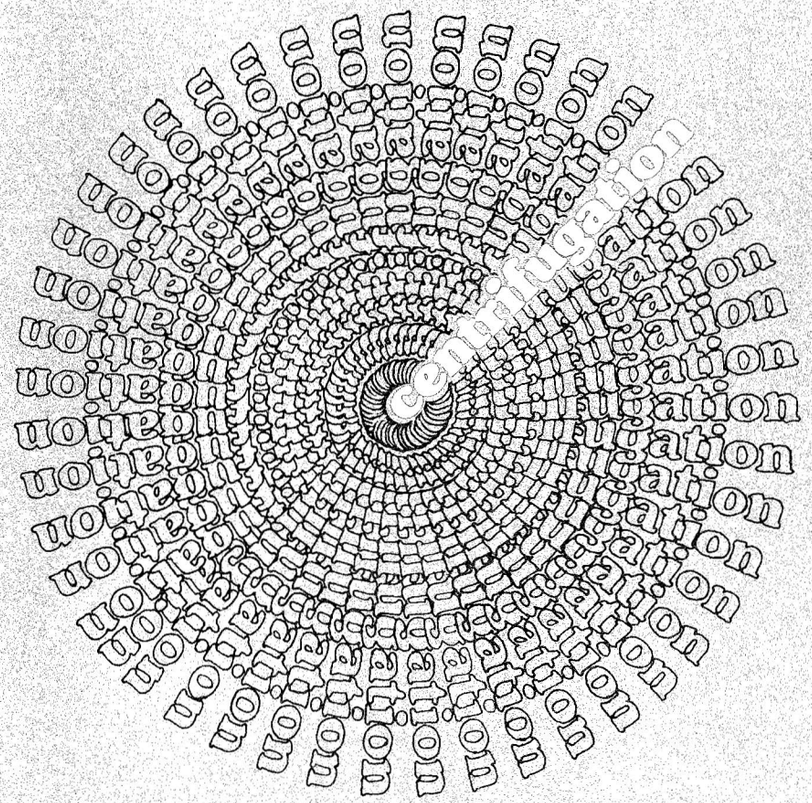
A Centrifuge Primer



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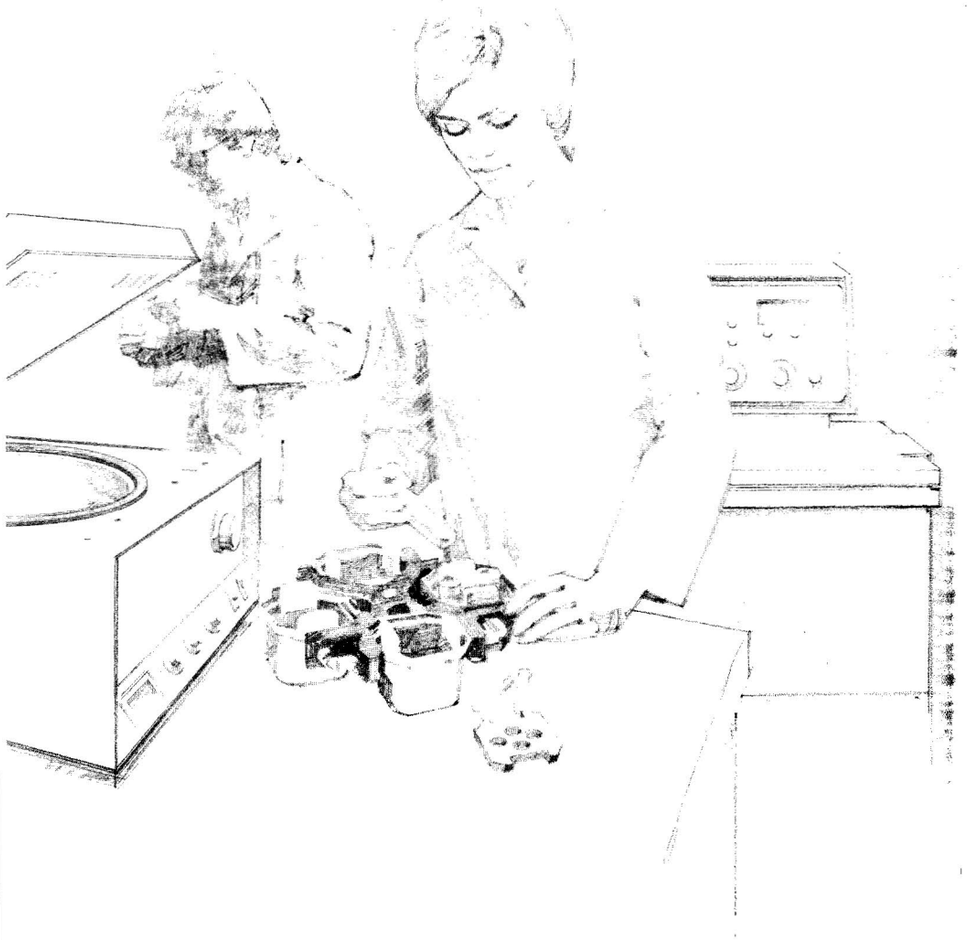
Preface

You may have operated a laboratory centrifuge before. Or perhaps this is your first day on a new job at a clinical lab or blood bank. In either case, you may have some questions about the centrifuge and its operating principles which we'll try to answer here.

This booklet is not a substitute for your instruction manual, which is essential reading for anyone who wants to use a piece of laboratory equipment safely and efficiently. We think you'll be a better centrifuge operator if you understand *why* separations can be made using centrifugal force. Directions for *how* to operate a centrifuge are in your manual. Please read it too!



The Centrifuge

All centrifuges have three basic components:

a rotor

a drive shaft

a motor

The rotor holds the tubes, bottles, or bags containing the liquids to be centrifuged. It is usually constructed of a high strength material such as an aluminum alloy or stainless steel. Different rotor types and sizes, interchangeable with one another, can be mounted on the drive shaft which connects to the motor. The motor provides the power to turn the rotor.

Usually, a cabinet surrounds and supports these parts, and also protects the operator should a tube break or any metal parts fail while the centrifuge is running. The operating controls and indicator dials for speed and time are mounted on the cabinet. Most centrifuges have a brake system to bring the rotor to a standstill shortly after the run is finished. Unlike the mechanical brakes on a car, the braking action is electrical: the current to the motor is simply reversed. Many centrifuges are also equipped with refrigeration to prevent delicate biological samples from getting warm.

As illustrated at the left, there are two centrifuge configurations: floor model and tabletop. The difference between the two is basically one of capacity; their operating principles are the same.

Operating Principles

During operation, the centrifuge rotor turns rapidly, up to 6000 revolutions per minute in the case of many laboratory centrifuges. This rotation generates a *centrifugal field* which can be used to make separations.

To visualize this centrifugal force field, imagine you have a stone tied to a string which you are whirling in a circle. The force you experience pulling against your hand is called *centrifugal force*. It arises whenever a body is made to move along a curved path, and is thus continuously deflected away from the direction it "prefers" to go—which is in a straight line. (The term centrifuge, in fact, means *to flee from the center.*)

If you whirl the stone faster, the pull becomes stronger. If you slow down or shorten the string, the pull decreases. Clearly, the strength of a centrifugal force field increases with the speed of rotation. It also increases with the distance from the center of rotation; the centrifugal force at a point 6 inches from the center is twice what it is at only 3 inches.

How can we describe and compare the strength of the fields generated by different size rotors and different operating speeds? The expression *relative centrifugal field* (RCF) serves this purpose. Just as length is measured in units of inches or millimeters, time in units of hours or minutes, the relative centrifugal field is measured in units also. It is expressed in multiples of the earth's gravitational field, abbreviated *g*.

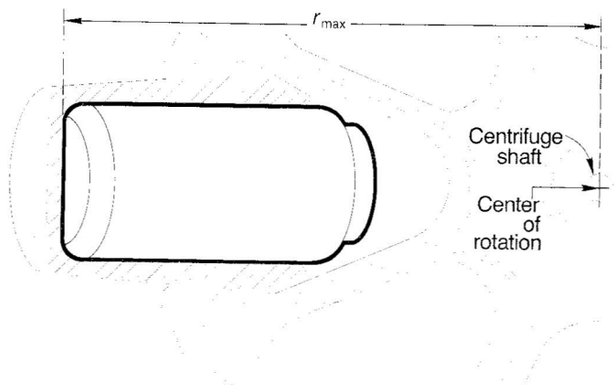
Calculating Centrifugal Fields

There's a simple formula for calculating the strength of a particular centrifugal field:

$$RCF = 1.12r\left(\frac{RPM}{1000}\right)^2$$

where *r* stands for the radius, which is the distance in millimeters (mm) from the center of rotation to some point within the rotor, and RPM is the speed of rotation in revolutions per minute (rpm). Sometimes radial distances are given in centimeters. Before using them in this equation, you must first convert them to millimeters (multiply by 10).

To find the maximum RCF of a rotor, you need to know its maximum speed and its *maximum radius* (r_{max}), the distance from the center of rotation to the bottom of the rotor cavity or bucket during centrifugation (see illustration below). Almost all centrifuge manufacturers publish this information for their rotors in their instruction manuals.



For example, the maximum RCF of the JS-4.2 rotor can be obtained from its maximum speed (4200 rpm) and its r_{max} (254 mm) as follows:

$$RCF = 1.12r\left(\frac{RPM}{1000}\right)^2 = 1.12 \times 254 \times \left(\frac{4200}{1000}\right)^2 = 5018 \times g.$$

If this same rotor is run at a lower speed, say 2000 rpm, the RCF it generates will also be lower:

$$RCF = 1.12 \times 254 \times \left(\frac{2000}{1000}\right)^2 = 1138 \times g.$$

Since the relative centrifugal field varies with the *square* of the rotor speed, you can see that any change in speed will cause a much greater change in RCF.

Separation by Sedimentation

How can a centrifugal field be used to separate particles from a mixture—blood, for instance?

Blood consists of plasma (which is a solution of water and many other compounds) and several kinds of particles in suspension, namely: red cells, white cells, and platelets. These cells are fairly large for biological particles —large enough, in fact, to settle out of the plasma if clotting is prevented and the blood is left standing in the 1-*g* field of the earth's gravity overnight. By using a centrifuge to generate an RCF of 1500 x *g*, we can speed up this sedimentation process and separate the cells from the plasma in approximately 10 minutes.

Why does this happen so quickly in a centrifugal field? Because the force which moves each cell away from the center of rotation is many times greater than the cell's own weight in the earth's normal gravitational field— 1500 times greater, in the example above.

Not all cells sediment at the same rate: large ones sediment faster than small ones. Thus, one kind of cell can be separated from another if there is a sufficient difference in their size and sedimentation rate.

Platelets, for instance, can be separated from red and white blood cells because they are so much smaller. It's only necessary to pick the right combination of centrifugal force and time. If blood is spun at 2900 × *g* for just 3 minutes, the platelets will not have time to move down with the heavier cells and can be collected from the top as platelet-rich plasma.

The process just described produces a *pellet* or sediment of particles in the bottom of the tube or other container. The liquid above the pellet is called the *supernatant*. As you can see from Figure 1, it is possible to collect a fairly pure fraction of the smallest particles from the supernatant. But the pellet of larger ones will always contain some of the smaller ones which were near the bottom of the tube before centrifugation began.

By centrifuging at various speeds and times, different size particles can be separated and collected from a mixture. This method is called *differential centrifugation.*

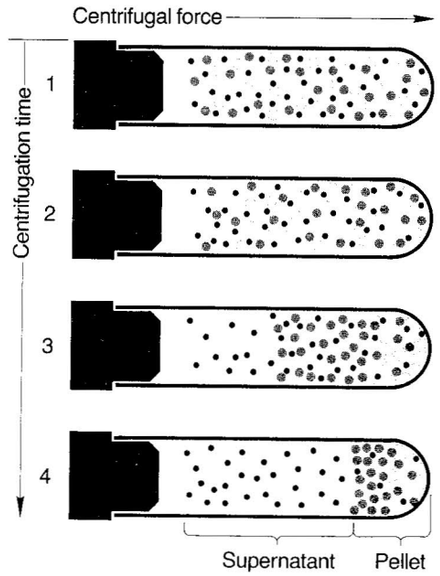


Figure 1. Sedimentation in a Centrifugal Field

Density Separations

There's another physical property of particles or cells which can also be exploited for the purpose of making separations: *density.*

Consider an apple, and a rock of exactly the same size and shape. A rock is a much more compact material than an apple, hence it sinks in water while an apple floats. It has more mass per unit volume, which is another way of saying its density is greater. Density is commonly expressed as grams per milliliter (g/mL); water has a density of 1 g/mL.

By applying centrifugal force, we can separate particles with small differences in density. It's only necessary to adjust the density of the liquid in which they will be sedimenting so that particles of one density will float, and particles which are more dense will sink.

This method is often used to separate lymphocytes, a type of white blood cell, which are so similar in size to many other blood cells that they can't be separated by ordinary sedimentation methods. However, their density is lower than the other cells. If a blood sample is layered over a liquid which has a density of 1.077 g/mL and then centrifuged, the lymphocytes will form a floating band, well separated from most other white and red cells which, being denser than 1.077 g/mL, sediment to the bottom of the tube. Plasma and platelets, the least dense of all, float to the top as illustrated in Figure 2.

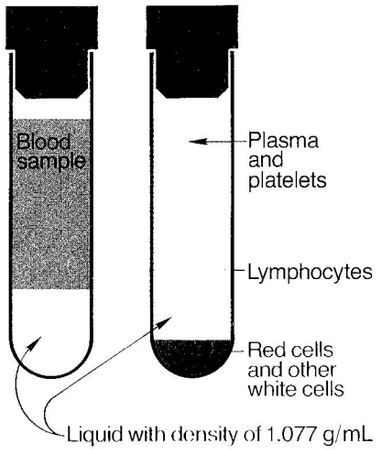
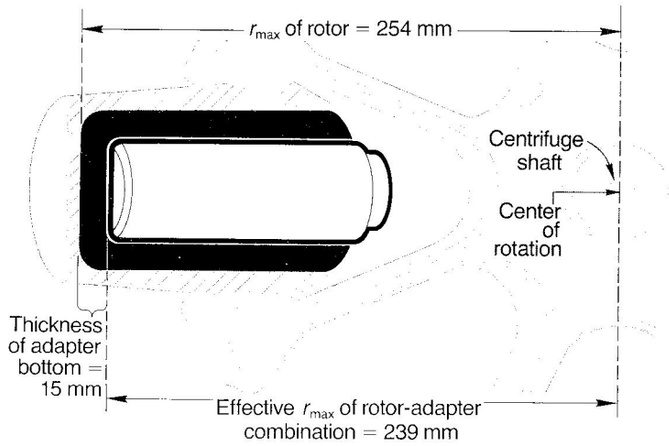


Figure 2. Density Separation of Lymphocytes. Tubes are shown before and after centrifugation.

Duplicating Centrifuge Run Conditions

Be wary of incomplete instructions, such as, "Centrifuge at 4000 rpm for 10 minutes." Unless a rotor is also specified, or its maximum radius given, there's no way to know what RCF is required to achieve that separation. For instance, a rotor such as the JS-5.2 with an r_{max} of 226 mm generates 4050 $\times$ *g* at the bottom of its buckets when running at 4000 rpm. The JS-4.2 rotor, however, has an r_{max} of 254 mm. When running at 4000 rpm it produces an RCF of 4550 $\times$ *g*. This difference of 500 $\times$ *g* seems small, but it is large enough to affect the results of certain separations if disregarded. The preparation of blood components or the pelleting step specified by radioimmunoassay (RIA) kits are examples of separations which require careful attention to the conditions of centrifugation.

Many adapters designed to carry a number of small tubes have bottoms which are 10-15 mm or more thick. Most separations won't be affected by this small reduction in the effective r_{max}. But if you need a more accurate calculation of RCF when using such adapters, you'll have to subtract the thickness of the adapter bottom from the r_{max} of the rotor to obtain the effective r_{max} for that rotor-adapter combination (see illustration below).



This is not to say that a run made in one rotor cannot be duplicated in a rotor with a different r_{max}. The same results can be achieved, but a change in rotor speed (or run time) must be made to compensate for the difference in r_{max}. See the nomogram for speed selection on page 20. By following instructions given there, you can estimate RCFs and speeds for rotors of various radii.

You can also calculate the proper speed to use by means of the equation already given on page 3. Let's say you want to follow a procedure written for a rotor with an r_{max} of 250 mm which calls for an RCF of 3430 $\times g$. You want to duplicate these conditions in the Beckman JS-5.2 which has an r_{max} of 226 mm. In this case you need to transpose the equation to solve for speed in rpm, so RCF = $1.12\,r(\text{RPM}/1000)^2$ becomes

$$\text{RPM} = 1000\sqrt{\frac{\text{RCF}}{1.12r}} = 1000\sqrt{\frac{3430}{1.12 \times 226}} = 3681 \text{ rpm.}$$

Thus, the JS-5.2 rotor will produce an RCF of 3400 $\times g$ if run at about 3681 rpm. If the original directions had specified the speed and radius of the rotor to be used, rather than the RCF, you would first have to find the RCF that combination generated, by means of the nomogram or the equation as shown on page 3.

Sometimes it may be better to change the length of time samples are centrifuged than to change the centrifugal force. You might want to duplicate a procedure calling for 10 minutes of centrifugation at 3000 g. Can you use a JR-3.2 rotor which attains a maximum RCF of 2300 $\times g$? Yes, but you'll have to run the samples a bit longer. The time required can be found with this equation:

$$t_1 = \frac{t_2 \times \text{RCF}_2}{\text{RCF}_1}$$

where

t_1 = run time needed for JR-3.2 rotor

t_2 = run time specified in procedure

RCF_1 = RCF of JR-3.2 rotor at maximum speed

RCF_2 = RCF specified in procedure

Thus, in our example,

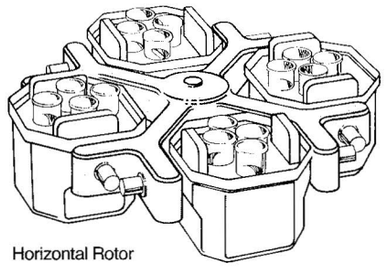
$$t_1 = \frac{10 \times 3000}{2300} = 13 \text{ minutes.}$$

Directions given for times of centrifugation usually correspond to the times to be set on the centrifuge's time control. This setting includes time for the rotor to accelerate and time at operating speed, but not deceleration time. The latter depends on the weight of the rotor, including its load, the type of brake system, and the brake setting selected by the operator. If a maximum brake setting is used, a fully loaded rotor takes somewhere between 1 and 3 minutes to decelerate.

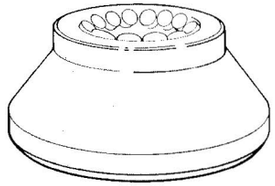
Sedimentation of particles in the sample continues during deceleration, of course, but the rate decreases as the rotor slows. Minimum deceleration times can be obtained by use of maximum brake settings. However, maximum braking in the final phase of rotor deceleration may be too abrupt when large diameter bottles or blood bags are in use; the result may be some undesirable stirring and resuspension of the sedimented material. The presence of this resuspended material can easily be mistaken for a poor separation.

A word of caution: before you change centrifugation conditions be very sure your particular sample will not be harmed by harder pelleting or by longer centrifugation times. Small changes usually cause no problem. However, some biological samples deteriorate if centrifuged too long, especially without refrigeration. And certain assays which are sold in the form of kits may be time-sensitive. When in doubt, follow the original instructions as closely as possible.

Rotors and Their Uses



Horizontal Rotor

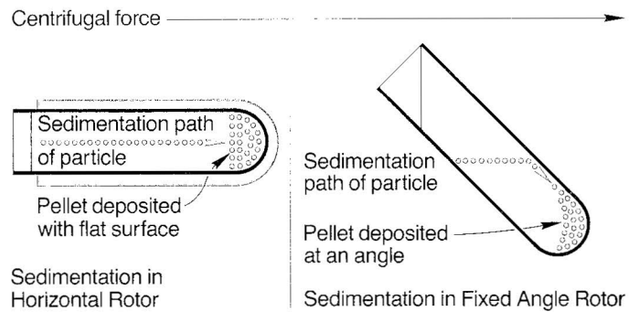


Fixed Angle Rotor

There are two types of rotors used in laboratory centrifuges: horizontal (also called swinging bucket) and fixed angle (or angle head).

Horizontal rotors are so-called because the buckets or racks which hold the centrifuge tubes are suspended in a manner which allows them to swing up into the horizontal plane when under the influence of a centrifugal field. Thus, when the centrifuge is operating, particles sediment along an unimpeded, radial path, away from the center of rotation, and deposit evenly on the bottom of the tube or other container. The flat upper surface of the sedimented material simplifies removal of the supernatant from a loosely packed pellet. By means of various adapters, more than one type or size of tube can be centrifuged together, provided the load is properly balanced. (Balancing is discussed in the next section.)

Fixed angle rotors hold the tubes at an angle to the axis of rotation. The angle varies with different rotors, somewhere between 25° to 40° being common. Although particles sediment along a radial path in these rotors also, they soon strike the opposite side of the tube where they slide down the wall to the bottom. The result is faster sedimentation than can be achieved in horizontal rotors which have a longer sedimentation pathlength. But because the bottom of the tube is not aligned with the



direction of the centrifugal force, particles will collect partly along the side of the tube. This can make the collection of a loosely packed pellet somewhat more difficult than when a horizontal rotor is used.

Within these two categories of rotors, various models offer different combinations of capacity and maximum RCF attainable. Horizontal rotors, in particular, have accessories which suit them to a wide range of applications. The buckets suspended from the rotor yoke can carry large containers such as blood bags or bottles. Adapters are available for these buckets so that a number of small tubes can be run simultaneously for applications such as radioimmunoassay (RIA). Horizontal rotors can also be equipped with racks or carriers, rather than buckets, suitable for spinning RIA tubes or microtest plates.

When quick pelleting of small particles is required, fixed angle rotors should be used. Because of their design, these rotors are capable of higher speeds than the horizontal type. Sedimentation of larger particles, such as cells, protein precipitates, antigen-adsorbent complexes, urinary crystals, etc., can be done at lower speeds with horizontal rotors. Maximum centrifugal force can be obtained with the latter if a wind-shielded version is used. (Wind-shielding improves rotor aerodynamics so that higher speeds are possible.) Density separation of cells is done best in a horizontal rotor of either type.

A Balanced Load

In order for a rotor to run smoothly and safely at its operating speed, the load it carries must be balanced. Examples of correct and incorrect loading are shown in Figures 4 and 5 on the following pages. A rotor can be properly balanced by following some simple rules:

1. A rotor must *never* be run with buckets missing, although opposing buckets may be left empty.
2. All opposing loads must balance within a certain weight as specified by the centrifuge manufacturer's instruction manual.
3. If opposing buckets are run with a *partial* load of tubes in their adapters, the tubes must be arranged symmetrically, both with respect to the pivotal axis of each bucket and across the center of rotation (see Figure 3). With some partial loads, it may be difficult or impossible to achieve the correct symmetry in both sets of opposing buckets. The simplest solution is to fill one or more tubes of the same size with water (or a denser liquid if necessary), and use them to balance the load symmetrically.

Most centrifuges are equipped with an imbalance detector which turns the centrifuge off before any eccentric rotation caused by a load imbalance can damage the drive shaft or bearings. However, the improper distribution of tubes in carriers or adapters can cause poor separations even if the imbalance isn't severe enough to trigger this detector. In these situations, the buckets won't pivot to the required horizontal position during the run (see Figure 5), resulting in poor density separations or remixing of sedimented material during deceleration. Also, the possibility of tube breakage during the run is greatly increased when the buckets are not horizontal at operating speed.

You may notice that the centrifuge vibrates when the rotor is accelerating or decelerating at low speeds. This is normal, and occurs as the rotor passes through a so-called critical speed range where any small vibrations are temporarily amplified. Your separations will not be disturbed during deceleration, because the centrifugal force is still high enough to stabilize them. However, you should not select an operating speed within the range where these exaggerated vibrations occur. Your instruction manual will tell you what speeds to avoid.

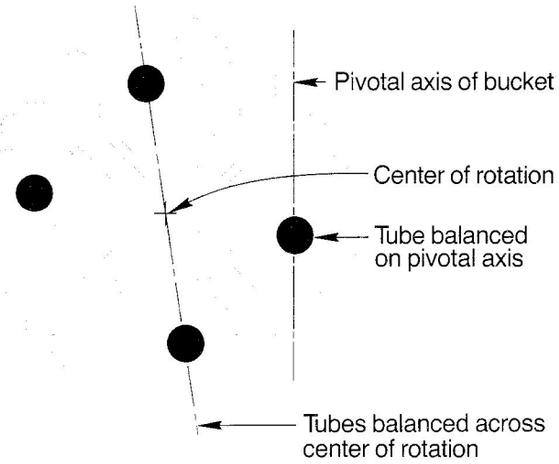
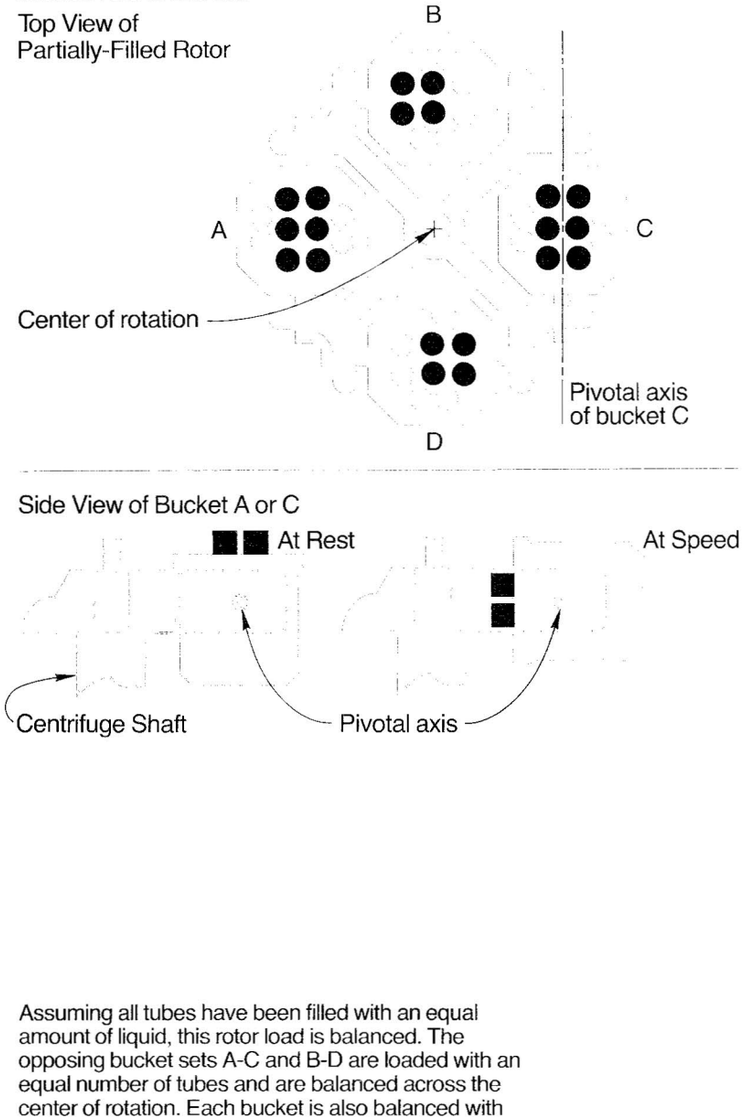


Figure 6. Correct Symmetry When Centrifuging a Partial Load of Tubes

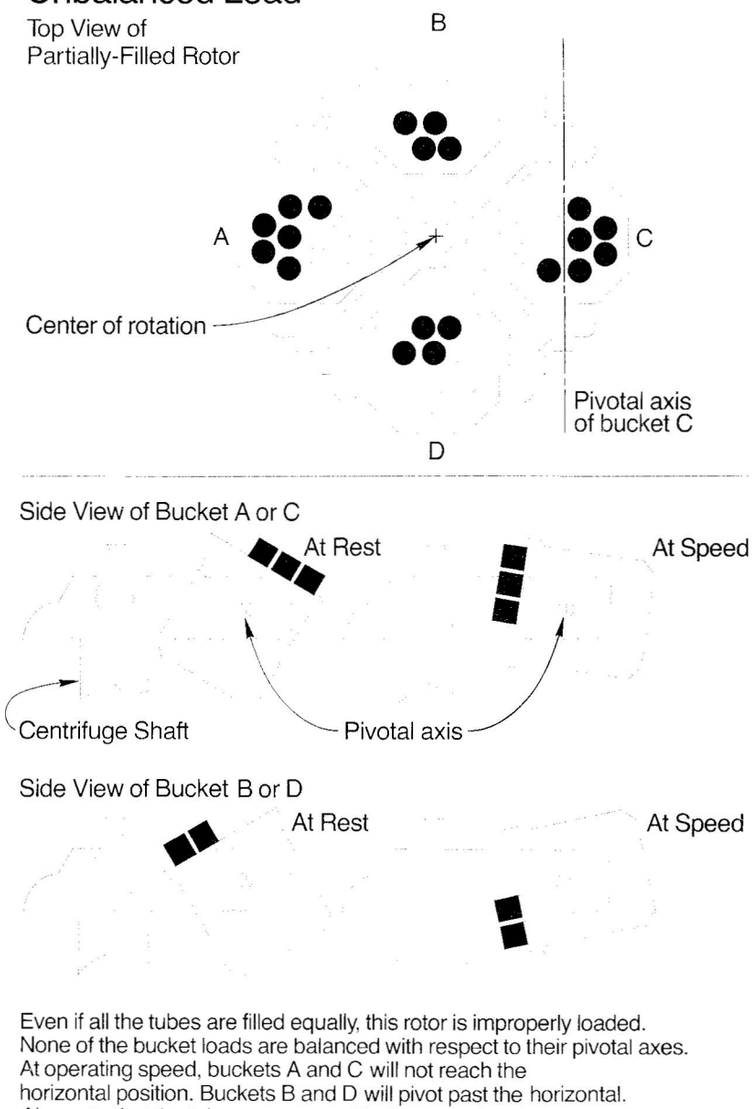
Balanced Load



Assuming all tubes have been filled with an equal amount of liquid, this rotor load is balanced. The opposing bucket sets A-C and B-D are loaded with an equal number of tubes and are balanced across the center of rotation. Each bucket is also balanced with respect to its pivotal axis.

Figure 4. Example of a Balanced Load

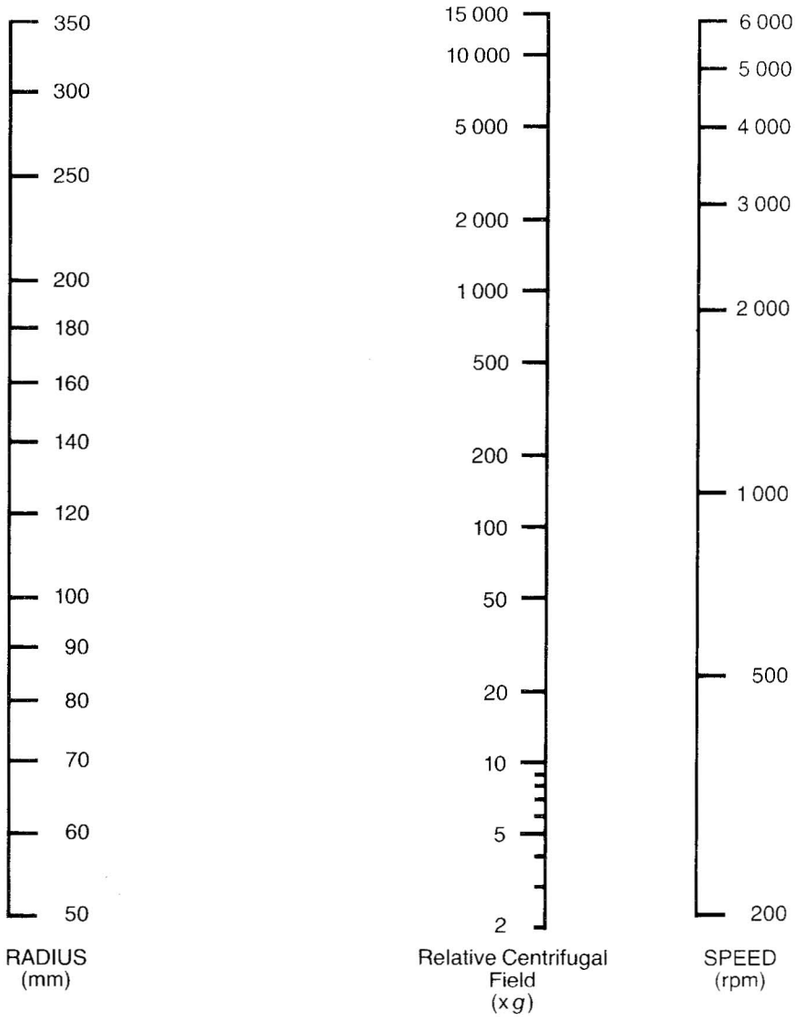
Unbalanced Load



Even if all the tubes are filled equally, this rotor is improperly loaded. None of the bucket loads are balanced with respect to their pivotal axes. At operating speed, buckets A and C will not reach the horizontal position. Buckets B and D will pivot past the horizontal. Also note that the tube arrangement in the opposing buckets B and D is *not* symmetrical across the center of rotation.

Figure 5. Example of an Unbalanced Load

Nomogram for Speed Selection



The centrifugal forces at a given radius are a function of run speed. To obtain a desired rotor force, align a straightedge through known values in any two columns. Read the required value from the third column intersect. Typical radii are given in the preceding table of Rotor Specifications.

Glossary

Some brief definitions of common terms relating to centrifugation are given here. Further discussion will be found on the page indicated after each definition.

Angle Head Rotor. Another name for a *fixed angle rotor.*

Centrifugal Force. In a centrifugal field, the force which pulls a particle away from the center of rotation (p. 2).

Density. Mass per unit volume (p. 6).

Density Separation. A centrifugal separation process based on differences in density between particles (p. 6).

Differential Centrifugation. A centrifugal separation process based on differences in size between particles (p. 5).

Fixed Angle Rotor. A rotor in which the tubes are held at an angle (p. 10).

Horizontal Rotor. A rotor in which the tubes are carried in buckets or racks that swing up to the horizontal position during centrifugation (p. 10).

Maximum Radius. The radial distance from the center of rotation to the bottom of the rotor cavity in a fixed angle rotor, or the bottom of the bucket during centrifugation in the case of a horizontal rotor (p. 3).

Pellet. The material sedimented to the bottom of the tube by centrifugation (p. 5). Also called the sediment.

Relative Centrifugal Field (abbreviated RCF). The ratio of a centrifugal field at a specific speed and radius to the earth's field of gravity. RCF = $1.12r(\text{RPM}/1000)^2$ where r is the radius in mm, and RPM is the speed of rotation in rpm (p. 2).

Sedimentation. The settling out of particles from a suspension in the earth's field of gravity. In the centrifuge this process is accelerated and the particles move away from the center of rotation (p. 4).

Supernatant. The liquid above the sedimented material following centrifugation (p. 5).

Swinging Bucket Rotor. Another name for a *horizontal rotor.*

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