Centrifuge

- **Definition**

A centrifuge is a laboratory instrument for the density-based separation of fluids, gas, or liquid. Centrifuges are used in daily life as well as in science and medical research. Cells, subcellular organelles, viruses, proteins, and nucleic acids can all be purified with it.[2]

2. Principle of Centrifugation

1) Many principles govern the mechanics of centrifugation, but the primary is centrifugal force. Centrifugal force is an outward push experienced during high-speed rotation, acting radially from the center of rotation and causing the movement and sedimentation of particles suspended in a sample.
2) The principle of the centrifugation technique is to separate the particles suspended in liquid media under the influence of a centrifugal field. These are placed either in tubes or bottles in a rotor in the centrifuge.
3) Sedimentation is a phenomenon where suspended material settles out of the fluids by gravity. The suspended material can be particles such as clay or powder. Example, tea leaves falling to the bottom in a teacup.
4) The particles having size more than 5 micrometres are separated by simple filtration process while the particles having size 5 micrometre or less do not sediment under gravity. The central force is useful to separate those particles.
5) **Centrifugal Force and Sedimentation** Caused by centrifugal force, sedimentation is the process by which denser particles settle at the bottom of a sample tube inside a centrifuge. The sedimentation rate is determined by the particles' size, shape, and density, with heavier and more dense particles moving faster. Differential sedimentation depends on the varied sedimentation rates of particles through a fluid.
6) **Sedimentation Rate and Pellet Formation** During rapid centrifugation, particles will sediment into a pellet at the base of the tube or container. This pellet is composed of heavy and dense particles suspended in the sample and can be easily separated for direct analysis after centrifugation.
7) **Other Factors Involved in Centrifugation** Another principle at work during centrifugation is buoyancy, which is influenced by the medium in which the

particles are suspended. Speed and time of centrifugation are also crucial factors to consider in experimental design, as increasing centrifugation's duration and speed increases the extent of separation. These factors can be determined for the optimal separation of desired particles or components of interest. The rotors' size and tubes can also impact efficiency and performance.

- **Centrifugation Techniques**

There are two types of centrifugation techniques, namely, preparatory centrifugation and analytical centrifugation. Preparatory centrifugation deals with the isolation and purification of components such as tissue, cells, subcellular structure, membrane vesicles, and other particles of biochemical interest. In contrast, analytical centrifugation is carried out to characterize purified biomolecules.

- **preparatory centrifugation**

Based on suspension, preparative centrifugation is divided into two different types. They are :

A. Differential Centrifugation

It separates particles based on shape, size, and density. A suspension of particles with varying densities or sizes will sediment at varying speeds, with the larger and denser particles sedimenting more quickly. Following a series of rising centrifugal force cycles on a suspension of cells, a series of pellets containing cells with a decreasing sedimentation rate will result.

Differential centrifugation utilizes multiple rounds of centrifugation at progressively higher speeds to separate components based on size and density. This allows for the separation of organelles and other cell components.

Differential centrifugation can have several steps, depending on the level of separation desired. For example, differential centrifugation can begin with a low-speed initial spin step. This slower spin allows debris, contaminants, whole cells, nuclei, and cell membranes to pellet, leaving a purified centrifugation supernatant. This supernatant proceeds to a second spin step, this time at a higher speed, called the medium speed step. The pellet that forms during this step is composed of organelles and the consequent supernatant contains the remaining small particles, proteins, and nucleic acids.

In this example, the supernatant from the medium-speed step undergoes a final spin at the highest speed. This ensures the separation of small ribosomal and viral particles along with proteins and nucleic acids. After centrifugation, the tubes from each step of the process can be divided into several fractions, each containing particles with similar densities for further analysis and processing.

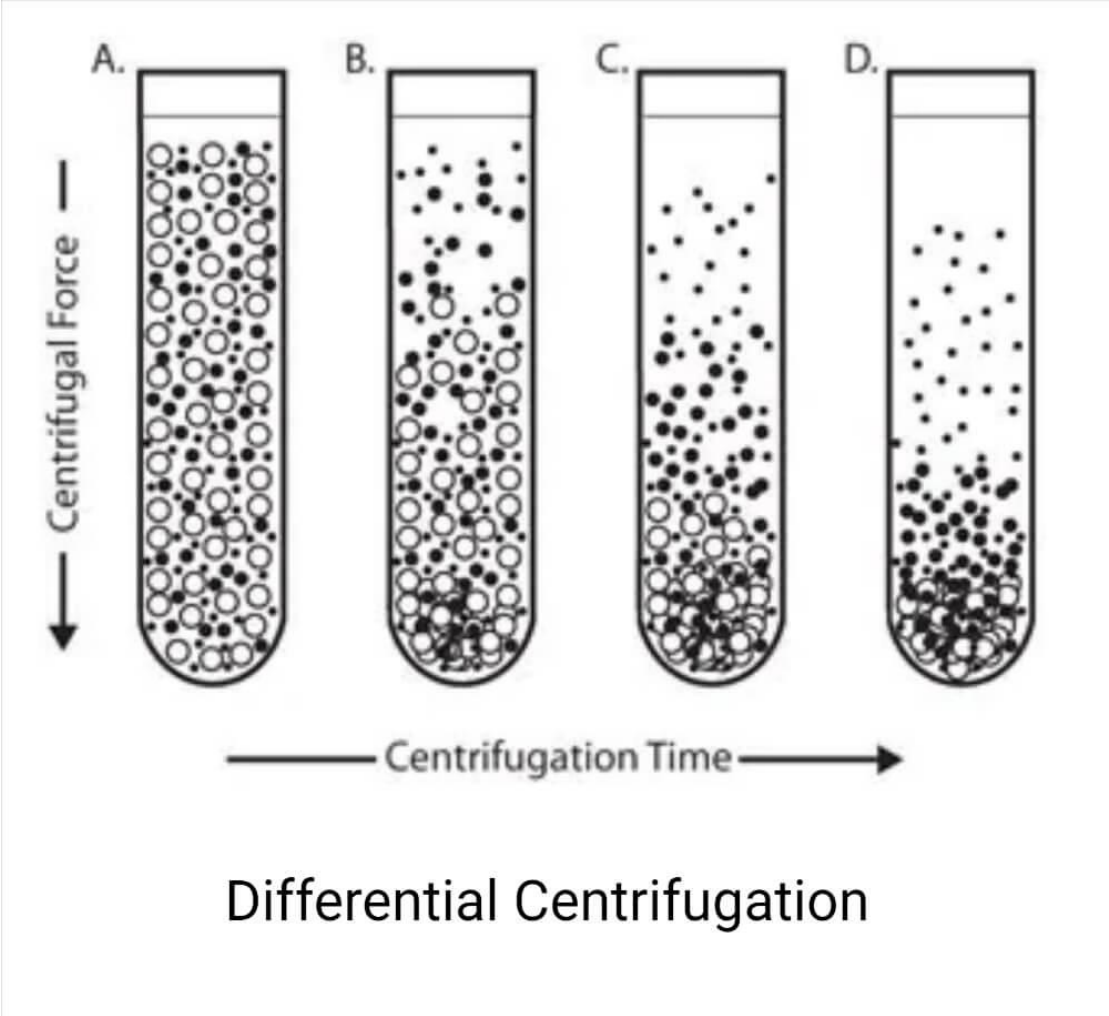

Fig 3: Differential centrifugation. Image Source: Merck KGaA. https://microbenotes.com/wp-content/uploads/2022/12/Differential-centrifugation.jpg

B. Density Gradient Centrifugation

Density-gradient centrifugation is an essential tool that allows for separating and defining the relative density of particles in a sample using a gradient of known density. A density gradient is created within a centrifuge vessel by layering solutions by known density. Compound CsCl is commonly used in this application to prepare a gradient.

The sample of unknown density is loaded onto the density gradient and centrifuged at a high speed until the particles migrate to their equilibrium positions. These positions align with the density of the particles themselves. This technique is especially useful in separating DNA, RNA, proteins, and nucleic acids, as buoyant density plays an important role in their response to the gradient**.**

It separates particles based on their buoyant density or sedimentation rate. A sample mixture is placed on the top of a preformed liquid density gradients such as CsCl for DNA banding and isolation of plasmids, nucleoproteins, and viruses; NaBr and NaI for fractionation of lipoprotein; Per coll, Ficoll, Metrizamide, Dextran for separation of whole cells and sucrose solution for the separation of DNase, RNase and Protease.

The two subtypes of density gradient centrifugation are rate-zonal and isopycnic centrifugation

b.1. Rate-zonal centrifugation

On top of a density gradient, the sample is overlaid as a small zone. Depending on their mass, particles travel under centrifugal force at various speeds. Size and mass are the main determinants of how quickly particles settle. As the band of particles descends through the density medium, zones with particles of comparable size develop as the faster sedimenting particles pass the slower ones.

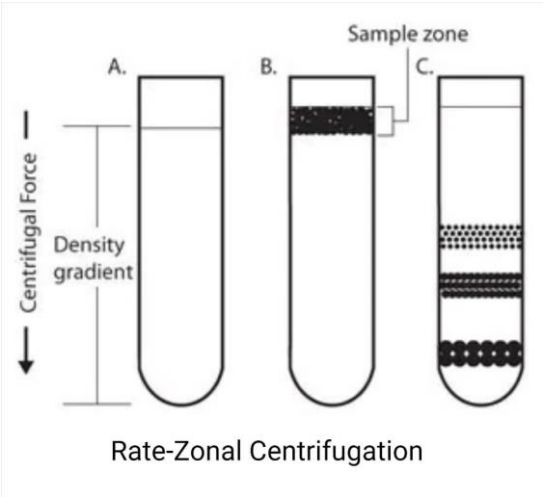

Fig 4 : Rate-zonal centrifugation. Image Source: Merck KGaA. https://microbenotes.com/wp-content/uploads/2022/12/Rate-zonal-centrifugation.jpg

b.2. Isopycnic centrifugation

Particles are separated exclusively based on their density in an isopycnic separation, also known as buoyant or equilibrium separation. It is necessary for the gradient medium to have a higher density than the particles that need to be separated.

Particles migrate under the influence of centrifugal force from a uniformly mixed sample and density gradient until their densities are equal to those of the surrounding medium. After centrifugation, particles of a certain density settle until their density equals that of the gradient media (i.e., the equilibrium position).

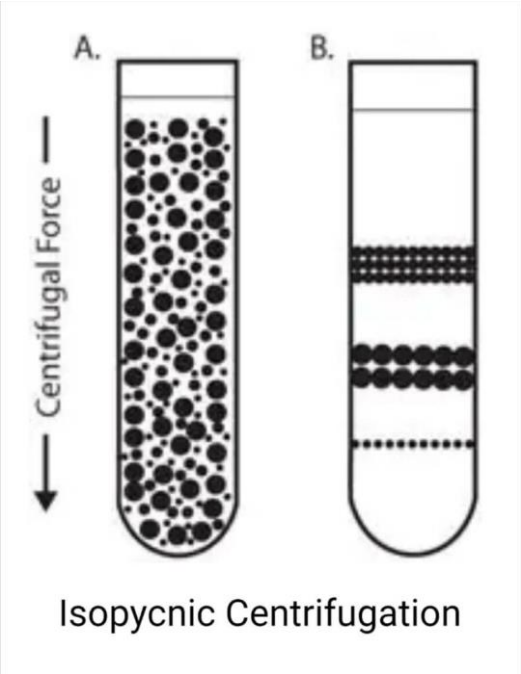

Figure: Isopycnic centrifugation. Image Source: Merck KGaA.

https://microbenotes.com/wp-content/uploads/2022/12/Isopycnic-centrifugation-792x1024.jpg

- **Analytical Centrifugation**

It aims to collect information to characterize the spun sample (sedimentation velocity, viscosity, concentration, etc.), determine the relative molecular weight of the solutes, purity of biomolecules, detect conformational changes of protein structure, etc.

- **Ultracentrifugation**

Ultracentrifugation provides maximum separation power and precision using high rotation speed and specialized rotors. Commonly used to separate macromolecules and

subcell components, there are two main types of ultracentrifugation: analytical and preparative.

- **Analytical Ultracentrifugation**

This is a technique used to study the hydrodynamic properties of small molecules, such as DNA. Analytical ultracentrifugation involves subjecting samples to high speeds and monitoring the reactions based on sedimentation velocity and equilibrium factors.

- **Preparative Ultracentrifugation**

Ideal for large-scale research, preparative ultracentrifugation purifies small molecules like DNA and proteins at high speed using unique rotors. This high-speed ultracentrifugation results in a pellet of dense particles.

- **Counterflow Centrifugation**

Also known as countercurrent centrifugation, counterflow centrifugation is a specialized technique that allows for the continuous separation of components based on density. Counterflow centrifugation differs from conventional centrifugation by sample and separation medium introduction and collection. In counterflow centrifugation, the sample and medium are introduced to opposing ends of the rotor and flow in opposite directions. The flow causes the components to separate based on density.

The sample is introduced to the rotor through a feeding tube and mixed with a separation medium to create a density gradient. The buffer or separation medium, generally composed of sucrose, is fed through the other end of the rotor. As the two products flow opposite each other, the particles migrate through the separation medium according to buoyant density. Heavier particles will move more quickly against the current, while lighter particles are swept up in the current. This design allows for the continuous flow of separation. The sample and medium will collect at their opposite ends for separate collection.

Counterflow centrifugation enables continuous separation and collection of components (as opposed to collection layers or pellets as required in conventional methods). Counterflow centrifugation is an essential tool for many research initiatives in cell separation, isolation, and purification.

Counterflow centrifugation is also integral for large-scale research and cell manufacturing demand. Because of counterflow centrifugation's continuous separation abilities, there is no need to stop the function between separation steps, which saves time and increases efficiency by providing an uninterrupted process. The size of rotor adaptors can be modified—as can the separation medium—to accommodate large sample sizes and high volumes continuously.

- **Parts of a Centrifuge**

Some of the common parts of the centrifuge are described below:

1) **Motor:** The motor is the powerful central component of the centrifuge that creates the spin.
2) **Rotor assembly:** A drive shaft and a rotor comprise the rotor assembly. The drive shaft provides support for the rotor components. The rotor head is attached to the motor, which bears the containers to house the tubes containing the sample to be centrifuged. It converts electrical energy to mechanical energy. Two rotors with different diameters can have the same rotational speed. Varying radii and angular momentum results in a difference in the acceleration of such rotors. Thus, relative centrifugal force (rcf) is regarded as the accepted standard unit for the rotation speed. There are mainly three types of rotors:

a) **Fixed angle rotors:** These rotors hold the tubes at an angle of 14 to 40° to the vertical such that particles travel a short distance while moving radially outwards and are used in differential centrifugation. The sedimentation takes place at the walls of the tubes at an angle since the sedimentation direction is the same as the direction of centrifugal force. The pellets (cluster of sediments) later settle at the corner of the base and the wall surface after colliding with the wall surface.

b) **Swinging bucket/ Horizontal rotors:** These rotors, along with the centrifuge tubes, swing out to a horizontal position during the time of acceleration such that particles travel a longer distance, thereby facilitating easier separation of supernatant from the pellet. These types of motors are employed in density gradient centrifugation.

c) **Vertical rotors:** These hold the tubes vertically, i.e., parallel to the motor axis, and the particles move shorter distances with shorter periods for separation. It is used for isopycnic and density gradient separation; however, it is not considered useful

for pelleting because the pellets are spread out along the entire outer wall of the tube by centrifugal force.

3) **Containers:** Several types of containers, such as test tubes, blood bags, cuvettes, centrifuge tubes, etc., are held in the rotors such that the sample rotates along as the rotor rotates.
4) **Control Panel:** It serves the purpose of controlling different parameters such as temperature, rotational speed (rcf or rpm), etc.
5) **Latch:** When a tube breaks, or there are other issues with the centrifuge while running, the latch keeps the lid closed.
6) **Lid:** The centrifuge will only spin if the lid is closed and locked to prevent mishaps.

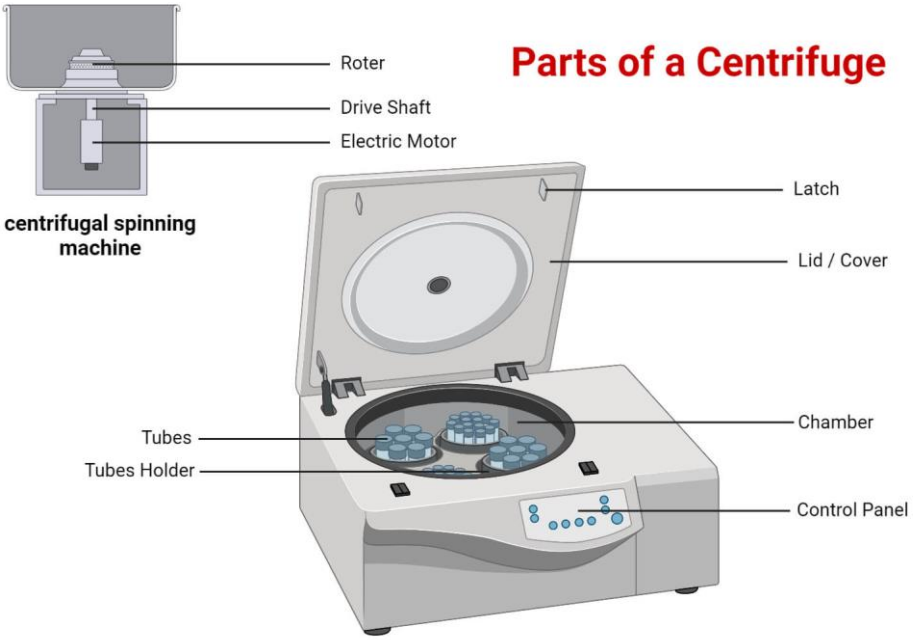

Fig 1: parts of centrifuge. https://microbenotes.com/wp-content/uploads/2022/12/Parts-of-a-Centrifuge.jpg

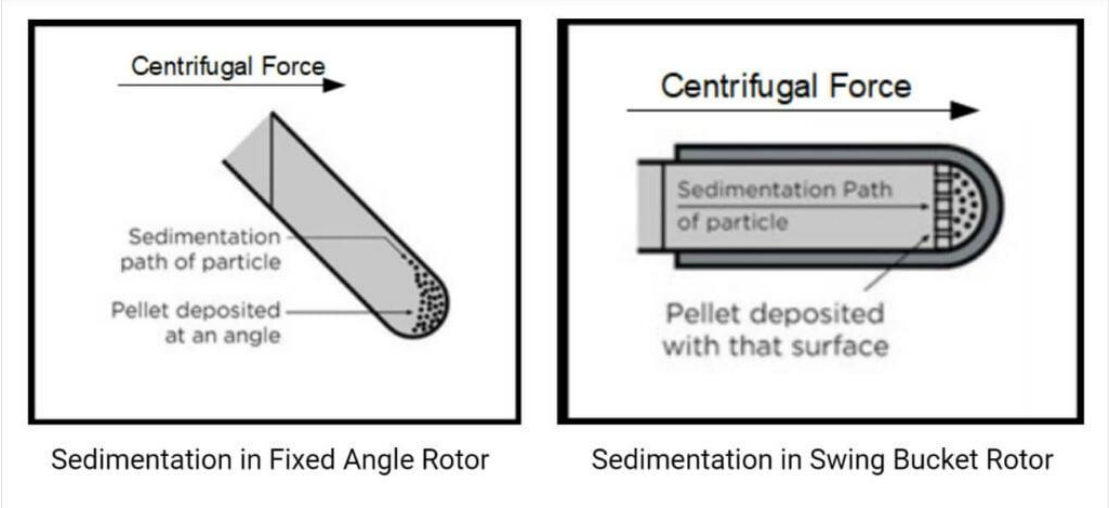

Fig 2: Sedimentation in Fixed Angle Rotor and Swing Bucket Rotor. Image Source: tec2med. https://microbenotes.com/wp-content/uploads/2022/12/Sedimentation-in-Fixed-Angle-Rotor-and-Swing-Bucket-Rotor-1024x470.jpg

- **Types of Centrifuges**

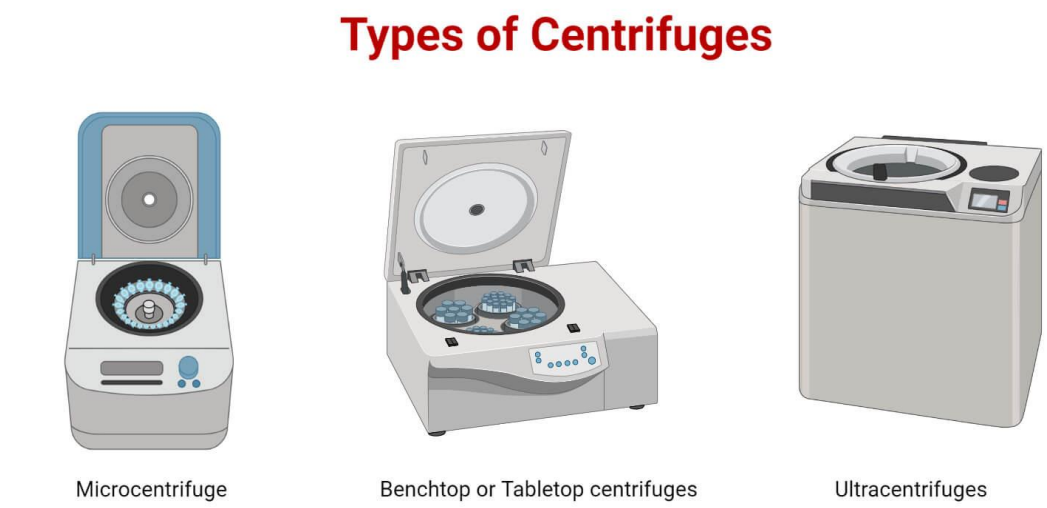

Fig 5: Types of Centrifuges. https://microbenotes.com/wp-content/uploads/2022/12/Types-of-Centrifuges.jpg

1) **Benchtop or Tabletop centrifuges**

- They can be handy for tiny labs with limited space because of their diminutive size.

- These are compact and are frequently employed in research and clinical laboratories.
- A tabletop centrifuge is furnished with a lid that covers the apparatus used to run the centrifuge and a rotor with racks for the test tubes.

2) **Gas centrifuges**

- These are used to separate molecules based on their masses and to separate gases based on their isotopes.
- Specifically, they are employed in the extraction and separation of uranium-235 and uranium-238.

3) **Haematocrit centrifuge**

- Haematocrit centrifuges operate between 7000 and 15000 rpm.
- The main purpose of hematocrit centrifuges is to calculate the volume-based erythrocyte percentage in blood. It is used to produce plasma for photometric analysis of the bilirubin concentration of neonatal blood.

4) **Microcentrifuge**

- They have a very small footprint and take up minimal room on the workstation because of their highly compact form.
- These work well with small tubes (up to 2.0 ml) and are frequently employed in biological applications.
- They are used to microfilter small amounts of aqueous samples and hold pelleted nucleic acids, proteins from solutions, and other substances.

5) **Refrigerated Centrifuges**

- These centrifuges run at their top speeds while keeping a constant temperature.
- It is used to analyze DNA, RNA, PCR, and antibodies because its temperature range is between -20 and -40 degrees Celsius.
- They are frequently used to collect sedimenting materials quickly, including yeast cells, chloroplasts, and more.

6) **High-Speed Centrifuges**

- A high-speed centrifuge is a type of centrifuge that can work at somewhat faster rates ranging between 15,000 and 30,000 revolutions per minute.
- High-speed centrifuges contain a device for regulating both the temperature and speed of the operation for the critical analysis of delicate biological molecules.
- These centrifuges employ three rotors: fixed angle, swinging bucket, and vertical.

7) **Low-speed centrifuges**

- These are frequently used in laboratories for routine particle sorting operated at a maximum speed of 4000-5000 rpm.
- There are few instances of temperature regulation, and they are often operated at room temperature.
- These centrifuges employ swinging bucket and fixed-angle rotor types.

8) **Continuous flow centrifuges**

- It enables the centrifugation of large quantities of samples without influencing sedimentation rates.
- They also have greater capacities, which saves time by eliminating the need to load and unload the sample repeatedly as is necessary with standard centrifuges.

9) **Ultracentrifuges**

- The ultracentrifuge is a highly developed and sophisticated centrifuge that can separate tiny molecules that conventional centrifuges can’t separate at a fast rate.
- Ultracentrifuge rotor speeds can range from 60,000 to 150,000 rpm.
- They run samples in groups or as continuous flow systems and are larger.

5. Centrifuge Operating Procedure

- Check the centrifuge to ensure it operates properly, is unharmed, and can move without restriction.
- After choosing the proper centrifuge tubes or containers, check them to ensure no blemishes or cracks. Any tubes or containers with damage or flaws should be discarded.
- Fill the preferred liquid in the tubes. Never overfill or underfill tubes or containers.
- Check that the centrifuge tubes are balanced; weigh each tube separately on a scale to confirm that their weights are equal. Avoid balancing solely on volume! This is especially true for solutions containing various sample types or varied sample concentrations.
- Screw the centrifuge tubes' lids on firmly.
- Before inserting the centrifuge tubes, ensure their exteriors are dry and clean.
- Keep the tubes in the centrifuge balanced.
- Shut the lid. Ensure that the lid fits tightly.
- Configure the run time and speed.
- When the centrifuge appears to be running and is running at maximum speed, do not leave the device. Check for any unusual vibrations or noises.
- Turn the centrifuge off and remove the sample as soon as you hear an odd noise or feel strong shaking. A typical cause of this is an improperly balanced centrifuge. Do not use the centrifuge until it has been repaired if the issue does not go away despite the centrifuge being properly balanced.
- After the centrifuge has finished its cycle, wait until it has stopped rotating before opening the cover. Never touch or open the centrifuge's lid before it has stopped rotating. Mechanical failure and harm might result from prematurely stopping the equipment.
- To allow the aerosols that were emitted during centrifugation to settle, it is best to wait at least 10 minutes after the rotation has stopped before opening the lid.
- Samples can be taken out of the centrifuge once it has stopped spinning.

6. Precautions

- Before the operation, always check that the centrifuge is on a suitable surface.
- Keep the lid closed while the rotor is operating.
- When the centrifuge shakes or vibrates, unplug it.

- Make sure tubes work with programs and settings before using them. The tubes used for the centrifuge should be in a set that is matched.
- Balanced neighboring and opposing loads should be used to load tubes symmetrically. Use mass instead of volume to balance the tubes.

7. Centrifuge Cleaning and Maintenance

Knowing how to clean your centrifuge is one of the most essential aspects of centrifuge care. Keep the following tips in mind for correct maintenance and cleaning practices.

1) Cleaning Your Centrifuge

Neglecting to clean your centrifuge could result in corrosion and imbalances, leading to more dangerous equipment failures. Clean your centrifuge daily, or at least once a week, for the best results. Follow these simple steps to clean your centrifuge correctly:

- Wipe it down after every use, especially around the test tube area.
- Remove the rotor and any container holders while cleaning.
- Clean it thoroughly, including the interior mechanisms.
- Use a mild detergent and warm water to clean and dry it thoroughly.
- Wipe any spills immediately.
- Avoid caustic detergents, and don't pour water into the bucket.
- Use a centrifuge lubricant for the bucket grooves and seals after cleaning.
- Disinfect the equipment regularly with an approved disinfectant.

2**). Inspecting Your Centrifuge**

Inspect your centrifuge often for signs of damage. Pay specific attention to the rotor for any cracks or corrosion. Damage to the rotor could cause your centrifuge to be uneven, resulting in breakage when rotating at high speeds.

3). Balancing Your Centrifuge

When using a centrifuge, ensuring it's balanced is critical. Imbalances can cause unnecessary movement and vibration in the machine, leading to wear and tear over time. For example, if you're spinning two test tubes, ensure they're opposite and equal in weight.

4) . Loading and Unloading Your Centrifuge

Rough handling can damage the rotor or tubes. Always load and unload tubes gently.

5) . Servicing Your Centrifuge

Just like any other complex equipment, your centrifuge needs regular tune-ups. Get a trained technician to check your unit regularly. Some signs of damage are only visible to professionals. A trained eye can discern and repair any minor issues before they become large and expensive, ensuring your unit is safe to operate.

Depending on the frequency of use, you should aim to have your unit checked and calibrated every six to 12 months.

8. Centrifugal force

8.1. Centrifugal force definition

Centrifugal force is an outward fictitious force that is experienced by an object moving in a circular path directed away from the center of rotation.

- The direction of this force is away from the axis of rotation and is parallel to the axis of rotation.
- Centrifugal force is equal in magnitude and dimensions with another force (centripetal) that acts towards the center of a circular path.
- It is termed a fictitious force because it only comes to play when there is a centripetal force. This force results due to the inertial property of the body moving in a circular path.
- The force does, however, depend on the mass of the object, the distance of the object from the center, and the speed of the rotation.
- The concept of centrifugal force has been used in various rotating devices like centrifuge rotors, banked roads, and centrifugal pumps.
- The unit of centrifugal force is Newton, and the dimensional formula is $M^1L^1T^{-2}$.

8.2. Centrifugal force formula

The formula to calculate the centrifugal force is given below.

If the velocity of the moving object is known, the centrifugal force can be calculated by the formula:

$$F = m \times \frac{v^2}{r}$$

Where **v** is the velocity of the moving body, **r** is the distance of the moving body from the center and **m** is the mass of the moving body.

If the angular velocity of the moving object is known, the centrifugal force can be calculated by the formula:

$$F = m \times \frac{(\omega r)^2}{r} \text{ or } F = m \times \omega^2 r$$

Where **ω** is the angular velocity, **r** is the distance of the moving body from the center and **m** is the mass of the moving body.

8.3. **How is centrifugal force calculated?**

For this, let us take the same example of stone at the end of the string tied to a pole. In order to calculate the centrifugal force experienced by the stone, the following steps can be followed:

1. Determine the mass (m) of the stone. Let's say the mass is 5 kg.
2. Determine the length of the string. Let's say the length is 10 m.
3. Determine the velocity of the stone. Let's assume that the tangential force is 5 m/s. The velocity can be either tangential velocity (v) or angular velocity (ω). If the velocity is the angular velocity, the tangential velocity can be calculated as :

v = ω × r

4. Calculate the centrifugal force by the given formula:

$$F = m \times \frac{v^2}{r}$$

$$F = 5 \times \frac{5^2}{10}$$

$$F = 12.5 \text{ N.}$$

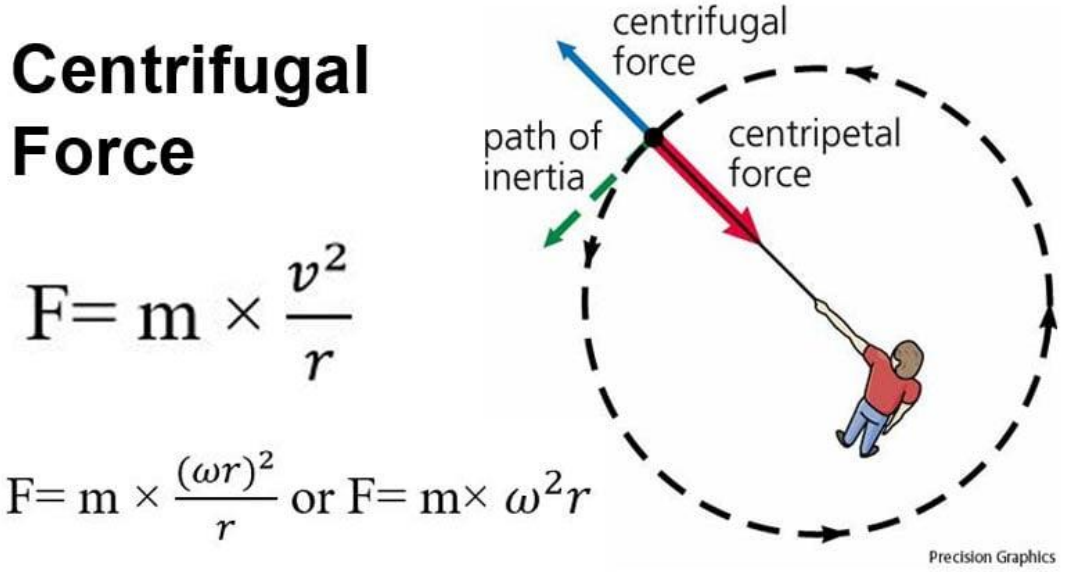

Fig 7: centrifugal force Image Source: Wikia.

8.4. **Applications of Centrifugal force**

Centrifuges operate on the principle of centrifugal force. The centrifugal force created due to the rotors induces a hydrostatic pressure gradient in the tubes directed perpendicular to the axis of rotation. This results in larger buoyant forces that push the less dense particles inwards while the denser particles are moved outwards. This principle allows the separation of particles on the basis of their densities.