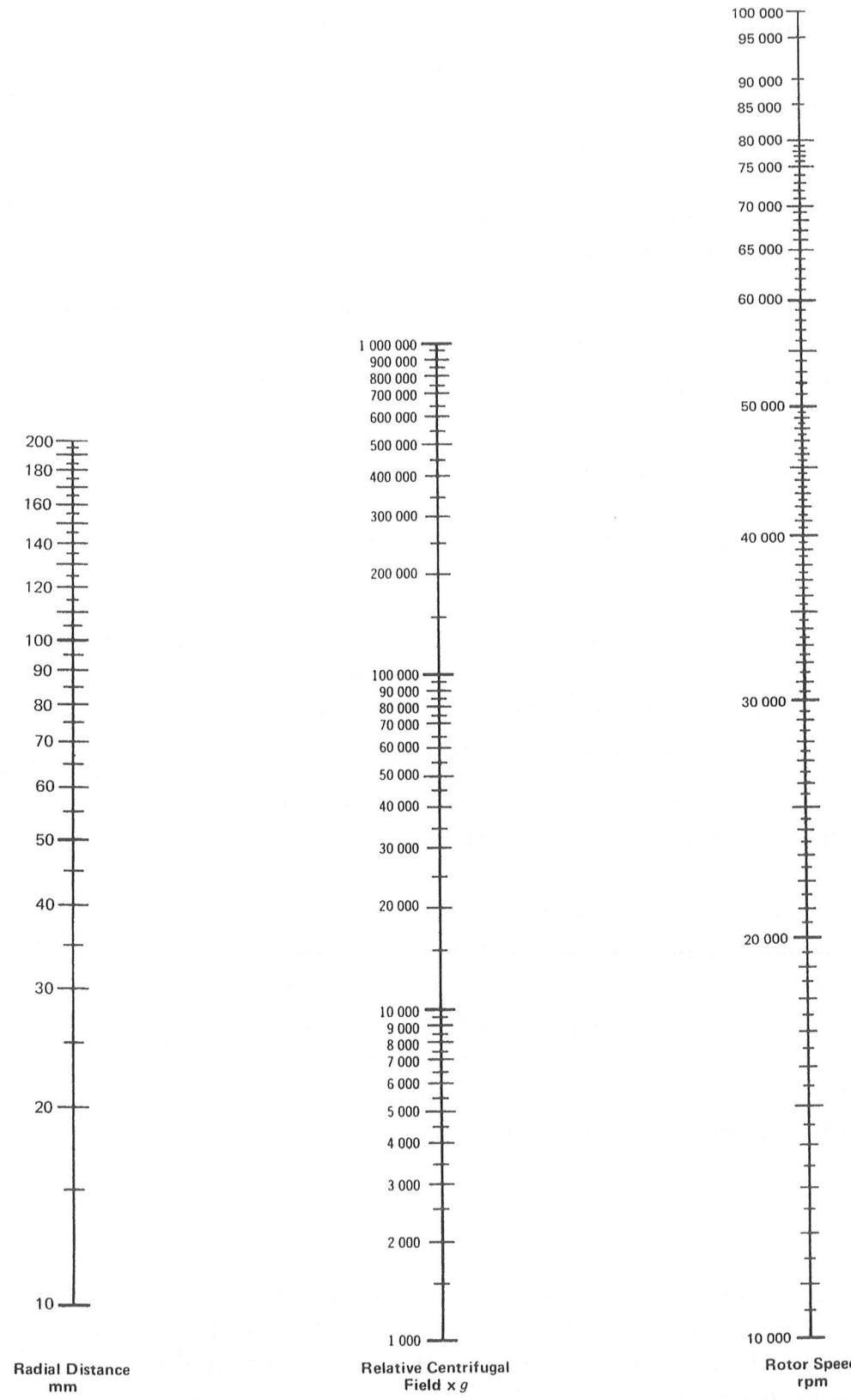

FIGURE 9.

Nomogram for computing relative centrifugal forces. (Reprinted, with permission, from Corning Science Products, Corning, New York.)

- Is your sample aqueous or organic? Is it biohazard material? The composition of the sample will influence the composition and the style of the tube.

  Aqueous samples will be fine in most plastics and glass. Organic materials are safe only in certain plastics, and in glass. Phenol extractions, for example, are usually spun in glass or in an inert material such as polypropylene.

  Special precautions must be taken for biohazard material. The top should be a screw or snap cap: The tube should never be left open.

- Temperature can affect the integrity of the tube. Clear polyallomer and Teflon FEP (not shown on chart) should be spun at maximum RCF only in a refrigerated centrifuge. Refrigerated centrifugation is not recommended for translucent polypropylene.

- Corex tubes (trademark Corex, from Corning Glass Works) are much stronger than regular glass tubes and are more resistant to heat, chemicals, and scratching. They are often used for collecting precipitated nucleic acids. They can be used at speeds up to 15,000–18,000, and may shatter above 18,000 rpm.

- Not all tubes can withstand all speeds. For low-speed spins, this doesn't matter. But as a tube is spun above approximately 5000*g*, it can shatter or crack from the centrifugal force. Never spin a tube above 5000*g* unless you are sure it is appropriate for that speed.

- Tubes may be round-bottomed or conical. For most cases, this doesn't matter. In a conical tube, spun in a swing-out rotor, the pellet will be a button on the bottom of the tube, and it is easier to remove the supernatant without disturbing the pellet. If you are spinning cells that don't like to be spun very hard and would therefore leave a soft, more diffuse pellet, this may be of some advantage. But swung in a fixed-angle rotor, the pellet in a conical tube may still be smeared along the side and on one side of the tube.

If you spin conical tubes, you will need an adapter, a rubber piece that conforms to the shape of the tube but is the same size as the holder or hole in the rotor. Some adapters just fit on the bottom of the tube. Without the adapter, which reduces centrifugal stress on the tube, the tube may shatter during the run.

Bottles are used for larger volumes. These are round-bottomed, conical, and flat-bottomed. As for tubes, there is an advantage to using conical bottles for bacterial or cell pellets. And also, as for tubes, adapters should be used with conical bottles. Adapters should be used for flat-bottomed bottles as well.

Microwell plates may be spun in a swing-out rotor in microplate carriers, or in a rotor that has been adapted for microplates. The carriers can hold a standard size microplate, and adapters can be used for smaller plates.

TABLE 1. A Quick Reference Chart to Tube Materials and Their Properties

Type	Optical property	Puncturable	Sliceable	Reusable	Sterilization methods	Chemical resistances[a]
Ultra-Clear	Transparent	Yes	Yes		Cold sterilization only, but *not* with alcohol.	Good tolerance to all gradient media *except* alkaline ones (>pH 8). Satisfactory for most weak acids and a few weak bases. Unsatisfactory for DMSO and most organic solvents, including all alcohols.
Thin-walled						
Standard tubes				Yes		
Quick-seal tubes				No		
Polyallomer	Translucent	Yes	Yes		All types can be autoclaved on a test tube rack at 121°C.	Good tolerance to all gradient media, including alkaline ones. Satisfactory for most acids, many bases, many alcohols, DMSO, and some organic solvents.
Thin-walled						
Standard				Yes		
Quick seal						
Thick-walled						
Tubes		No	No	Yes		
Bottles		No	No	Yes		
Polycarbonate	Transparent	No	No		Cold sterilization recommended, but *not* with alcohol. Can be autoclaved at 121°C but tube life can be reduced	Good tolerance to all gradient media *except* alkaline ones (>pH 9). Satisfactory for some weak acids. Unsatisfactory for all bases, alcohols, and most organic solvents.
Thick-walled						
Tubes				Yes		
Bottles				Yes		
Polypropylene	Translucent	No	No		Can be autoclaved at 121°C.	Good tolerance to all gradient media, including alkaline ones. Satisfactory for many acids, bases, and alcohols. Unsatisfactory for most organic solvents.
Tubes				Yes		
Bottles				Yes		
Stainless steel	Opaque	No	No		Can be autoclaved. Dry thoroughly before storage.	Good tolerance to many organic solvents. Marginal with many gradient media and salts. Unsatisfactory for most acids and many bases.
Tubes				Yes		
Polyethylene	Translucent	No	No		Can be autoclaved at 121°C.	Good tolerance to a wide range of chemicals. Suitable for use with strong acids and bases. Unsatisfactory for most organic solvents.
Tubes				Yes		
Corex/Pyrex	Transparent	No	No		Can be autoclaved at 121°C.	Good tolerance to a wide range of gradient media. Corex has greater resistance to alkalis and acids.
Tubes				Yes		
Bottles				Yes		

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[a]Chemical resistances are described in general terms, and are not meant to express or imply any guarantee of safety based on these recommendations or resistances. If there is any doubt about a particular solution, it should be tested under actual operating conditions to evaluate the performance of a tube material. High vapor pressure inflammable solvents should not be handled in close vicinity to centrifuges because of possible ignition by sparking switches, relay contacts, or motor brushes.

- Caps must fit tightly, to minimize contamination of the inside of the centrifuge from the aerosols generated during the spin. Of course, no biohazard material must be spun without caps. But even seemingly innocuous material can mess the centrifuge and cause problems if not removed promptly.

  Cover Corex tubes with a square of parafilm, not aluminum foil or cotton plugs. If a snap-cap is available for ultracentrifuge tubes, use it.

  Heat-sealed tubes have only a nipple opening, and are used to spin hazardous substances in high speed and ultracentrifuges. The tubes are sealed shut before the run. These tubes are used for spinning hazardous substances such as cesium chloride gradients: The only downfall to their use is that the tube must be sliced or pierced to remove the contents, procedures which can also be hazardous.

Can disposable tubes and bottles be reused? Generally, yes. Can they be autoclaved? Generally, yes. However, tubes not designed for repeated autoclaving will break down, and you don't want that to happen during a run. If you reuse disposables, use them only for two or three low-speed spins, and then discard them.

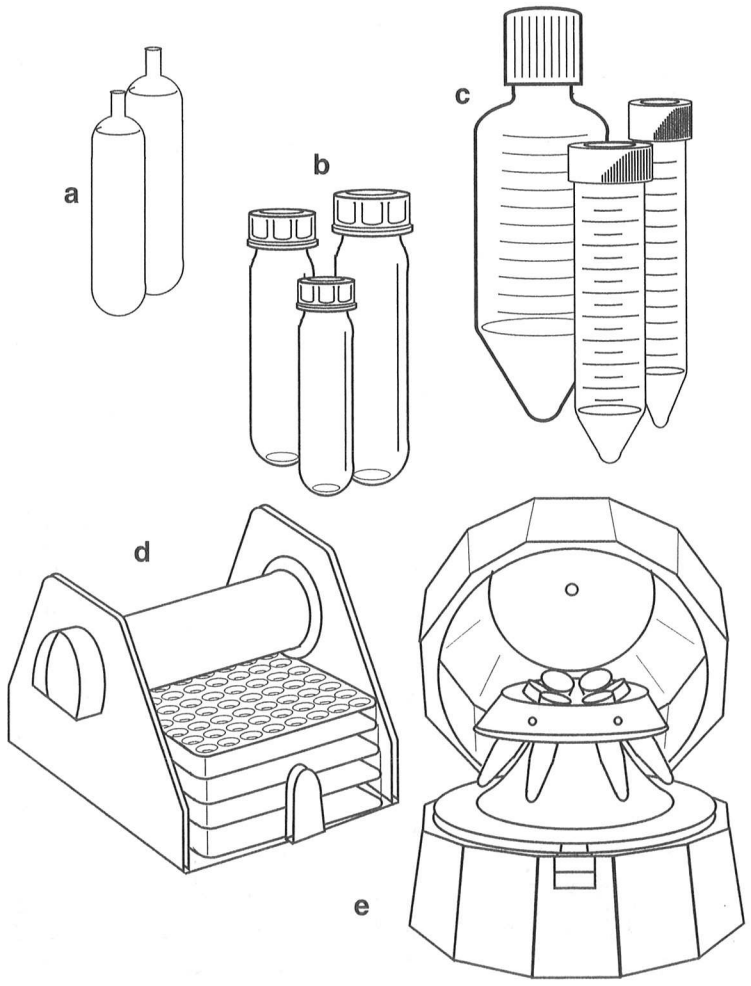

FIGURE 10.

Quick seal tubes (*a*) are heat-sealed and provide containment for biohazard material. Oak ridge style tubes (*b*), made of polyallomer, Teflon FEP, or polysulfone can be used for ethanol precipitations of large volume. Polypropylene 15-ml and 50-ml conical tubes and 250-ml bottles are excellent for pelleting cells (*c*). Microplate rotors or carriers (*d*) allow the microplates used for cell culture, biochemical assays, and DNA sequencing to be spun in an all-purpose centrifuge. A mini-centrifuge (*e*) only spins to a maximum of approximately 2,000*g*, but it can pellet cells and samples for electrophoresis.

Removing Supernatants

There are several ways to remove supernatants, with advantages and disadvantages to each.

- **Pouring off the supernatant (decanting)**

  Advantage: Quick.
  Disadvantage: Creates aerosols, may dislodge pellet.

- **Aspirating the supernatant**

  Advantage: Gentle, doesn't disturb pellet. Not messy, fewer aerosols.
  Disadvantage: Slow for large volumes.

Decanting supernatants from tubes and bottles

1. Set up an Erlenmeyer flask to use as a receptacle for the supernatant. If you will be spinning biohazard material (such as supernatants from cells or bacteria), add bleach to the bottom of the flask. Add an amount of bleach that is 10% of the volume you will be spinning and pouring.
2. Carry the bottle to the hood or bench where you will be working. Carry the bottle or tube at the same angle you have pulled it out of the rotor. There are tube racks that will maintain this angle.
3. Pour, keeping the pellet on top. If the pellet is firm, pour in one motion. Add wash liquid immediately, as many pellets should not be left to dry out. If you see the pellet starting to break up, stop pouring immediately. Try to aspirate the fluid around the pellet. If you are going to wash the pellet, don't try to remove supernatant at the risk of disturbing the pellet: You will have better luck after washing.
4. Allow the supernatants to incubate with the bleach for at least 30 minutes before pouring them down the drain.

Decanting supernatants from microplates

The entire plate should be quickly inverted: Partial inversion would allow dripping to occur from well to well. If there are cells or a reaction immobilized on the bottom of the wells, the plate may be quickly and sharply inverted onto a piece of paper towel on the bench.

There are automated microplate washers that can wash and remove liquid from all the wells. These are particularly useful for ELISAs.

Aspirating supernatants from tubes and bottles (See Chapter 9 for more on aspiration.)

You can protect the tip of the pasteur pipet by slipping a 100-µl pipettor tip over the end.

1. Attach a pasteur pipet into the tubing of the aspirator. Turn on the aspirator, and adjust so it is pulling only a gentle vacuum.
2. Hold the open tube at an angle, with the pellet on the upper side.
3. Insert the tip of the pasteur pipet or tube just below the meniscus on the lower side of the tube.
4. Move the tip toward the base of the tube as the fluid is withdrawn, using gentle suction to avoid drawing the pellet into the pipet tip. Keep the tip away from the pellet.
5. Aspirate the walls of the tube to remove any adherent drops of fluid. With practice, you will be able to gently shake and cajole every drop of fluid away from the pellet by tilting and shaking the tube. You can then carefully aspirate the drops. It is usually desirable to remove as much of the supernatant as possible.

*If you are aspirating with a pipet and a bulb or pipettor, be careful not to blow air **into** the supernatant. This could disturb the pellet.*

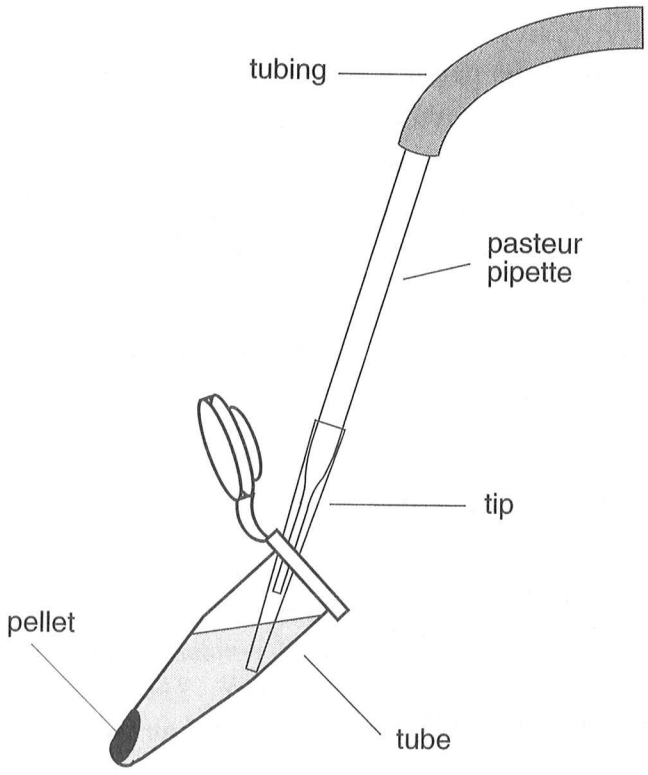

FIGURE 11.
Avoid the pellet as you aspirate closer toward the bottom of the tube. (Redrawn, with permission, from Sambrook et al. 1989.)

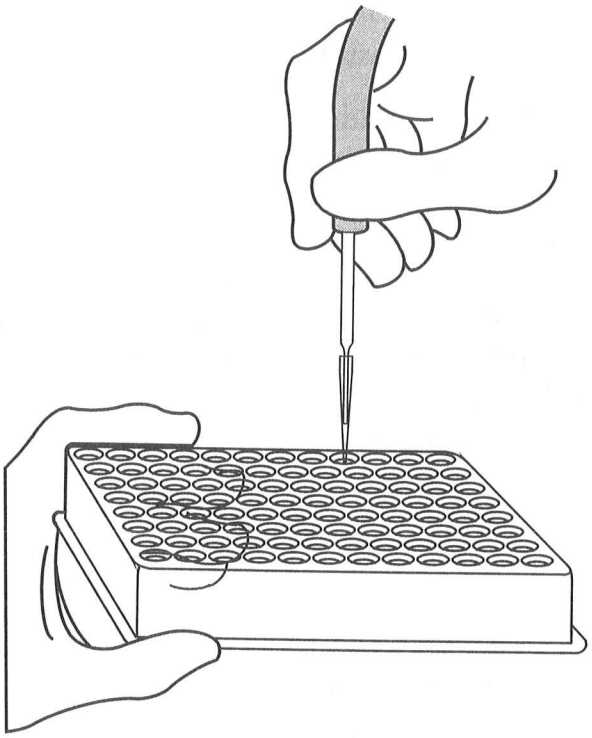

FIGURE 12.

Keep the microplate tilted toward you at an angle of about 30°. As you aspirate, bring the tip straight down, and you will avoid contact with the bottom of the well.

Aspirating supernatants from microplates. You can quickly move from well to well with an aspirator, removing supernatants or medium. Tilt the plate toward you so the tip, if it contacts the well, will strike the side of the well and not the bottom where cells and reaction products will be concentrated.

Washing Pellets

- Washing the pellet will remove impurities, such as unwanted solvent or culture medium. It should be regular practice to wash pellets.
- The quality of the final preparation is helped by multiple washes. For cells or bacteria, one wash may suffice to spare the cells the trauma of another wash:

If no pellet is formed or is very loose:
- The spin may not have been fast enough or long enough.
- The suspension fluid is very thick. For example, cells may take much longer to sediment in serum than in culture medium.
- There may not be enough material to spin down.

Try to spin longer and faster. Attempts to remove medium around a loose pellet, and add a less dense medium such as a buffer may work. But the efficiency is low, recovery is low, and this should only be done to preserve a very precious material.

However, you must take into account the use the cells will have. If you need them for an enzymatic reaction, it is probably necessary to wash twice.

- Yes, you must *resuspend* the entire pellet in the wash fluid before you centrifuge! If you don't, washing will not be effective. The impurities will be trapped inside the pellet as well as outside, and you might as well do nothing as merely wash the outside of the pellet.
- After you have resuspended the pellet in the wash solution,which is usually a buffer of neutral pH, spin at the same speed at which you originally pelleted the material. But spin for half the time as for the original spin.

If the pellet is loose, or must be actually dried, it can be subjected to vacuum drying in a Speed-vac (see Chapter 12).

Spinning Infectious or Dangerous Samples

Centrifugation is one of the riskiest procedures to perform with hazardous material. Aerosols are generated, and there is always a risk of tube breakage.

- Always use tubes you are sure of. Check carefully for cracks or chips; discard any tube that doesn't look prefect. Although disposable tubes can actually be reused, this isn't the time to do it.
- Use canisters with covers.
- Open the rotor in a hood.
- Open the tubes in a hood.

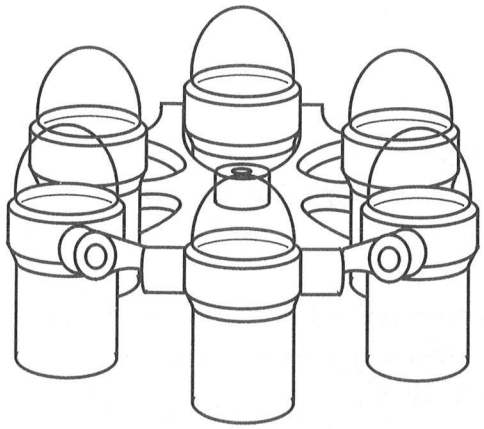

FIGURE 13.

Many buckets or canisters on trunnion-type swinging-bucket rotors have covers and O-rings that will contain biohazardous or dangerous materials.

GRADIENTS

Gradients are used to separate complex solutions and isolate a particular molecule.

Common density media. Density media have different densities, viscosities, concentrations, and osmolarities. Some cells cannot tolerate some media and may suffer a loss in viability or function, so you should always ask around before using a particular medium for your cells.

- **Percoll™.** A synthetic colloidal suspension of polyvinyl pyrrolidone-coated silica. Designed for sedimentation centrifugation. In a fixed-angle rotor, it will spontaneously form linear gradients. Good for organelle and cell isolation.

- **Ficoll™.** A sucrose polymer, made by Pharmacia. Tends to be less viscous than other media at high density. Used for cell isolation.

- **Metrizamide (Nygaard).** A nonionic derivative of metrizoate. Used for cell isolation.

- **Sucrose.** Sucrose solutions are used to isolate organelles, and are seldom used for cells.

- **Cesium chloride.** A salt used for isopycnic separations, most commonly for the isolation of plasmid DNA.

Making gradients. Gradients can be made by hand, by centrifugation, with a pump, or with a gradient maker. Discontinuous gradients, or step gradients, are often made by hand, since all one needs to do is to gently layer the lesser density media upon the more dense. An alternative method is to use a long-needled syringe to add layers of increasing density to the bottom of the tube.

Continuous gradients, with a smooth range of densities, can be made by centrifugation. For example, a gradient is formed during the centrifugation of cesium chloride, when isolating plasmid DNA. A gradient maker, which mixes the density media with diluting medium and pumps the increasingly less concentrated solution into a tube, can be used to make a linear or (with programmable models) step gradient.

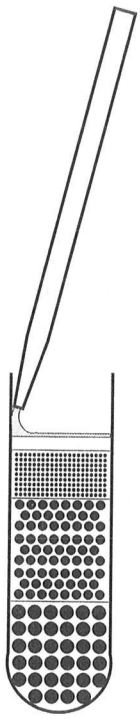

FIGURE 14.

To make a step gradient, use a syringe or pipet to slowly layer the less dense solution upon the more dense. Allow the solution to gently run down the side of the tube just above the surface, so it won't disturb the gradient.

Removing cesium chloride bands. If you run a gradient at all, it may well be to isolated plasmid DNA by centrifugation through CsCl. Have someone walk you through the entire procedure before you attempt any part.

After the cesium chloride band has been removed from the tube, the ethidium bromide (EtBr) must be removed from the DNA. This is usually done by extraction with organic solvents, and protocols are available in most molecular biology manuals.

You should check with EHS about the disposal of EtBr. It is not okay to merely dump some bleach into the EtBr. In most labs, the EtBr solutions are decontaminated in the lab, and the EtBr is then disposed of in hazardous waste.

CENTRIFUGE AND ROTOR MAINTENANCE

Centrifuge maintenance. Safe use of the centrifuge (as described earlier in the chapter) is the best way to maintain the centrifuge.

- **Wash any spills inside the centrifuge immediately.** Use water and a mild detergent. If it is a refrigerated centrifuge, don't allow the wash fluid to freeze: Dry well with paper towels after washing.

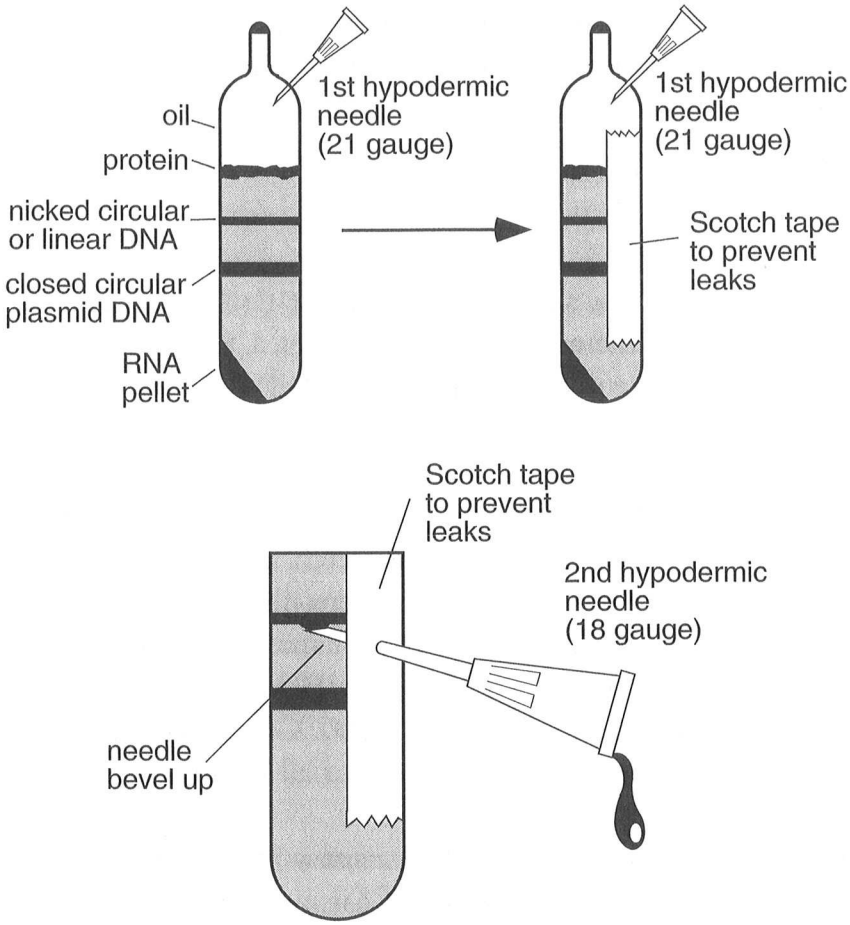

FIGURE 15.

Collection of plasmid DNA from CsCl gradients containing ethidium bromide. (Redrawn, with permission, from Sambrook et al. 1989.)

- **Call a serviceperson if the "Brushes" light comes on.** Brushes, part of the motor, become worn and must be replaced periodically.
- Don't panic immediately if the centrifuge doesn't start. Various safety devices work to ensure that a centrifuge shouldn't run until all settings are correct. If a centrifuge won't start, check that:
  1. The centrifuge is plugged in, and the control panel lighted (if it can be).
  2. The speed is set. Sometimes people turn the rpm to 0 when they are finished, and that is what you are getting—0 rpm.

Many labs have an equipment service plan for all centrifuges (not including microfuges). Basically, this is an insurance plan: The department or laboratory pays a company a set sum, and the company is responsible for payment for all breakdowns. Some service contracts will even cover maintenance. Before you call the manufacturer or a serviceperson to fix a problem centrifuge, check whether you have a service plan.

3. The centrifuge is at the correct temperature. If the temperature is set at a particular temperature, and it hasn't yet been reached, it may wait until that temperature is achieved.
4. The door is closed and the lock engaged.

Rotor Maintenance. Rotors are subjected to a great deal of force, and seemingly minor flaws can become major at high *g*-forces. Once the integrity of the structure is broken, the rotor can very quickly fail. Preventive maintenance is the only way to avoid problems.

- *Observe the speed and sample density ratings for each rotor.* Centrifugal force can cause stress of the metal, making it stretch and change in size, and inappropriate speed is the major culprit. Every ultracentrifuge rotor has a maximum speed, and it is part of the rotor name: For example, the SW28 can achieve a maximum of 28,000 rpm. However, it is recommended that the SW28 not be used above 25,000. Check the manufacturer's manual to find the recommended speed for all rotors.

- *Don't use a rotor past the time covered by the warranty.* Warranty times are based on a certain number of runs or hours of use, or to a certain period of time, after which the company considers use to be unsafe. Usually only high-speed and ultracentrifuge rotors are warrantied in this way.

- *Have the rotor inspected periodically,* according to the manufacturer's suggestion.

- *Keep the cavities and buckets of the rotor clean.* Moisture, chemicals, or alkaline solutions such as cesium chloride and other salts can cause metal surfaces to corrode. Rotors should be cleaned after every use.

> *Never clean the cavities and buckets with an ordinary bottle brush, with sharp wire ends. These brushes can damage the rotor. Use plastic-coated brushes only.*

Cleaning rotors

1. Remove the buckets from swinging-bucket rotors. The body of the rotor should never be immersed in water, as the hanger mechanisms are hard to dry and may rust. The entire fixed-angle rotor can be rinsed, but should not be immersed in water.

2. Rinse each bucket or cavity with water. Be very careful when inverting fixed-angle rotors. If there has not been a spill, rinsing is usually sufficient.
3. If radioactive counts are not removed, or there has been a spill in a bucket, wash the bucket with a mild detergent. Ask the dealer to recommend a detergent. Most solutions used for radioactive decontamination are highly alkaline, and should not be used on a rotor.
4. Rinse with distilled or deionized water.
5. Air dry the buckets or fixed-angle rotor upside down, resting on a paper towel.
6. Store the rotor in a dry place. All fixed-angle rotors should be stored upside down, with lids or plugs removed. Swinging-bucket rotors should be stored with the buckets in place, but with the bucket caps removed.

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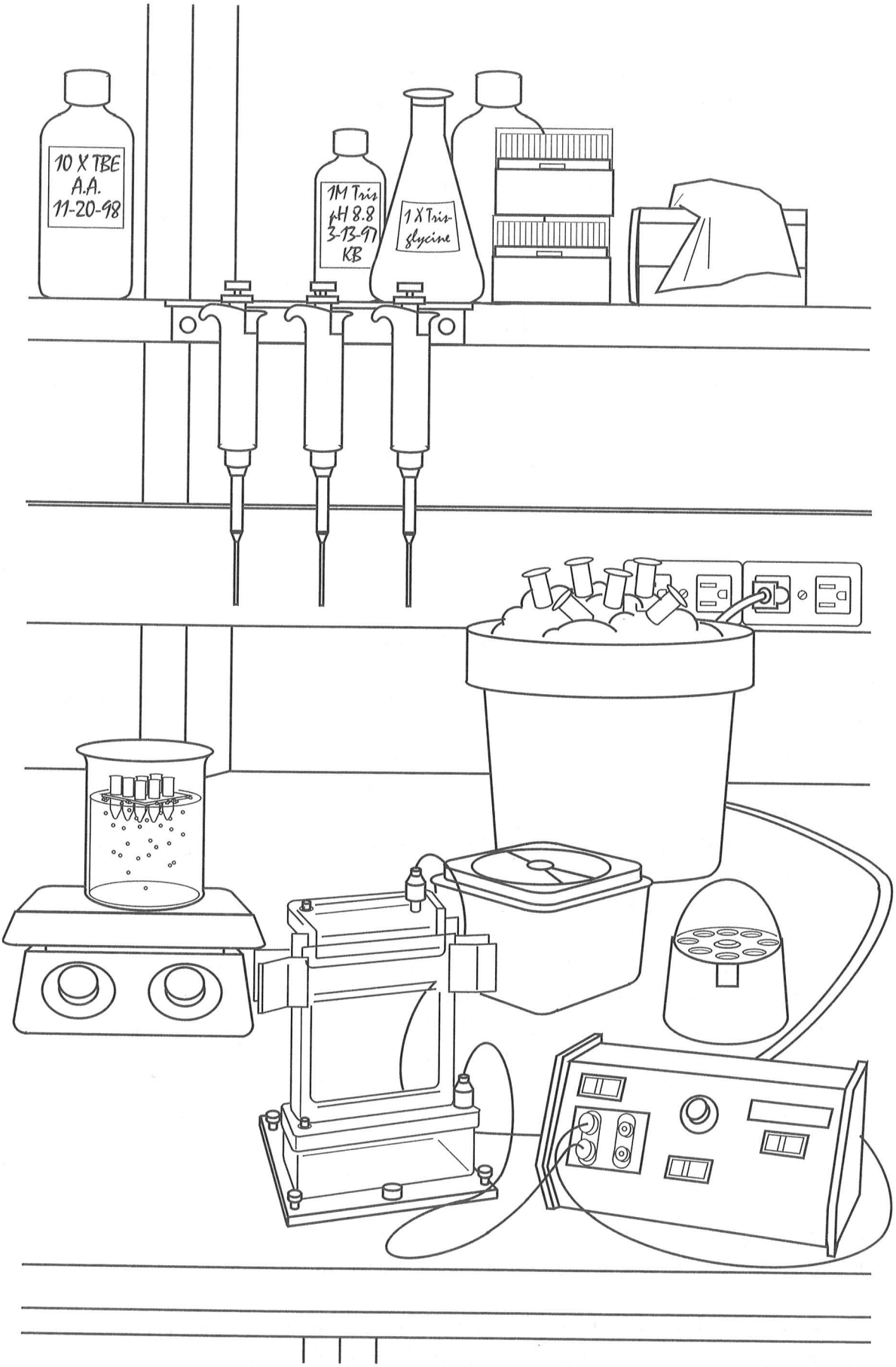
10 X TBE
A.A.
11-20-98
1M Tris
pH 8.8
3-13-97
KB
1 X Tris-
glycine