# Centrifugation: a guide to equipment use and maintenance

With increased emphasis now placed on work-based learning and the need for trainees and support workers to receive a solid grounding in laboratory techniques, *The Biomedical Scientist* once again goes back to basics. This first in a series of articles sees Peter Riddle consider the use and maintenance of laboratory centrifuges.

The centrifuge is ubiquitous in biomedical laboratories and a basic knowledge of the theory of centrifugation is more than useful. Centrifuge performance can be classified as low-speed, high-speed and ultra-speed. Usual applications include the separation of serum or plasma from red blood cells, the separation of precipitated solids from the liquid phase of a mixture, or the separation of liquids of varying density.

## PRINCIPLES OF CENTRIFUGATION

Particles suspended in a fluid move, under the influence of gravity, towards the bottom of a vessel at a rate that depends, in general, on their size and density. Centrifugation is a technique designed to utilise centrifugal forces, which are greater than the force of gravity, to speed up the sedimentation rate of particles. This is achieved by spinning the vessel containing the fluid and particles about an axis of rotation so that the particles experience a force acting away from the axis. The force is measured in multiples of the Earth's gravitational force and is known as the relative centrifugal field (RCF) or, more commonly, the '*g*' force.

**Table 1.** Calculations used to convert rpm to RCF, and *vice versa*.

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|---|
| RCF = 11.18 x r $(rpm/1000)^2$ |
| rpm = 299.07 $\sqrt{RCF/r}$ |
| r: radius (cm) |

## RELATIVE CENTRIFUGAL FIELD

The RCF generated by a rotor depends on the speed of the rotor in revolutions per minute (rpm) and the radius of rotation (ie the distance from the axis of rotation). The equations that permit calculation of the RCF from a known rpm and radius of rotation, and calculation of the rpm from a known RCF and radius are shown in Table 1. The RCF value can also be obtained using a nomogram (Fig 1). Using a straight-edged ruler, line up the known rotating radius (distance from the centre of the rotor to the bottom of the centrifuge bucket) on the left with the known rpm on the far right and read the RCF value where the line crosses the graph in the centre. Most manufacturers include a nomogram in the instruction manual; however, most modern centrifuges now have the facility to swap the figure displayed on the control panel between rpm and RCF, making manual calculation unnecessary.

## LOW-SPEED INSTRUMENTS

Low-speed centrifuges have maximum rotor speeds of less than 10,000 rpm, which do not require the rotors to be run in a vacuum, and there are instruments with a temperature control facility. Most instruments now include a sensor that will detect any imbalance when the rotor is running and cut off power to the drive mechanism if imbalance is present. Low-speed instruments are used to separate serum or plasma from red blood cells, and to harvest and purify chemical precipitates, intact cells, nuclei, large mitochondria and large plasma-membrane fragments.

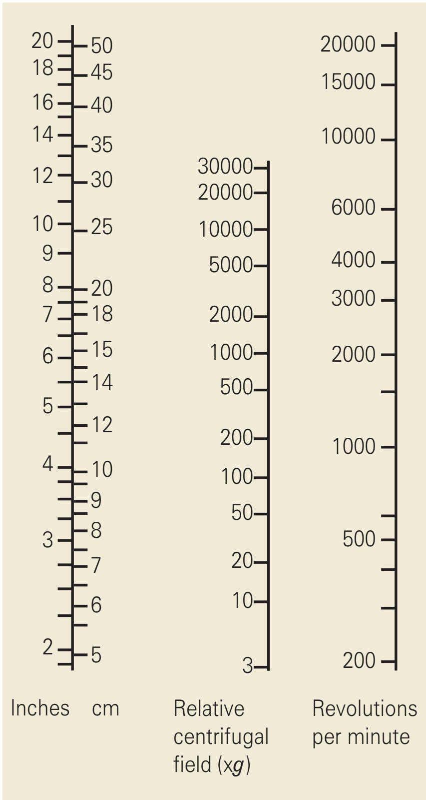


**Fig 1.** Nomogram used to convert revolutions per minute to relative centrifugal field (x***g***), and *vice versa*.

## HIGH-SPEED INSTRUMENTS

In general, high-speed centrifuges are capable of rotor speeds up to 21,000 rpm, although the new generation of super-speed instruments are capable of rotor speeds of 30,000 rpm, in which RCFs of 120,000 x*g* are possible. These instruments require refrigeration systems to overcome the heat generated by the friction of the spinning rotor, and the higher-speed machines must incorporate vacuum systems. High-speed centrifuges are used in the separation of a number of cell constituents and in the isolation and purification of viruses.

## ULTRACENTRIFUGES

Ultracentrifuges are capable of speeds in excess of 30,000 rpm and RCFs of over 600,000 x*g*. They can be used in the isolation and purification of membrane components such as the endoplasmic reticulum and Golgi membrane, endosomes, ribosomes, DNA and RNA. Once again, refrigeration and vacuum systems are necessary.

RSP

Owing to their aerodynamic construction, fixed-angle rotors can attain much higher speeds.

## INSTRUMENT COMPONENTS

**Rotor**

The design of most centrifuges allows the drive system to accept rotors of different sizes and capacities, although most instrument rotors are now capable of accepting a large range of tube sizes through the use of adaptors. Rotors have three basic designs: horizontal, in which the tubes are carried in buckets that can swing outwards to a horizontal position and can operate at speeds to about 3000 rpm; fixed angle, in which the sample tubes are held at a fixed angle to the vertical position and can attain much higher speeds (approximately 7000 rpm) because of the aerodynamic construction of the rotor; and vertical, in which the tubes are fixed in the vertical position. In general, the horizontal rotor offers advantages to the clinical laboratory because sedimentation of large particles (eg red blood cells) is efficient at low force and because a flat sediment is produced.

The load on the rotor should always be balanced before operating the centrifuge, particularly when using high-speed instruments in which the buckets and caps are often numbered so that they can be matched on opposite sides of the rotor.

The load must be balanced both by equal mass and by centres of gravity across the centre of rotation. Thus, it is important not to run the centrifuge with buckets, carriers or shields missing from the unit, and not to exceed the maximum rated speed of the rotor in use. Most modern rotors have microprocessor-controlled automatic rotor identification so that it is impossible to set the speed beyond the safety limit for that rotor.

**Motor**

In general, centrifuge motors are high-torque, series-wound DC motors, the rotation of which increases as the voltage is increased. The rotor shaft is driven directly or through a gyro, although occasionally a pulley system is used. Electrical contact to the commutator is provided by graphite brushes, which gradually wear down as they press against the commutator turning at high speed, and thus should be replaced at specified intervals. Modern centrifuges have induction drive motors that have no brushes to change. The shaft of the motor turns through sleeve bearings located at the top and bottom of the motor. Most instruments contain sealed bearings that are permanently lubricated, while others require periodic application of oil or grease.

The speed of the centrifuge is controlled by a potentiometer that raises and lowers the voltage supplied to the motor. The calibrations on the speed control are often only relative voltage increments and should never be taken as accurate indicators of speed. Therefore, periodic recalibration is required.

**Imbalance detector**

Some instruments have an internal imbalance detector that monitors the rotor during operation, causing automatic shutdown if rotor loads are severely out of balance.

**Tachometer**

A tachometer indicates the speed in rpm. Most modern centrifuges use electronic tachometers, in which a magnet rotates around a coil to produce a current that can be measured.

**Safety lid**

Modern centrifuges must have a door-locking mechanism to prevent the lid from being opened while the instrument is running. If there is a power failure or the safety latch fails for some reason it may be necessary to trip the door-locking mechanism manually to retrieve the samples. Manufacturers' instructions should be checked for the exact procedure required.

**Refrigerator**

A centrifuge generates heat as it rotates and if samples are temperature labile then a refrigerated centrifuge should be used. Some centrifuges enable the rotor and chamber to be precooled before a run.

**Braking system**

Braking devices are incorporated to provide rapid rotor deceleration. Modern instruments have an electrical braking system that functions by reversing the polarity of the electrical current to the motor. Other machines may have a mechanical brake.

## CENTRIFUGE TUBES

It is advisable to use a conical-bottomed tube in a swing-out bucket rotor for the sedimentation of cells. This tube type will retain the pellet of cells more effectively as the supernatant is removed. All tubes for use with high-speed rotors are round-bottomed. Pyrex glass tubes can withstand forces of around 2000 x*g*, while Corex tubes can be used up to 12,000 x*g*. Polycarbonate or polyallomer are the most common plastic tubes in use but great care must be taken when using organic solvents. Manufacturers usually provide extensive information about solvent, salt and pH resistance, as well as sterilisation procedures.

## PREVENTIVE MAINTENANCE

- If the bearings on the upper and lower ends of the motor shaft are not of the sealed type then they should be lubricated as per the manufacturer's instructions.
- Brushes should be removed regularly and checked for wear; they should be replaced if they are worn to more than one-half of their original length. When reinserting used brushes, replace them in the same orientation. New brushes should be broken in by slowly accelerating the unloaded unit to mid-speed and then allowing it to run for a period of time.
- The rotor, buckets and shields or carriers should be examined for signs of mechanical stress (eg cracks, corrosion).
- Some manufacturers etch the expiry date on the rotor and this should be checked periodically.
- Regularly lubricate the contact areas between the centrifuge buckets and the pins.
- Regularly check the condition of the O-ring on the tie-down nut on top of the rotor, and replace it if worn or damaged.
- Always follow a manufacturer's specific instructions. ■