



Bioinstrumentation and Biostatistics

Microscopy and Centrifugation

By

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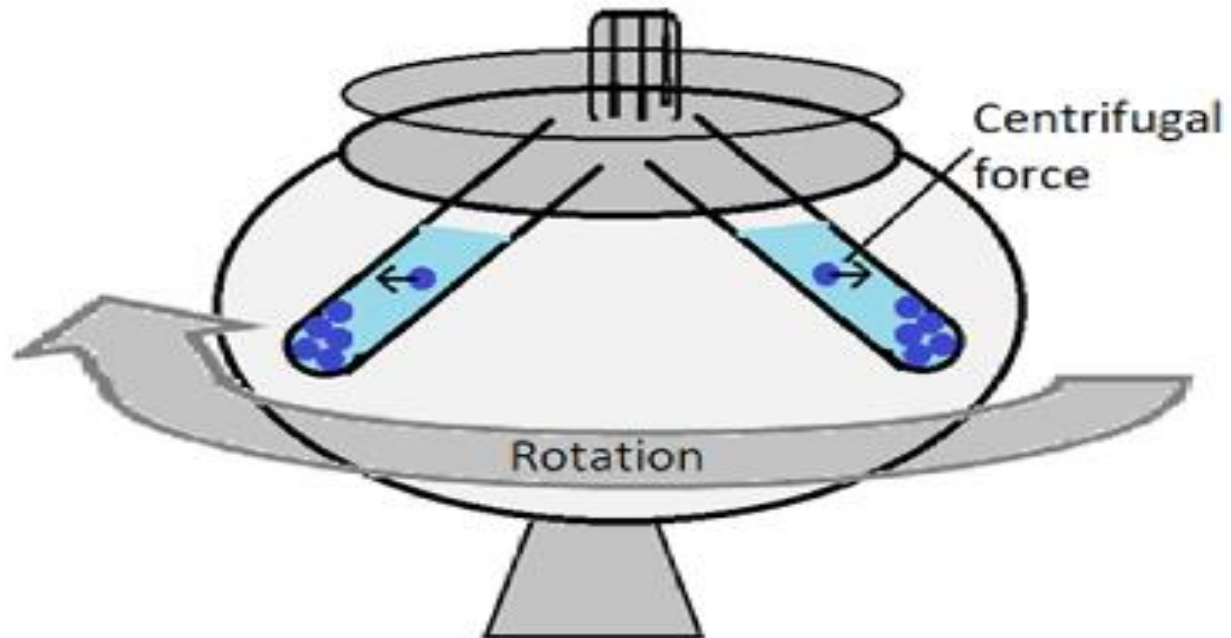
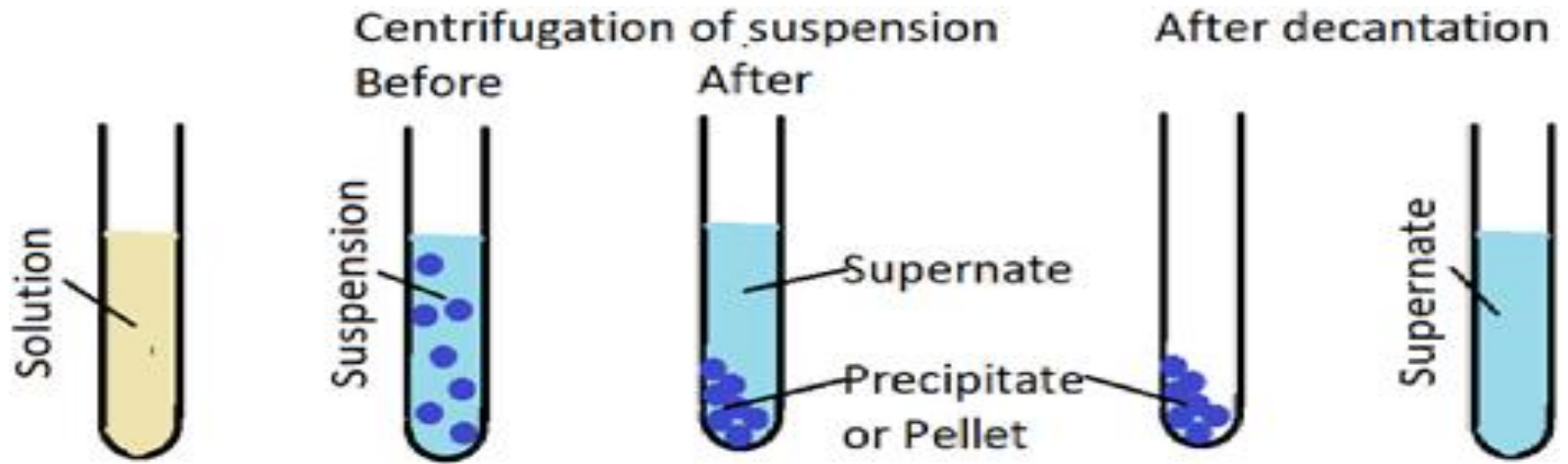
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Centrifugation

- Centrifugation is a process which separates or concentrates materials suspended in a liquid medium.
- The theoretical basis of this technique is the effect of gravity on particles in suspension. 2 particles of different masses will settle in a tube at different rates in response to gravity.
- The centrifugal force is proportional to the rotation rate of the rotor. The centrifuge consists of a rotor and closed in a refrigerated chamber by an electric motor.

Centrifugation process



Definition of Centrifugation

It is a unit operation working for separation separating the consequent present in a dispersion with the help of centrifugal force.

It is a technique which involves the application of centrifugal force to separate particles from a solution according to their size, shape, density, the viscosity of the medium and rotor speed.

Principle of Centrifugation

- The centrifuge involve the principle of sedimentation.
- 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.
- Sedimentation is a phenomenon where suspended material settles out of the fluids by gravity.
- 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.

Types of Centrifugation

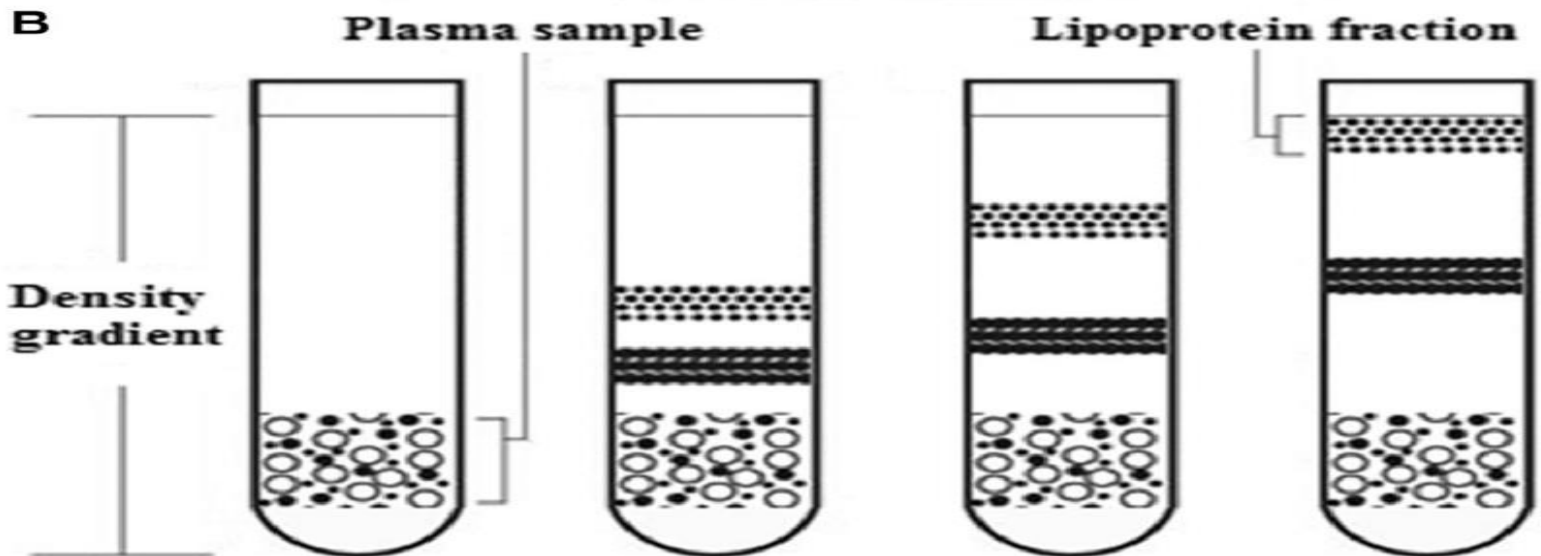
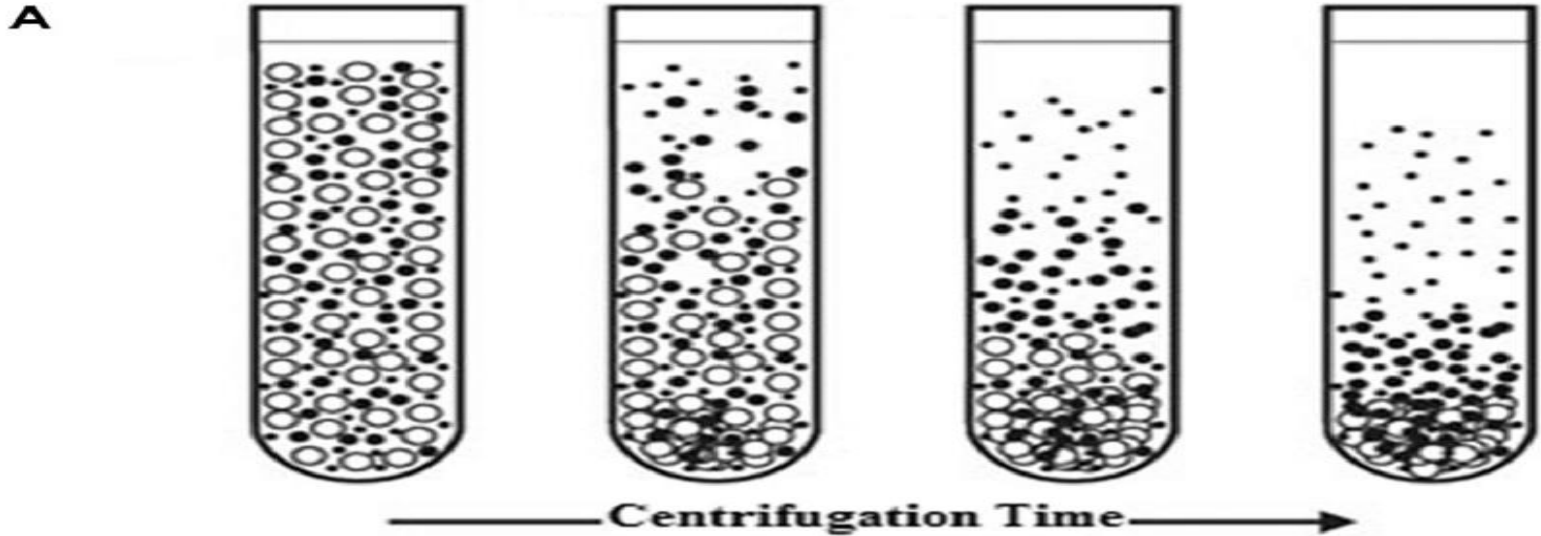
- **Differential Centrifugation**

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

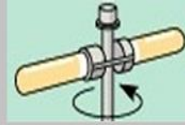
- **Rate zonal centrifugation**

In rate zonal centrifugation the solution has a density gradient. The sample has a density therefore greater than all the layers the solution. The sample is applied in a thin zone at the top of the centrifuge tube on a density gradient. Under centrifugal force, the particles will begin segmenting through the gradient. The particles will begin segmenting in separate zones according to their size, shape and density.

(A) Differential (B) Rate zonal centrifugation

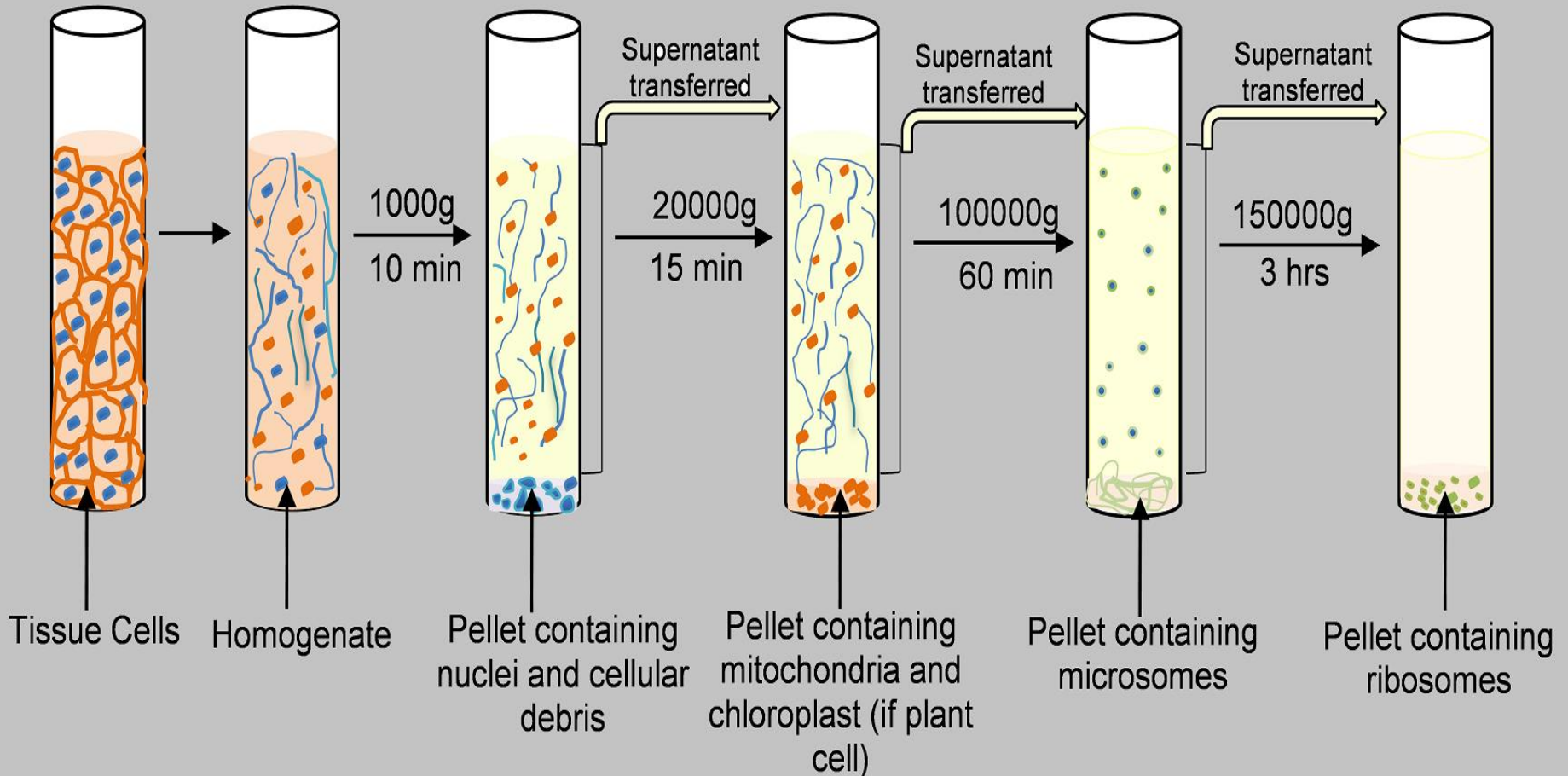


Differential centrifugation



Homogenization

Differential Centrifugation (in each step)



Types of centrifuges

- Analytical centrifuges

The analytical centrifuge is more or less like the preparative centrifuge.

- Preparative centrifuges

Preparative centrifugation is concerned with the actual isolation of biological material for subsequent biochemical investigations. preparative centrifugation methods can be divided into two main techniques depending upon the medium of suspension in which the separation is carried out in suspending medium which is homogenous are known as differential centrifugation while those carried out in a suspending medium having density gradients are known as gradient centrifugations.