Chapter 7

Centrifuge

GMDN Code	**15115**	**10778**	**10778**
ECRI Code	**15-115**	**15-117**	**15-116**
Denomination	**Centrifuges, standing, low velocity, non-refrigerated, for blood bank**	**Centrifuge, standing, refrigerated**	**Standing centrifuge**

The word *centrifuge* comes from the Latin word *centrum* which means *centre* and *fugere* which means to escape. The centrifuge is designed to use the centrifugal force generated in rotational movements to separate the constitutive elements of a mixture. There is a wide range of centrifuges capable of serving specific industry and research needs. This chapter focuses on standing centrifuges normally used in public health and clinical laboratories.

PHOTOGRAPH OF CENTRIFUGE

Photo courtesy of Beckman Coulter

PURPOSE OF THE CENTRIFUGE

The centrifuge uses centrifugal force (the force generated when an object rotates around a single point), for separating solids suspended in a liquid by sedimentation, or liquids of diverse density. The rotational movements allow forces much greater than gravity to be generated in controlled periods of time. In the laboratory, centrifuges are generally used in processes such as the separation of solid components from biological liquids through sedimentation and in particular of blood components: red cells, white cells, platelets among others and for conducting multiple tests and treatments.
There are several kinds of centrifuges. The most widely used in public health, surveillance and clinical laboratories are the table-top centrifuge, the ultracentrifuge, the haeamatocrit centrifuge and the standing centrifuge.

OPERATION PRINCIPLES

Centrifuges represent a practical application of Newton's law of motion. When a body of mass [m] turns around a central point [O], it is subjected to a *centripetal* force [N] directed towards the rotation axis with a magnitude $N = m\omega^2R$, where [m] is the mass of the body, [R] is the radius and ω is the angular speed. Centrifuges possess a rotating axis on which is mounted a rotor with sample receiving compartments. Tangential speed is defined by the following equation: $VT=\omega R$.

When the system spins at a speed of ω radians per second, the samples are subjected to the *centrifugal* force **Fp** of the same magnitude as **N**, but in an opposite direction. The figure shown below[1] features a diagram of the concept, of its actual application and of the obtained result. This **Fp** force acts on particles in the substance centrifuged, causing them to separate as a result of differences in density. Denser particles will settle at the bottom of the tube in shorter periods of time, while lighter ones require longer periods of time, settling onto those of greater density. The relationship between the centrifugal acceleration [$\omega^2 r$] to a given radius [r] and the force of gravity [g] is known as the *relative centrifugal field or [RCF]*[2].

$$RCF = \frac{r\omega^2}{g}$$

The RCF is the tool which allows rotors of different specifications to be compared when equivalent centrifugal effects are required.

COMPONENTS OF THE CENTRIFUGE

The most important components of a centrifuge are the following[3]:

The electric/electronic control which generally has the following elements:

1. On and off control, operation time control (timer), rotation speed control (in some centrifuges), temperature control (in refrigerated centrifuges), vibration control (safety mechanism) and brake system.
2. Refrigeration system (in refrigerated centrifuges).
3. Vacuum system (in ultracentrifuges, not shown in the figure).
4. Base
5. Lid/cover
6. Casing
7. Electric motor
8. Rotor. There are different types of rotors. The most common are the fixed angle, the swinging buckets, the vertical tube and the almost vertical tube types, which are explained next.

Sectional diagram of a centrifuge (numbers correspond to descriptions in the text above)

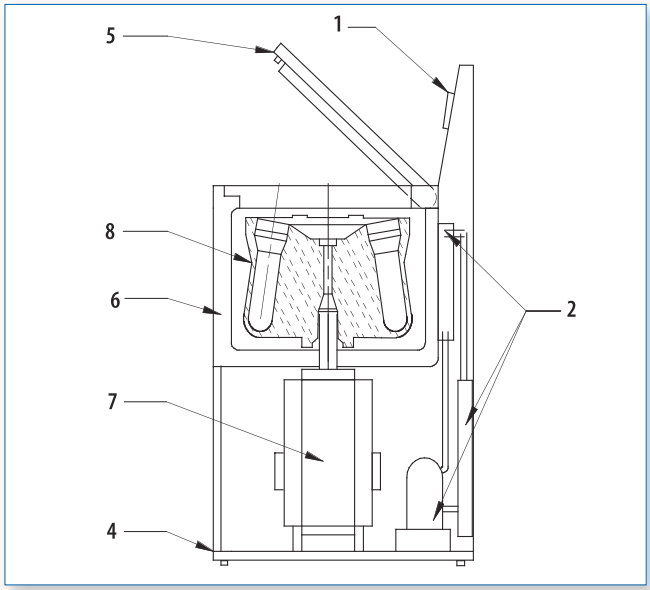
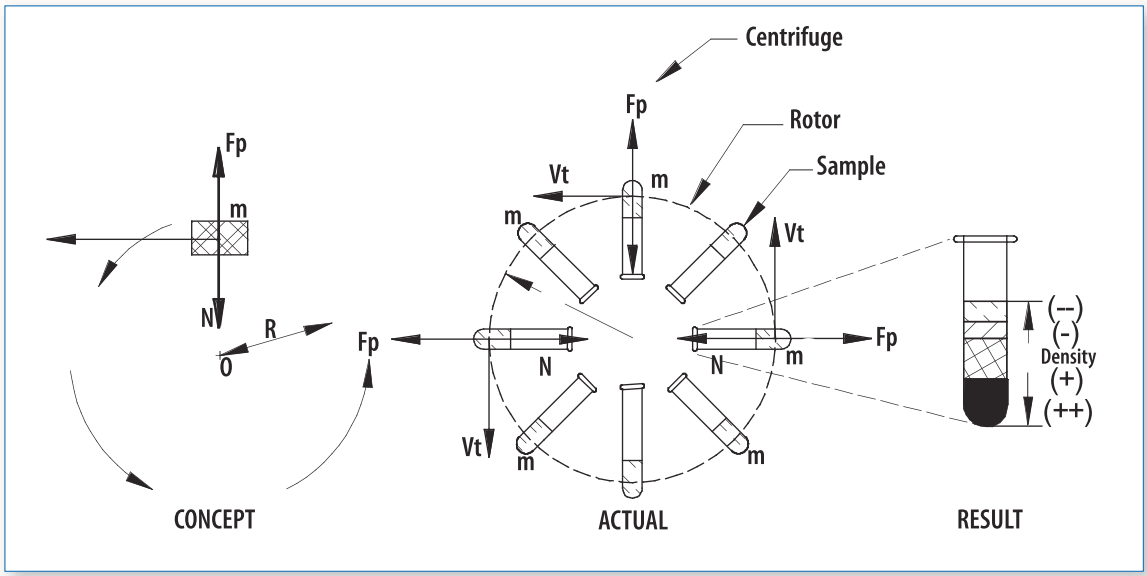


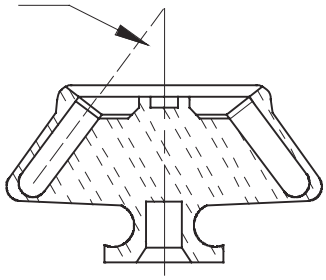
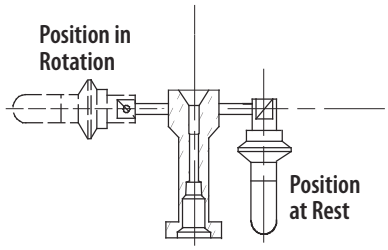
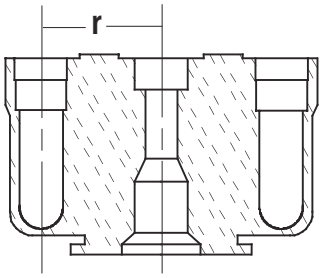
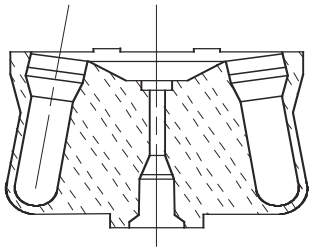
Figure 20. Centrifugal force concept



[1] Newton's law of movement, together with the explanation of the inertia marks of reference can be consulted in books on physics, chapters on uniform circular movement.
[2] RCF. Relative Centrifugal Field.
[3] The numbers identifying each component correspond to those in the sectional diagram of the centrifuge.

Types of rotors

Centrifuges use many different types of rotors. Among the most commonly used are the following:

Type of rotor	Characteristics	Transversal cross-section
Fixed angle rotors.	These are general purpose rotors. They keep tubes at a fixed angle [α] which by design, is specified between 20 and 45 degrees. They are used for sediment sub-cellular particles. The angle shortens the trajectory of the particles and the centrifugation time compared to the swinging buckets rotors.	
Swinging buckets rotors.	These are used for carrying out isopycnic studies (separation by density) and rate-zonal studies (separation by sedimentation coefficient), where maximum resolution of the zones is required for the sample.	
Vertical tube rotors.	This type of rotor keeps tubes parallel to the rotational axis. Thus, separate bands are formed across the tube's diameter, not its length. These rotors are used for carrying out isopycnic studies and in some cases, zonal limit separations where a short centrifugation time is important. These rotors use specially designed tubes.	
Almost vertical tube rotors.	This type of rotor is designed for gradient centrifugation when some sample components do not participate in the gradient. The small angle of these rotors reduces the centrifugation time in comparison to fixed angle rotors.	

Normally, manufacturers specify rotors to be used in centrifuges by providing specialized publications of tables with the following information:

1. ***Type of rotor.*** Specifies the type of rotor for which the technical information is being provided.
2. ***Nominal capacity of the rotor.*** Defines the capacity in litres or litre submultiples. For example: 6 litres; 250 ml, etc.
3. ***Maximum speed.*** This indicates the maximum speed at which this particular rotor should be operated in revolutions per minutes (RPM).
4. ***Maximum Relative Centrifugal field*** (RCF) obtained by that type of rotor.
5. ***k Factor,*** the sedimentation coefficient, defined by the following equation:

$$k = \frac{\ln(r_{max}/r_{min})}{\omega^2} \times \frac{10^{13}}{3600}$$

Where:
ω= angular speed in radians per second
r_{max} = maximum radius in mm, measured in the centrifugation tube
r_{min} = minimum radius in mm, measured in the centrifugation tube
The time required for sedimentation can be calculated in hours using this factor.

6. Information on the compatibility of the rotor with other models of centrifuges from the same manufacturer.

Recently manufactured centrifuges have incorporated numerous improvements into their design to provide greater safety and longer operational life. Among advances mentioned are controls based on microprocessors. By means of *software* controlled by a keyboard, these have several different operational programs in memory. According to the type of rotor being used and procedure conducted, these programs control the centrifugation time, the required temperature, the rotor's revolutions, the acceleration and deceleration, alarms warning the operator about any anomaly during operation.

Manufacturers have also incorporated induction motors (without brushes) in centrifuges. These have the advantage of electronically controlling currents and magnetic fields regulating the rotor's speed which reduces the frequency of maintenance. Operation and maintenance of such equipment must be carried out according to the manufacturer's recommendations.

INSTALLATION REQUIREMENTS

Centrifuges require the following for normal operation:

1. An electrical connection with a capacity suitable for the equipment providing stable single phase or triphase type voltage (depending on the model and specification given by the manufacturer). In general, centrifuges use 110V or 220 V/60 Hz.
2. A clean, dust free environment with a firm levelled floor.
3. If the centrifuge is refrigerated, it needs a free space on the side of the condenser for adequate heat transfer.
4. A cabinet in which the centrifuge accessories such as the alternate rotors can be kept.

ROUTINE MAINTENANCE

The routine maintenance required by a centrifuge depends on multiple factors such as the incorporated technology, usage intensity, training of users, quality of the electrical feed and environmental conditions. The following are general recommendations regarding adequate use and most common maintenance for guaranteeing correct operation. The routines or specialized repairs will depend on manufacturers' recommendations for each brand and model. Always disinfect the rotor bowl, centrifuge head, buckets and trunnion rings as applicable before any servicing of centrifuges used to prepare clinical or infectious samples.

Priority recommendation. Verify that only qualified personnel trained and familiar with the use, care, risks and handling of the centrifuge operates it. It is the laboratory directors' responsibility to supervise and take necessary precautions so that personnel operating centrifuges understand the implications of working with such equipment.

APPROPRIATE MANAGEMENT AND STORAGE RECOMMENDATIONS[1]

Rotors

1. Register the date of purchase of each one of the rotors, including information related to the serial and model number.
2. Read and understand the rotor manuals, equipment and tubes before use. Comply with indications for use and care specified by the manufacturer.
3. Use rotors only in centrifuges for which these have been manufactured. Do not interchange rotors without verifying the compatibility with the centrifuge.
4. Register operation parameters for each rotor in a log book in order to determine its remaining operational life and to acquire its replacements when needed.
5. Use the recommendations regarding maximum speed and sample density from the manufacturer. Each rotor is designed for supporting a maximum level of effort; these specifications must be followed rigorously.

[1] http://www.sunysb.edu/facilities/ehs/lab/cs.shtml

6. Obey the recommendation related to reducing the operation speed when working with high density solutions in stainless steel tubes or plastic adaptors. Manufacturers provide the related information.
7. Use titanium rotors if working with saline solutions frequently.
8. Protect the rotors' coating in order to avoid the metal base from deteriorating. Do not use alkaline detergents or cleaning solutions which can remove the protective film. The rotors generally made of aluminium [Al] are covered by a film of anodized aluminium which protects their metal structure.
9. Use plastic brushes when cleaning the rotor. Metal brushes scratch the protective coating and generate sources for future corrosion. Corrosion is accelerated in operation conditions and shortens the rotor's operational life.
10. If there are spills of corrosive substances, wash the rotor immediately.
11. Air dry the rotor once cleaned and washed with water.
12. Store vertical tube rotors and almost vertical tube rotors with the larger side facing downwards and without their covers.
13. Store rotors in a dry area. Avoid leaving them in the centrifuge.
14. Store swinging buckets rotors without the compartments' covers.
15. Lubricate spiral and O-rings, according to the manufacturer's recommendation.
16. Observe recommendations related to guaranteed times and operational life of each type of rotor.
17. Avoid using rotors whose operational lives have ended.
18. Use a shield if working with radioactive material.
19. Load or unload rotors inside a biological safety cabinet if working with materials classified as Biosafety level II or higher.
20. Never try to open the cover of a centrifuge while it is functioning and never try to stop the rotor by hand.

Tubes

Tube care includes aspects such as filling of the tubes, adequate temperature selection, centrifugation speed limitations, washing and sterilization. The principle recommendations are the following:

1. Wash tubes, adaptors and other accessories by hand using a 1:10 mild detergent solution in water and a soft textured brush (not metallic). Avoid using automatic dishwashers.
2. Avoid using alcohol and acetone since such liquids affect the structure of the tubes. Manufacturers recommend the solvent to be used with each type of centrifugation tube material.
3. Avoid drying tubes in a drying oven. Dry always with a stream of hot air.
4. Verify if the tubes are reusable or not. If they are disposable, use them only once.
5. For sterilizing, it is necessary to verify the material from which the tube is made, as not all can stand sterilization by heat. Glass tubes are normally sterilized with vapour at 121 °C for 30 minutes.
6. Store tubes and bottles in a dark, fresh, dry place, far from chemical vapours or ultraviolet radiation sources.
7. Verify maximum filling levels and the sealing of thin wall tubes in order to avoid collapse inside the rotor by the action of the centrifugal force. Comply with manufacturers recommendations.

Preventive maintenance

Warning: Never carry out a technical intervention in a centrifuge if it has not been previously decontaminated.

The most important maintenance routines performed on a centrifuge are the following:

Frequency: Monthly

1. Verify that the centrifuge external components are free of dust and stains. Avoid affecting the rotor with spills. Clean the rotor compartment using a mild detergent.
2. Test that the rotors' connecting and adjustment mechanisms are in good condition. Keep the points lubricated as the manufacturer recommends.
3. Verify the locking /safety mechanism of the centrifuge's cover. This is fundamental in guaranteeing operators' safety as this mechanism keeps the cover of the centrifuge closed while the rotor is turning.
4. Check the lubrication state of elements such as for O-rings as the manufacturer recommends. Always use lubricants according to the manufacturer's instructions (frequency and type of lubricants). In recently manufactured centrifuges, there are sealed ball bearings which do not require lubrication.
5. Verify the state of gaskets and watertight joints.

Frequency: Annually

1. Verify that electronic cards are clean and well connected.
2. Test operation controls needed for selection of the different parameters of the centrifuge: speed, time, temperature, alarms selectors and analogous or digital instruments.
3. Verify compliance with electrical standards. Use an electric safety analyzer: earth resistance test, escaping current test.
4. If the centrifuge is refrigerated, test the temperature by using an electronic thermometer. The temperature must not vary by more than ± 3 °C.
5. Examine the exactitude of the time controls. Use a timer. The time measured must not vary by more than ± 10 % of the programmed time.

6. Verify the actual rotation speed against the selected one using a normal load. The testing is done with a tachometer or a photo tachometer. If the hatch is not transparent, the procedure indicated by the manufacturer must be followed.
7. Confirm the functioning of the brake system.
8. Verify the functioning of the refrigeration system in refrigerated centrifuges. The following are the most important activities:
   a) Check the selected temperatures. These should not vary by more than 3 °C from the temperatures measured on the digital thermometer.
   b) Verify the state of the air intake filter. If the filter is obstructed, clean or substitute with an equivalent.
   c) Conduct a detailed cleaning of the diffusing wing of the condenser to eliminate the filth deposited. This maintains the heat transference rate according to the design specifications. If abnormal functioning is detected, seek assistance from a specialized service technician.

Note: Avoid spilling liquids on control keys. The keys must be operated with the fingertips: The operator should avoid using fingernails, as this can result in the perforation of their protective membrane.

Every six months:

Verify the state of the motor's brushes, if the centrifuge has a motor with brushes. Substitute with new ones (with the same specifications as the original) if necessary. Perform this routine every six months.

Tools and required instrumentation

In order to carry out the maintenance inspections normally required for a centrifuge, the following tools or instruments are necessary:

1. A key for tightening and slackening the rotor's nuts.
2. An electrical safety analyzer or an instrument for measuring escaping current.
3. A timer.
4. An electronic thermometer with exactitude of 0.5°C for refrigerated centrifuges.
5. A tachometer or photo tachometer.

TROUBLESHOOTING TABLE

Rotors[1]

PROBLEM	PROBABLE CAUSE	SOLUTION
Severe vibration.	The rotor is unbalanced.	Balance the rotor's load. Fill all the opposite tubes with the same level of liquid of same density.
		Distribute the weight of the opposite tubes symmetrically.
		Load fixed angle or vertical tube rotors symmetrically.
	The speed selected is near the rotor's critical speed range.	Select a rotation outside of the critical speed range.
	The rotor is incorrectly mounted.	Verify the rotor's assembly. Test that it is well adjusted.
	There is a lack of lubrication in the rotor's supports.	Lubricate the pivoting axis according to the manufacturer's recommendation. For e.g. each 250 centrifugation procedures.
Rotor covers, canister or cubes difficult to loosen after centrifugation.	A vacuum is being produced during centrifugation.	Open the ventilation line in the upper part of the rotor or bucket to eliminate the vacuum.
	The rings are contaminated with filth, dried lubricants or metallic particles.	Perform routine cleaning of the rings and lubricate. Use recommended products recommended by the manufacturers.

[1] *Rotors and Tubes for Beckman Coulter J2, J6 and Avanti® J series centrifuges, User's Manual,* Palo Alto, California, The Spinco Business Center of Beckman Coulter, 2001.

Tubes

PROBLEM	PROBABLE CAUSE	SOLUTION
The tubes leak.	The covers are badly secured.	Adjust the covers.
	The tubes are too full.	The meniscus must be lower in order to prevent leaks.
	The maximum recommended level has been exceeded in the open tubes.	Verify the volume and speed recommendations for the centrifugation.
	A deficient seal is presumed in the rapid seal tubes.	Press lightly, after heat sealing (only if the contents are not affected). If leaks are visible, seal again.
The tubes are cracked or broken.	The tubes can be broken or become fragile if they are used below the recommended temperature.	If the sample is frozen, warm to 2 °C before centrifuging. Evaluate how the tubes behave at low temperatures before centrifuging.
	The tubes become fragile with age and use.	Discard expired tubes, use new ones.

Various systems

PROBLEM	PROBABLE CAUSE	SOLUTION
The main switch is in the on position but the centrifuge is not functioning.	There is no power to the instrument.	Verify the power supply.
The centrifugue cover cannot be opened.	The centrifuge is off.	Turn the centrifuge ON. Press the handle and open the cover.
The balance indicator is activated.	The load to be centrifuged is unbalanced.	Balance the load to centrifuge.
	The centrifuge is not levelled.	Level the centrifuge.
There is a vibration at low speed.	The rotor adjustment mechanism is slack.	Correctly adjust the fastening system.
	The load is unbalanced.	Verify the balance of the load to be centrifuged.
	The selected speed is close to the rotor's resonance point.	Select a more elevated rotation speed or use a different type of rotor.
There are fluctuations in the rotation speed.	The transmission belts are in a bad condition (*).	Turn off the centrifuge. Verify the condition and state of the belts. The belts must be tempered.
The rotation speed does not reach the selected speed.	The brushes are defective.	Turn off the centrifuge. Verify the condition of the brushes. If this is the problem, put new brushes with the same specifications as the originals.
	The speed control calibration is maladjusted.	Adjust the speed control calibration.
The chamber is cold but the rotor is warm.	The temperature is incorrectly selected.	Verify the temperature selection.
The display which signals the state of the brushes is on.	The brushes are in a bad condition.	Turn off the centrifuge. Verify the condition of the brushes. Substitute the brushes by others with the same specification.

(*) Valid procedure in centrifuges with potential belt transmission system.

BASIC DEFINITIONS

Anodized coating. A hard, thin layer of aluminium oxide, which is deposited on the surface of a rotor by means of electrochemical processes with the aim of preventing corrosion. The coating is often finished in various colours.

Angular speed. The turning rate of a body measured in radians per second. It is calculated using the following formula:

$$\omega = \frac{2\pi \times \text{rpm}}{60}$$

Where:
rpm = revolutions per minute
π = constant with a value of 3.1416

Brush. A device that transmits electrical energy between the external electrical connection (cables in a static state) and the internal components (in rotation) of a motor. In general, brushes are manufactured in very soft textured graphite and, in motors, must be changed regularly (every six months).

Centrifugal force. Apparent force equal and opposite to the centripetal force, driving a rotating body away from the centre of rotation and caused by the inertia of the body. It is one of the components of the inertia vector, which equals the set of forces acting on a body. Its magnitude is always [m x a_n] and its direction radial, moving away from the centre.

Density. A body's mass by volume unit, generally expressed in gram per cm^3.

$$D = \frac{m}{V}$$

Isopycnic separation. A method for separating particles based on the density of the particle's flotation. It is known as sedimentation in balance. The speed of a particle due to differences in density is given in the formula:

$$v = \left(d^2 (\rho_p - \rho_c) \Big/ 18\mu \right) \times g$$

Where:
v = speed of sedimentation $\left(\frac{dr}{dt}\right)$

d = diameter of the particle
ρ_p = density of the particle
ρ_c = density of the solution
μ = viscosity of the liquid medium
g = gravitational force

Radian. A unit of angular measure equal to the angle subtended at the centre of a circle by an arc equal in length to the radius of the circle. It is expressed as the ratio between the arc formed by the angle with its vertex in the centre of the circle, and the radius of that circle.

RCF (Relative centrifugal field or force). A relationship between the centrifugal acceleration and a specific speed and radius, [$r\omega^2$] given with the normal gravity acceleration. It is calculated by means of the following equation:

$$\text{RCF} = \frac{r\omega^2}{g}$$

Where:
R = radius in mm
ω= angular speed in radians per second $\omega = \frac{2\pi \times \text{rpm}}{60}$

g = Standard gravity acceleration = 9 807 mm/s^2

Resonance. A situation in which a mechanical system vibrates as a response to a force applied at the system's natural frequency.

Sedimentation. Particles from a suspension settling at the bottom of the liquid as a result of the action of the gravitational force. During centrifugation, this process is accelerated and particles move away from the rotational axis.