CENTRIFUGES

Types

Three general types of centrifuge are available: *swinging-bucket,* or *horizontal-head, centrifuges: fixed-angle,* or *angle-head, centrifuges:* and *ultraceritrifuges.* These are available as floor or table models, allowing the laboratory to purchase the instrument that best suits its needs.

Centrifuges are used in the clinical laboratory to separate substances of significantly different masses or densities. The two substances to be separated can be a solid (particles) and a liquid or two liquids of different densities. Centrifuges are used in the chemistry laboratory primarily to separate clotted blood or cells from serum or plasma and body fluids. Although the choice of a specific relative centrifugal force (RCF) to carry out these separations is not crucial, a force of 1000 to 1200 x *g* for 10 ± 5 min is recommended. In some instances, more time may be necessary.

Swinging bucket, or horizontal-head, rotors hold the tubes in a vertical position when the centrifuge is at rest: the tubes move to and remain in a horizontal position when the rotor is in motion. During centrifugation, particles constantly move along the tube while it is in the horizontal position, distributing the sediment uniformly against the bottom of the tube. After
centrifugation is complete and the rotor has ceased turning, the surface of the sediment is flat with a column of liquid above it.

Fixed-angle rotors keep the tubes at a specified angle, 25 to 52 degrees to the vertical axis of rotation. During centrifugation, particles move along the side of the tube to form a sediment that packs against the side and bottom of the tube. The surface of the sediment in this case is parallel to the shaft of the centrifuge. As the rotor slows and then stops, gravity may cause the sediment to slide down the tube, forming a poorly packed pellet. Fixed-angle rotors are used when rapid sedimentation of small particles is required. The design of these rotors is more aerodynamic. allow ing operation at speeds higher than those achievable with a swinging-bucket rotor. This capability allows microhematocrit centrifuges to operate at 11.000 to 15.000 revolutions per minute (rpm). with an RCF as high as 14.000 x g.

Ultracentrifuges are high-speed centrifuges that use fixed-angle or swinging-bucket rotors. They are often refrigerated to counter the heat generated as a result of friction. A small air-driven ultracentrifuge, the Airfuge (Beckman Coulter Inc., Spinco Division. PaloAlto. CA) is a miniature air turbine with a small rotor operating at 90.000 to 100.000 rpm, generating a maximum RCF of 178.000 x g. This type of centrifuge has been used to separate chylomicrons from serum, allowing accurate analyses to be performed on the clear infranatant. It has also been used to fractionate lipoproteins,perform drug-binding assays, and prepare tissue for hormone-receptor assays. Analytical ultracentrifuges are used to determine sedimentation coefficients of proteins, allowing assessment of molecular weights.

Centrifuge Components

All centrifuges have a motor, drive shaft, and head or rotor, which may be in the form of a chamber with a cover. A power switch, timer, speed control, tachometer, and brake are the components that control the centrifuge. When necessary, refrigeration units are included. Some centrifuges are equipped with an alarm that sounds when a malfunction such as a tube imbalance occurs. Some centrifuges automatically shut down under these conditions, preventing tube breakage and the potential for exposure to biohazardous agents. All modern centrifuges have a required safety latch that prevents the operator from opening the instrument before the rotor has stopped.

Swinging-bucket rotors use pairs of buckets or carriers that swing freely. The carriers are designed to accept a variety of cushioned inserts, allowing centrifugation of small tubes or large bottles. Different fixed-angle rotors are required for different-sized containers.

The motor in a large centrifuge is usually a direct-current, heavy-duty, high-torque, electric motor. In smaller centrifuges the current is usually alternating. Power is transmitted to the rotor by the commutator and brushes. The rotor shaft is usually driven by a gyro system, and the bearings are usually sealed, minimizing vibration and the need for lubrication. Centrifuge speed is controlled by a potentiometer that modulates the voltage supplied to the motor. Speed is also determined by the mass of the load in the rotor. The tachometer measures rotor speed in rpm. The brake decelerates the rotor by reversing the polarity of the current to the motor. The timer permits the rotor to reach a preprogrammed speed: the rotor then decelerates without braking after a set time has elapsed.

Refrigerated centrifuges are used when the heat generated during centrifugation could cause evaporation or denaturation of protein or leakage of cellular components in the sample. The temperature can be controlled between -15° and 25° C, allowing centrifugation at higher speeds and for prolonged periods.

The selection of centrifuge tubes and bottles is of importance. Plastic tubes (polystyrene, polypropylene) have a higher speed tolerance and can withstand RCFs as high as 5000 x g. Tubes with tapered bottoms, which form more compact pellets, may be required under certain conditions such as preparing urine sediment for microscopic analysis and some radioimmunoassay procedures. The tubes must fit snugly in the carriers; small tubes in too large a carrier result in improperly packed pellets. The top of the tube must not protrude so far above the carrier that the rotor is impeded. Balancing of tubes within the earners is crucial. Newer centrifuges automatically decelerate and shut down when carriers are improperly balanced. Improper balancing can cause the centrifuge to vibrate, disrupting the formed pellet. Whenever possible, tubes containing biohazardous materials should be centrifuged with the caps or stoppers in place to minimize aerosols.

Maintenance and Quality Assurance

Daily cleaning of the inside surfaces of the centrifuge with a tenfold dilution of household bleach or an equivalent disinfectant is crucial. When tube breakage occurs, the portions of the centrifuge in contact with the blood or other potentially infectious agent must be immediately decontaminated. The centrifuge bowl should be cleaned with a germicidal disinfectant. and the rotor heads and buckets should be autoclaved. All broken glass or plastic must be carefully removed and disposed of appropriately.

Centrifuge speeds that are routinely used should be checked periodically using a reliable photoelectric or strobe tachometer in accordance with CAP inspection guidelines. The measured and rated speeds should not differ by more than *5%* under specified conditions. The accuracy of the centrifuge timer should also be checked and verified according to CAP inspection guidelines. The temperature of refrigerated centrifuges should be checked at least monthly under standardized conditions. The agreement between the measured and expected (or programmed) temperature should be within *2° C.*

Manufacturer's instructions for lubrication, maintenance and replacement of brushes should be followed. Failure to replace worn brushes may cause the motor to fail and require replacement. All maintenance function checks must be recorded, and all corrective actions documented.

Principles of Centrifugation

The speed of a centrifuge is expressed in rpm. whereas the RCF generated is expressed as a number times the gravitational force, *g*. The relationship between rpm and RCF is expressed by the equation

$$\mathbf{RCF = 1.12 \times 10^{-5} \times r \times (rpm)^2}$$

where *r* is the radius of the centrifuge expressed in centimeters and is equal to the horizontal distance from the center of the centrifuge bucket to the rotor shaft, and 1.12×10^{-5} is an empirical factor. The RCF applied to a tube in a swinging-bucket rotor may be considerably greater than that applied to the same tube in a fixed-angle rotor because the tube never reaches a horizontal position under the latter condition. For this reason, it is preferable to process serum separator tubes with swinging-bucket horizontal rotors, which operate at higher RCFs.