

Centrifuge

- Separate solid matter from a liquid suspension by means of centrifugal force.
- Accelerates gravitational separation of substances that differ significantly in their masses.
- Exerting a force greater than that of gravity.
- suspended materials are deposited in the order of their weight.



Historical Background

- **Antonin Prandtl** has the credit of inventing of a centrifuge in the 1860s for separating milk and cream.
- Further explored by **Dr. Friedrich Miescher** in 1870s,.
- Early models were manually operated, but modern centrifuges are automated and highly precise.
- They have become indispensable in medical diagnostics, research, and biotechnology.

Components

- **Basic components**

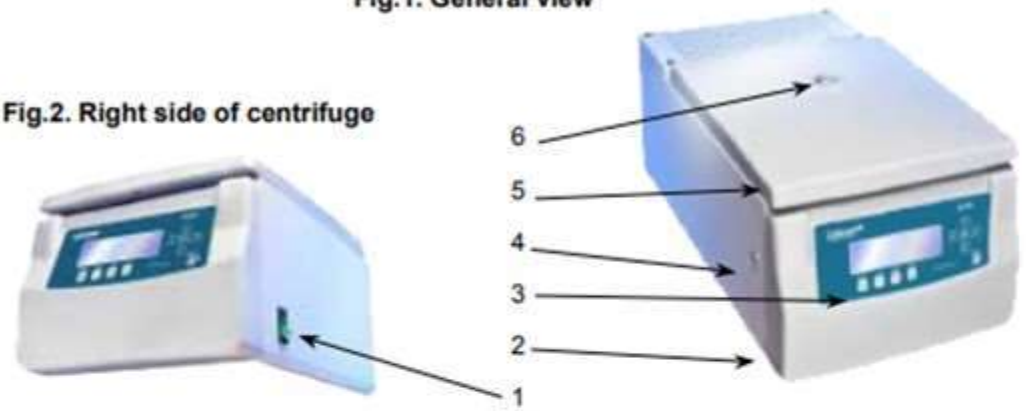
- 1. Central Shaft:** - It is a part that rotates when spinning is effected manually.
- 2. Head:** - It is a part that holds the bucket and connected directly to the central shaft or spindle.
- 3. Bucket or tube:** - Are portions that hold test tubes containing a given sample to be spine

- 1.** A rotor or centrifuge head
- 2.** A drive shaft
- 3.** Motor
- 4.** Hanging buckets
- 5.** Power switch
- 6.** Timer
- 7.** Speed/gravity control
- 8.** Tachometer
- 9.** Brake
- 10.** Protective shield to minimize aerosol
- 11.** Safety lock

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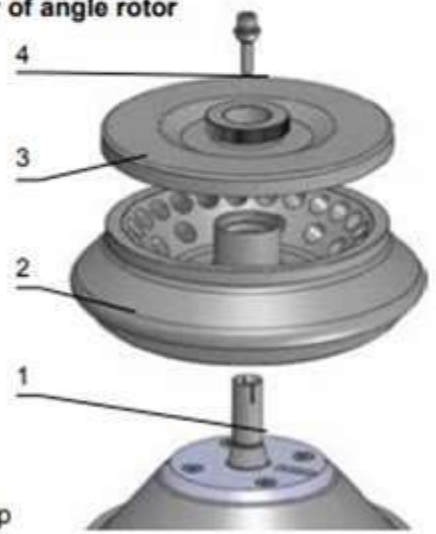
Fig.1. General view

Fig.2. Right side of centrifuge



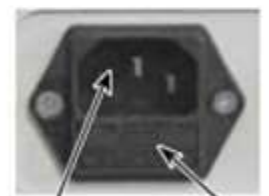
- 1. Power switch
- 2. USB
- 3. Control panel
- 4. Point of emergency lid opening
- 5. Lid
- 6. Inspection glass

Fig.3. Assembly of angle rotor



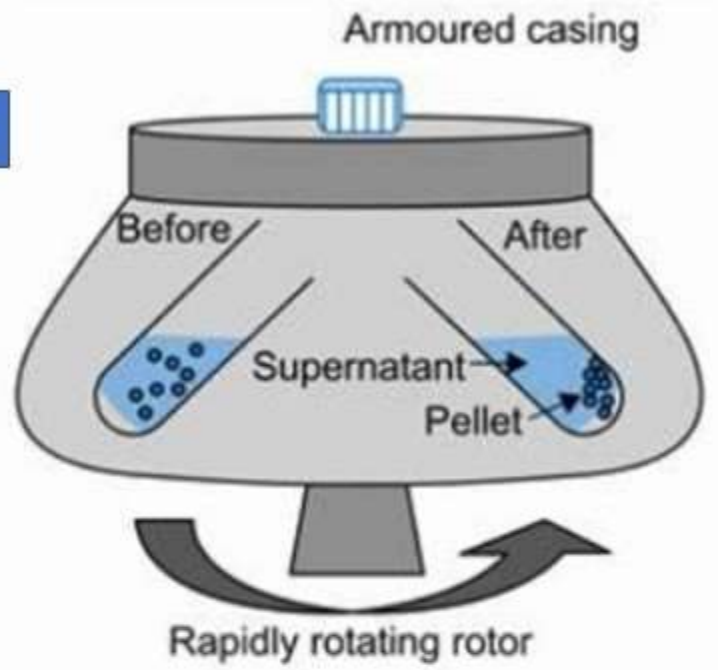
- 1. Motor axle
- 2. Rotor
- 3. Rotor lid
- 4. Complete clamp

Fig.4. Mains socket back of the centrifuge



- 1. Main socket
- 2. Fuse socket

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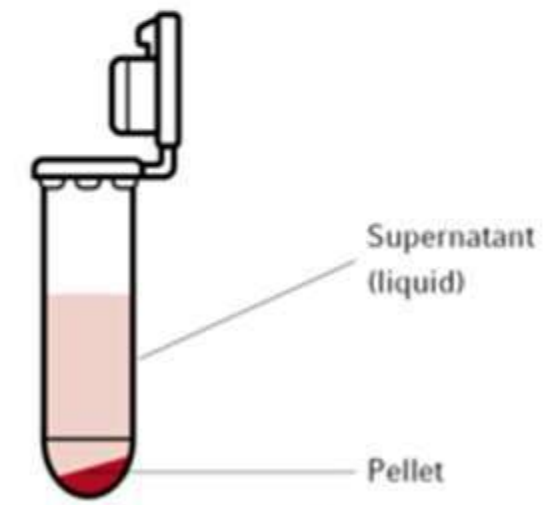


Fig. Centrifuge: General View, Centrifugation procedure and components separation

Principle of Centrifuge (Centrifugal Force)

to separate particles in a sample based on their density using centrifugal force.

- **Centrifugal force:**

The force that pushes outward when a centrifuge spins at high speeds. This force causes particles to move and settle.

- **Sedimentation:**

The process by which denser particles settle at the bottom of a sample tube.

- **Density gradient:**

The layers of particles that separate based on their density.

Most techniques requiring centrifugation will usually specify the required relative centrifugal force (RCF) expressed in gravity.

For example, an RCF of 2000 x G refers to a force 2000 times the force of gravity. Most centrifuge manufacturers specify both the RPM and G.

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

Where; **RCF** = relative centrifugal force.

r = radius from the shaft to the tip of the centrifuge tube.

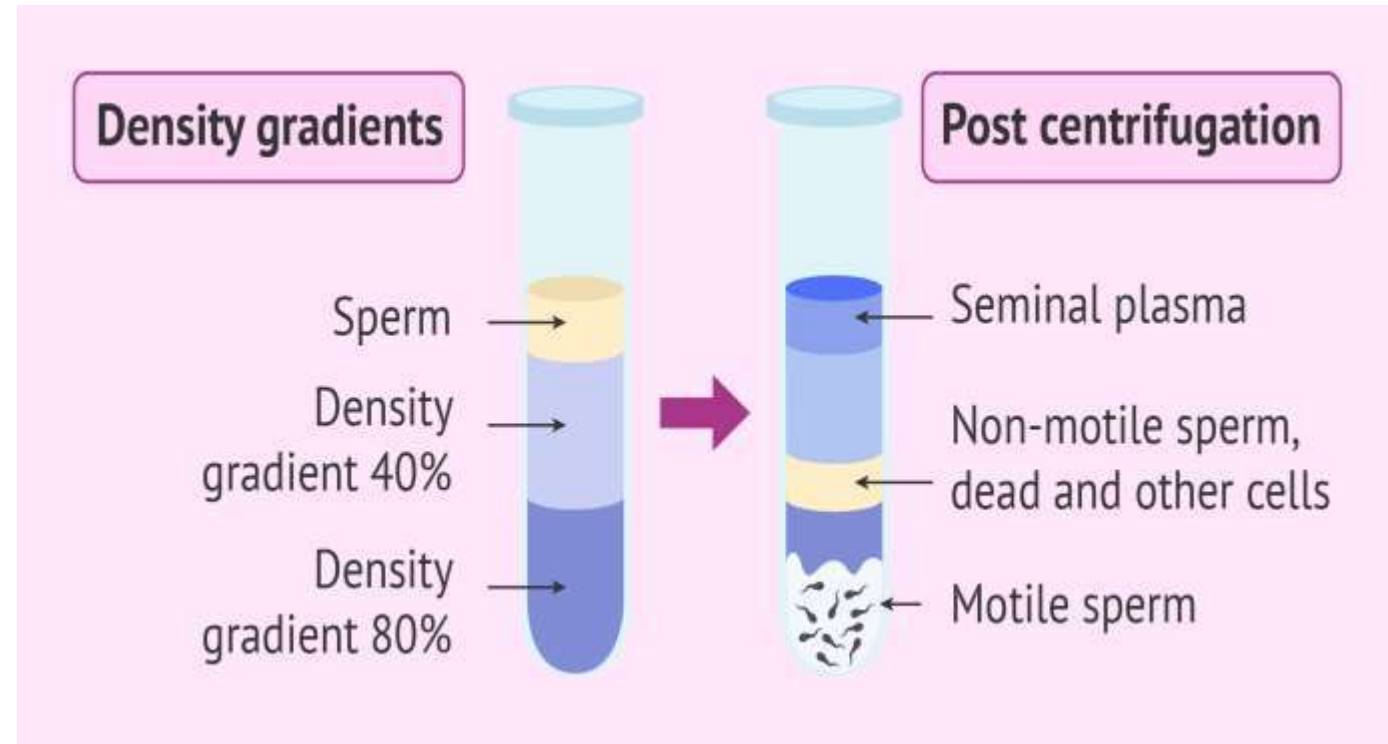
rpm = Revolution per minute.

g = Gravitational force.

Types of Centrifugation Techniques

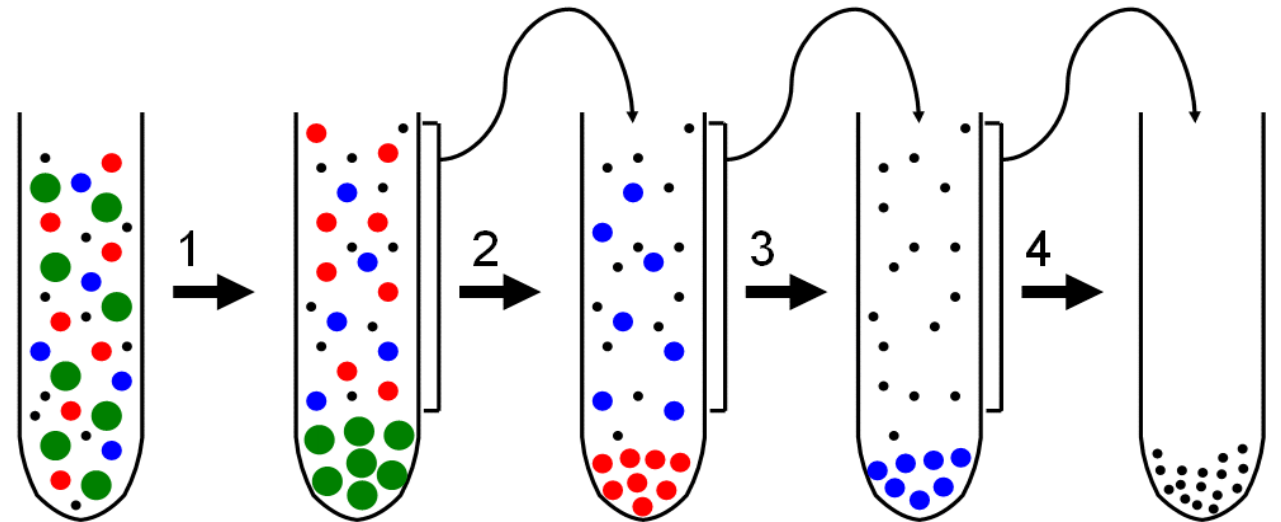
1. Density gradient centrifugation:

- separate substances based on size, shape, and density.
- Meselson and Stahl invented a specific type of density gradient centrifugation, called isopycnic centrifugation
- used a solution of cesium chloride to separate DNA molecules based on density alone
- Molecules settle down under a centrifugal force until they reach a medium with the density the same as theirs (**Density Gradient Reagents**)



2. Differential Centrifugation

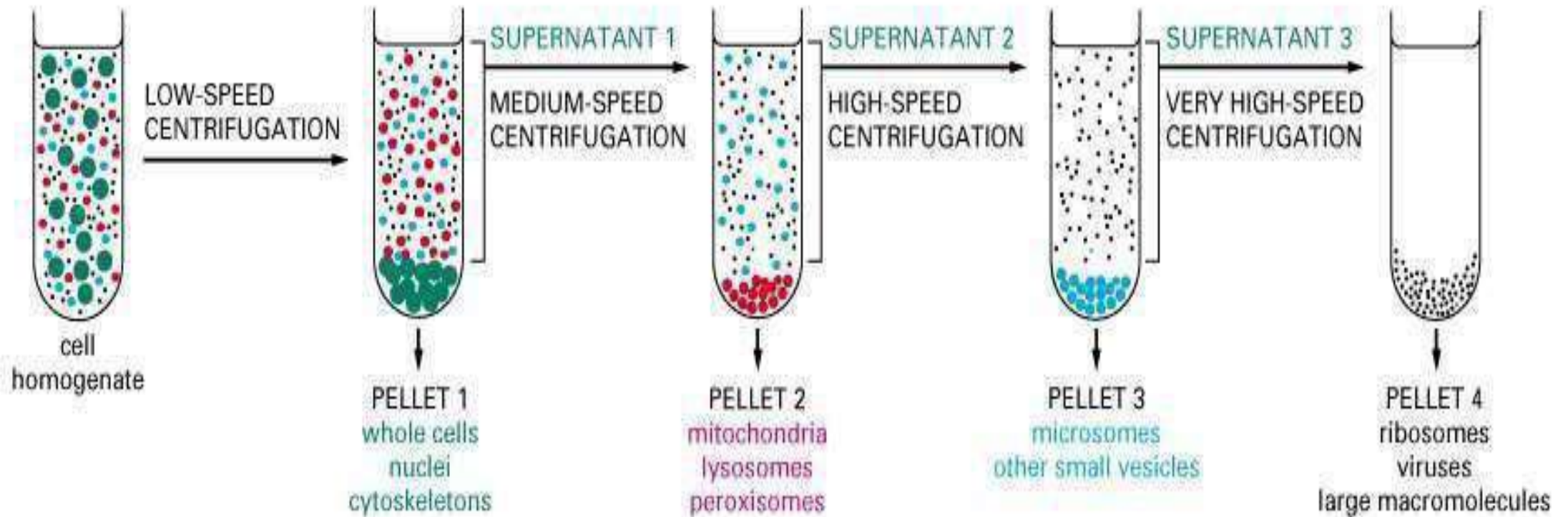
- Differential centrifugation is a common procedure in microbiology and cytology used to separate certain organelles from whole cells for further analysis of specific parts of cells.
- In the process, a tissue sample is first homogenized to break the cell membranes and mix up the cell contents.
- The homogenate is then subjected to repeated centrifugations, each time removing the pellet and increasing the centrifugal force.



DIFFERENTIAL CENTRIFUGATION

Repeated centrifugation at progressively higher speeds will fractionate cell homogenates into their components.

Centrifugation separates cell components on the basis of size and density. The larger and denser components experience the greatest centrifugal force and move most rapidly. They sediment to form a pellet at the bottom of the tube, while smaller, less dense components remain in suspension above, called the supernatant.



3. Ultra Centrifugation:

- Centrifugal force created by very rapid rotation (50,000 revolutions per minute or more).
- Produced in 1940 by SPINCO. An important tool in biochemical research .
- E.g. Nuclei from mitochondria in cell homogenates, and one protein from another in complex mixtures, lipoproteins and ultra-microscopic particles
- heat is generated during their operation, as a result of friction, so they are always provided with a refrigerated



Centrifuge Rotor

Rotating unit of the centrifuge, which has fixed holes drilled at an angle. Test tubes are placed inside these holes and the rotor spins to aid in the separation of the materials.



swing-bucket Rotor



fixed-angle Rotor



vertical rotor



Types of Rotor

Special types

1. Immunofuge or Serofuge:

Used in immunohematology. It is horizontal head centrifuge with a fixed tube size head and fixed speed. It is commonly used in blood bank.

2. Cytospin:

Horizontal head centrifuge having fixed speed and time. allow the cells in fluid phase to settle down on a glass slide. It is used for cytology.

3. Blood bag centrifuge:

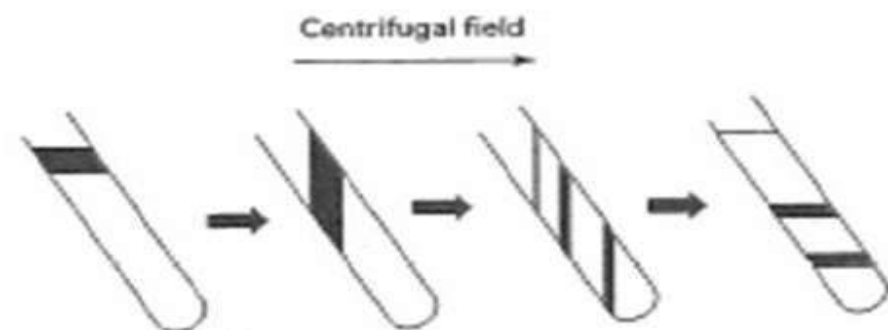
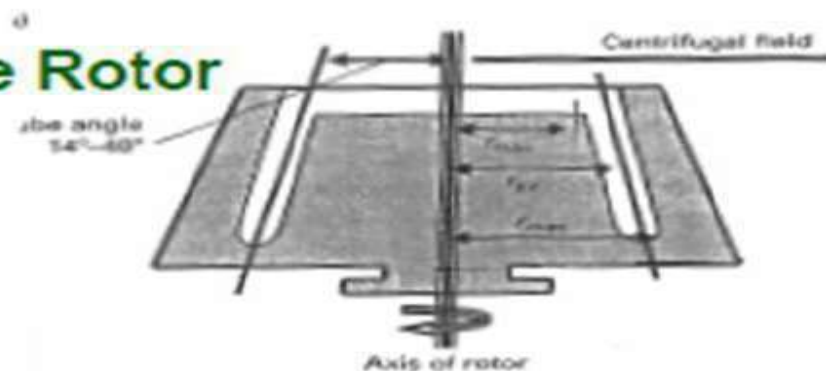
Horizontal head centrifuge but is provided with large buckets, used in preparation of blood components i.e. packed red cells, platelets and plasma etc.

4. Gerber centrifuge:

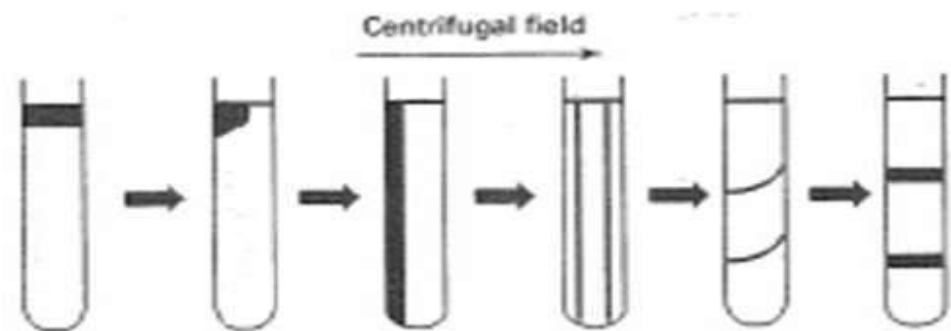
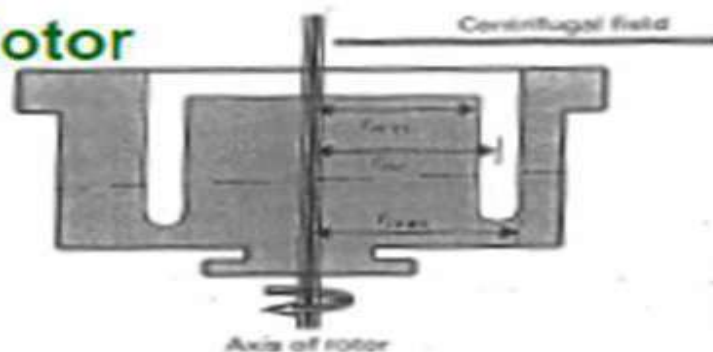
This is a special centrifuge. It can hold and spin the Gerber tube, a special glass tube for milk analysis.

Centrifuge Rotors (MBM3.3.2)

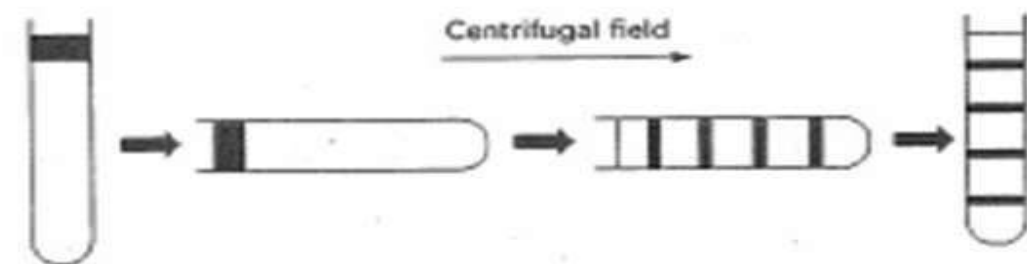
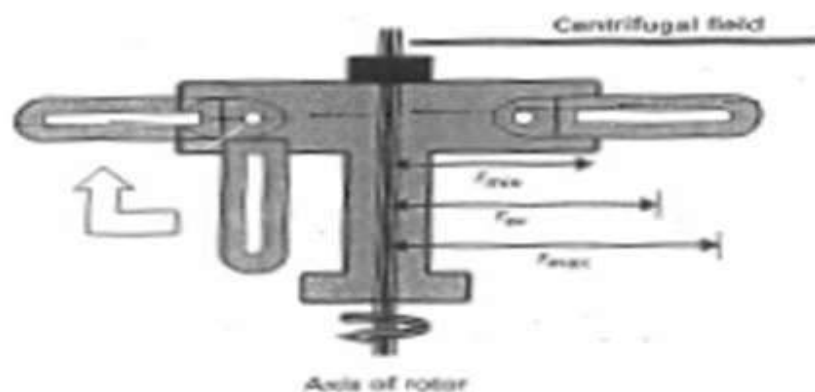
Fixed Angle Rotor



Vertical Tube Rotor



Swinging Bucket Rotor



Operation of the Centrifuge

- Tubes recommended by the manufacturer of the centrifuge should be used.
- The rotor must be properly balanced.
- Specimen tubes should be placed on opposite pans of a balance and equalized in weight.
- Tubes filled with water may also be used to equalize the weights.
- The total weight of each rack should not exceed the limits stated by the centrifuge manufacturer.
- Imbalance of the rotor causes wear and tear in the centrifuge and more frequent breakages of the tubes.
 - The lid should be closed and locked.

Operation of the Centrifuge

- Required time for centrifugation should be adjusted with the timer knob.
- Increasing spinning speed gradually is important
- The centrifuge should then be switched on and allowed to attain speed for required centrifugation force, which is set by speed / gravity knob.
- Lid should not be opened until rotor has completely stopped.

Maintenance of Centrifuge

- Cleanliness of a centrifuge is important in minimising the possible spread of infectious agents such as hepatitis viruses or mycobacteria.
- In case of breakage the racks and the chamber of the centrifuge must be carefully cleaned.
- Any spillage should be considered a biohazard and dealt accordingly.
- Broken glass embedded in cushions of the tube holders may be a continuing cause of breakage if cushions are not inspected and replaced in the cleanup procedure.

- The speed of a centrifuge should be checked at least once every 3 months, by stroboscopic light or a vibrating read external tachometer of known accuracy.
- The centrifuge timer should be checked weekly against a reference timer such as stopwatch and the difference should not be more than 10%.
- The temperature of a refrigerated centrifuge should be measured monthly under reproducible conditions and should be within 2°C of the expected temperature.

stroboscopic light As a flashing light, primarily used to create effects for a concert, or on an emergency vehicle. On a “continuous” mode, where the flashes are spaced in such a way that it's almost impossible for the naked eye to tell they are flashing.



A **tachometer** is an instrument which measures the rotation speed of a shaft or disk. It is designed to measure the revolutions per minute (RPM) of a moving object.



Centrifuge Safety

- Ensure that centrifuge bowls and tubes are dry.
- Ensure that the spindle is clean
- Ensure that the rotor is properly seated on the drive shaft.
- Make sure that tubes or containers are properly balanced in the rotor.
- Do not exceed the rotor's maximum runspeed.
- Close the centrifuge lid during operation.
- Make sure that the centrifuge is operating normally before leaving the area.
- Make sure that the rotor has come to a complete stop before opening the lid.