Date: 24 November, 2020

Postgraduate course, Monsoon semester, 2020

VMC 609: Techniques in microbiology and immunology

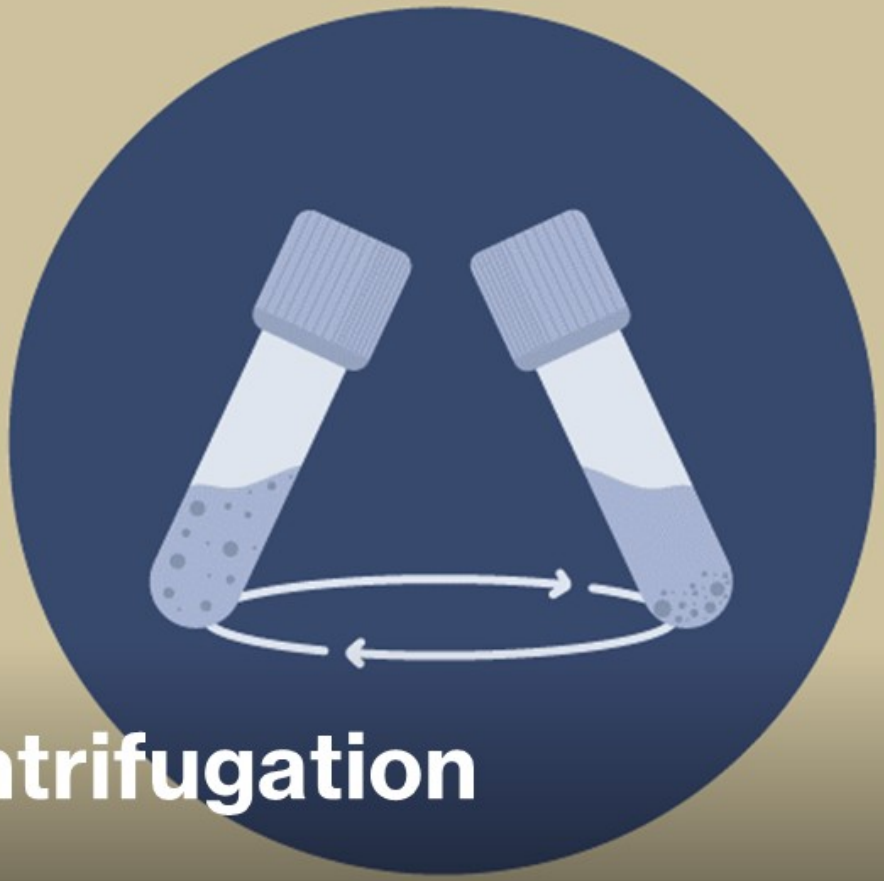
Topic: Concentration and purification of animal viruses (PART III)

DR MANOJ KUMAR

ASSISTANT PROFESSOR

DEPARTMENT OF VETERINARY MICROBIOLOGY

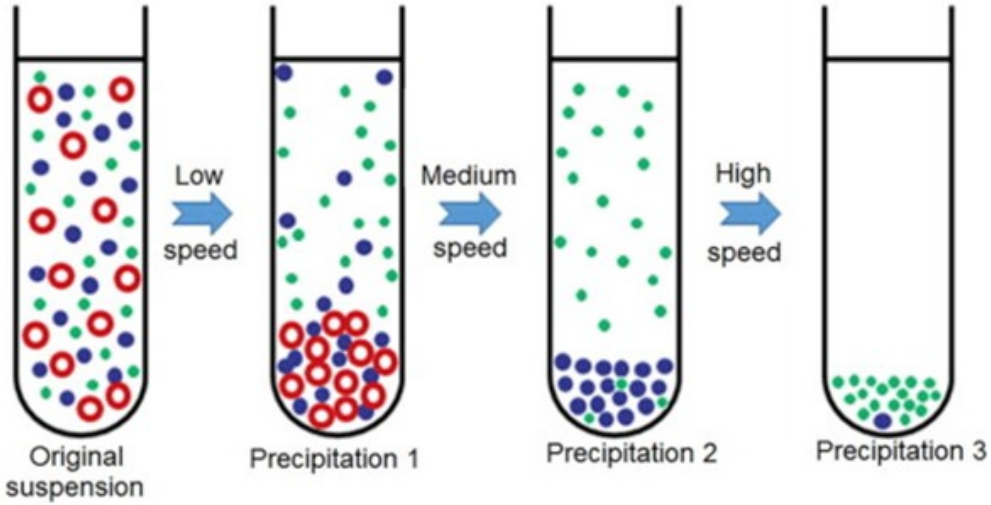
BIHAR VETERINARY COLLEGE, PATNA.



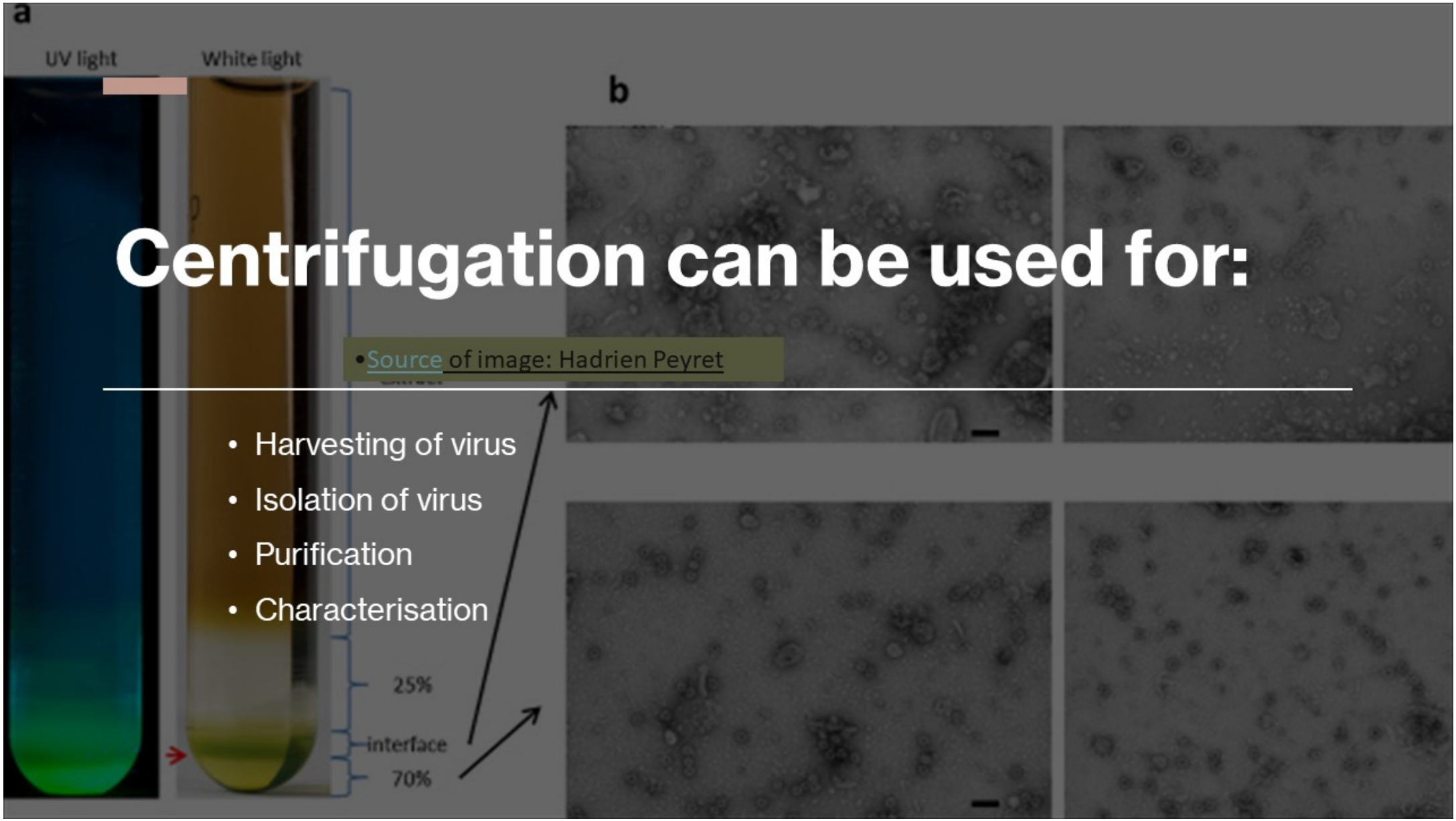
Centrifugation

Type of centrifugation

- **Low-speed centrifugation** - used for primary purification
  - enables cells and cell debris removal by sedimentation.
- **High speed centrifugation** - used for secondary purification



Source: PengsongLi

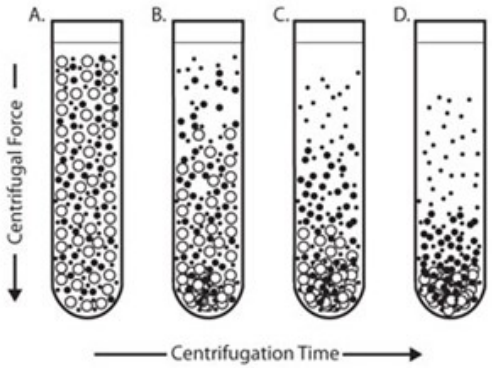


Centrifugation can be used for:

- Source of image: Hadrien Peyret

- Harvesting of virus
- Isolation of virus
- Purification
- Characterisation

Principles of ultracentrifugation

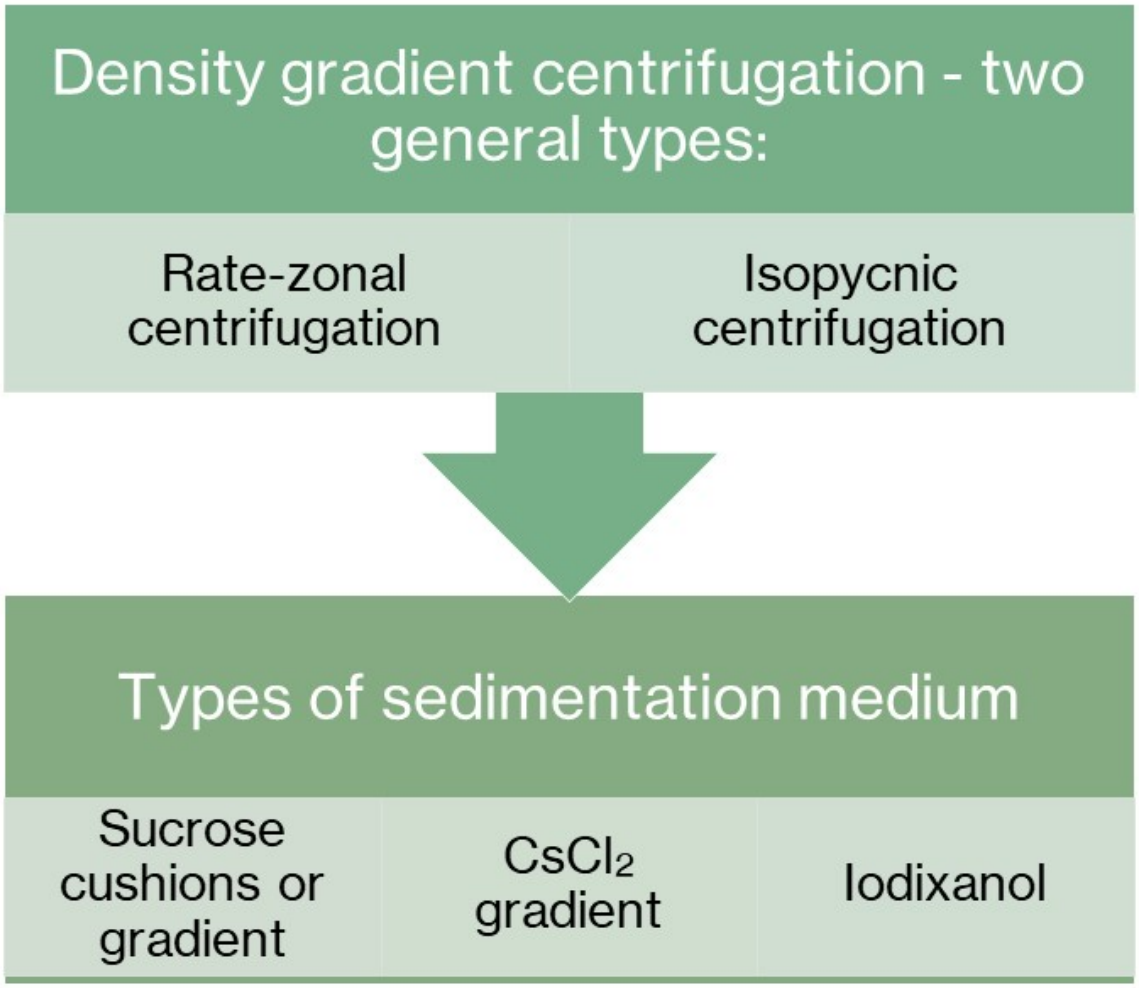


Particles of different densities or sizes in a suspension will sediment at different rates, with the larger and denser particles sedimenting faster followed by less dense and smaller particles..

These sedimentation rates can be increased by using centrifugal force.

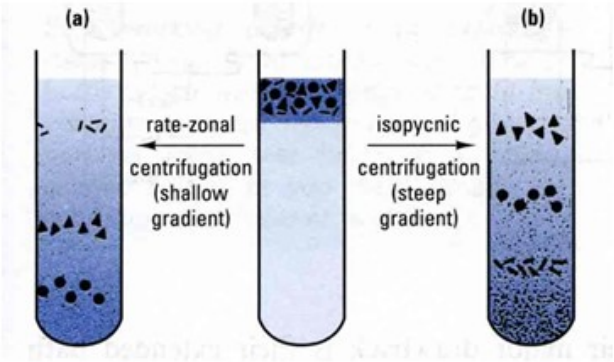
A suspension of cells subjected to a series of increasing centrifugal force cycles will yield a series of pellets containing cells of decreasing sedimentation rate.

Ultracentrifugation



- Based upon differences in
- -Size, Shape, Density of particles
- -Density and viscosity of a medium and centrifugal field
- Technique is used to separate enzyme, hormone, DNA-RNA hybrids, ribosomal subunits
- Can separate proteins with similar density and different molecular mass easily

Rate-zonal centrifugation



- Rate-zonal centrifugation
  - separates viruses primarily based on differences in size mass, shape & density of virus
  - result in different sedimentation rates.
  - a virus sample is layered as a narrow zone on the top of continuous density gradient.
  - Viruses separate from other impurities by forming a band that contains only the target virus
  - some viruses (e.g. herpesvirus, rotavirus etc.) may lose infectivity during density gradient centrifugation.

Isopycnic centrifugation

- Isopycnic centrifugation (Isodensity or equal density)
  - Depends totally upon buoyant density of the particle and not its shape and size and is independent of time, size of particle.
  - Separates viruses based solely on differences in the buoyant density rather than size
  - Virus sample are either overlaid on or placed under prepared density gradient (either continuous or discontinuous).

Contd...

- Alternatively, to ease the sample handling, some gradient materials (e.g. CsCl, Cs_2SO_4, iodixanol) is directly dissolved into virus sample and gradient are self-generated during ultracentrifugation.

- Viruses separate from other impurities by forming a band that contains only the target virus

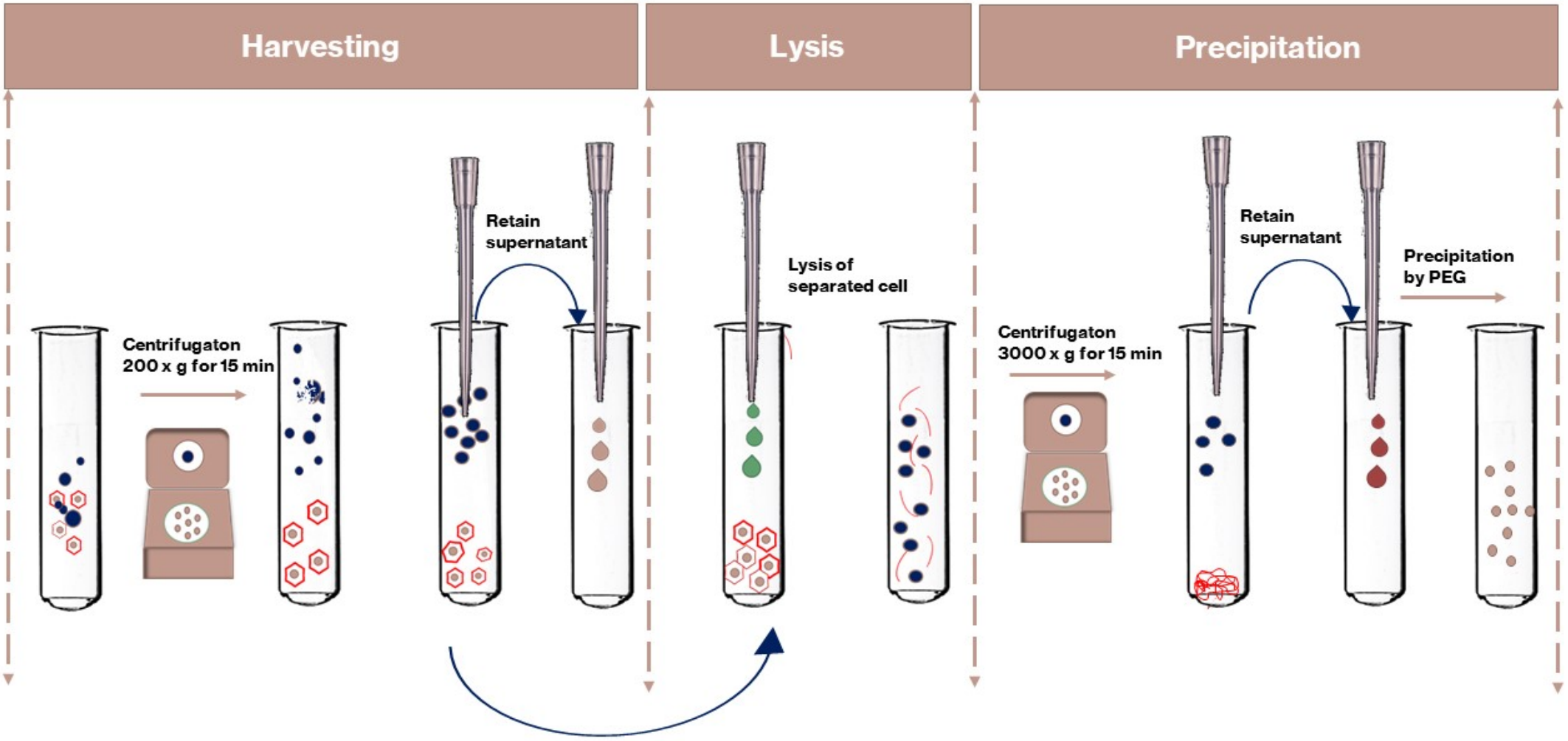
Contd...

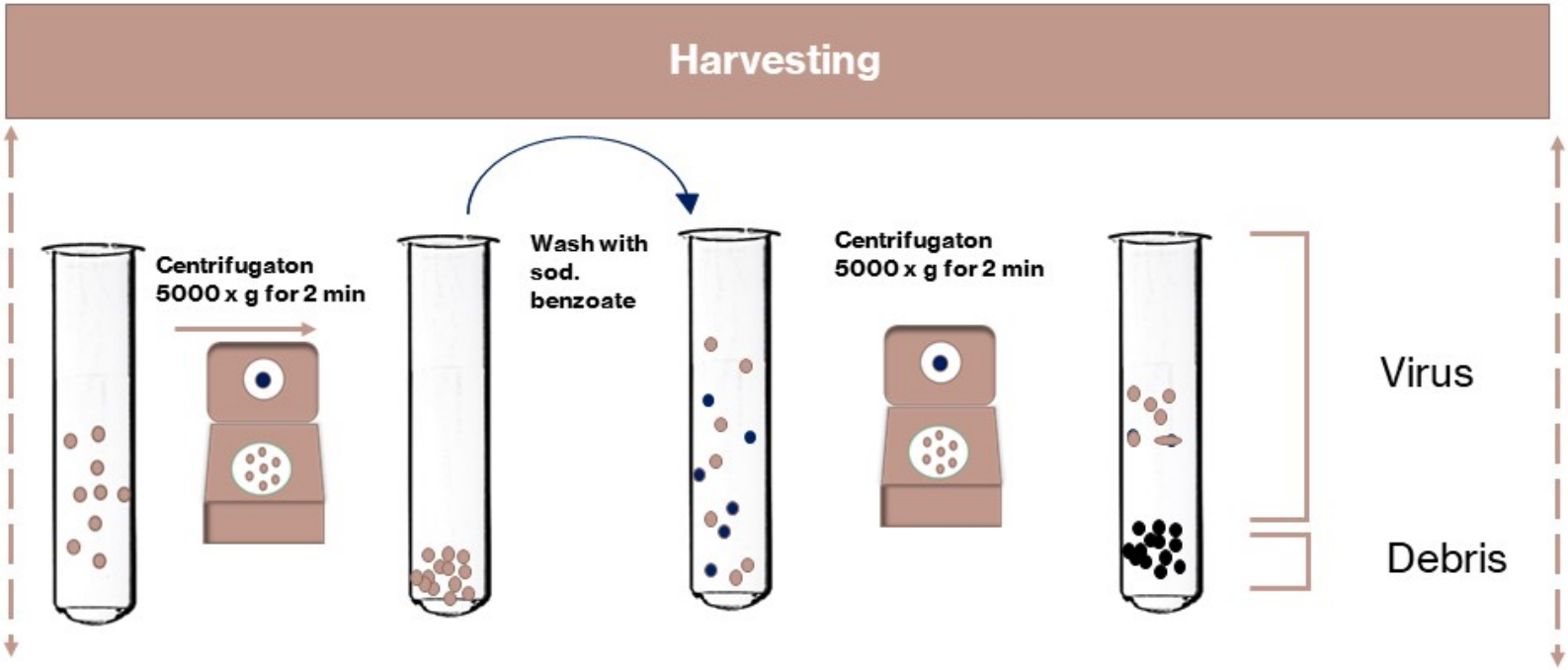
- Particles are distributed homogenously throughout the tube prior to centrifugation.

- During centrifugation the gradient is allowed to establish itself. Sample particles distribute and band in a series of zone at their respective isopycnic positions.

- Sedimentation occurs when buoyant density of the particle equals density of gradient.

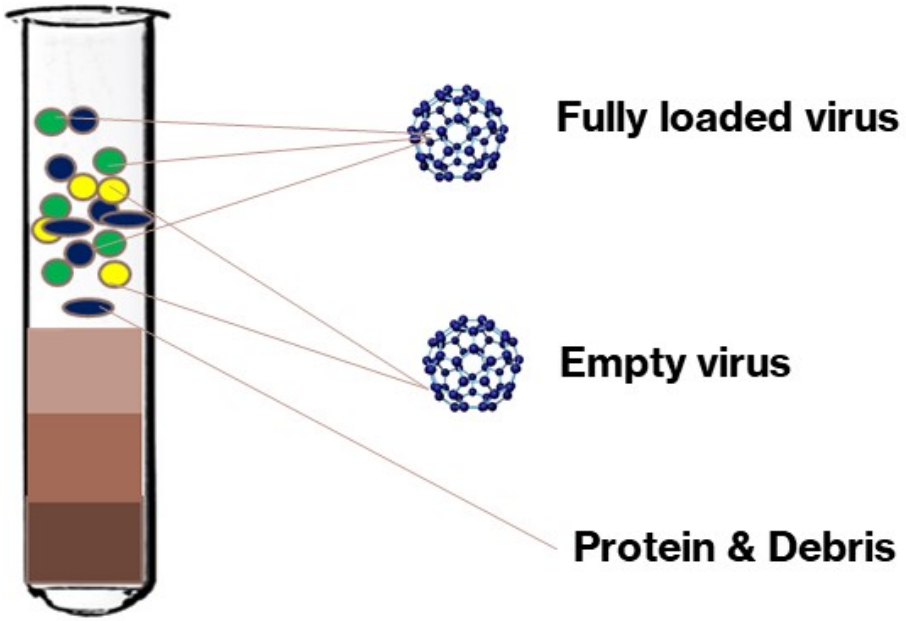
Rate-zonal centrifugation



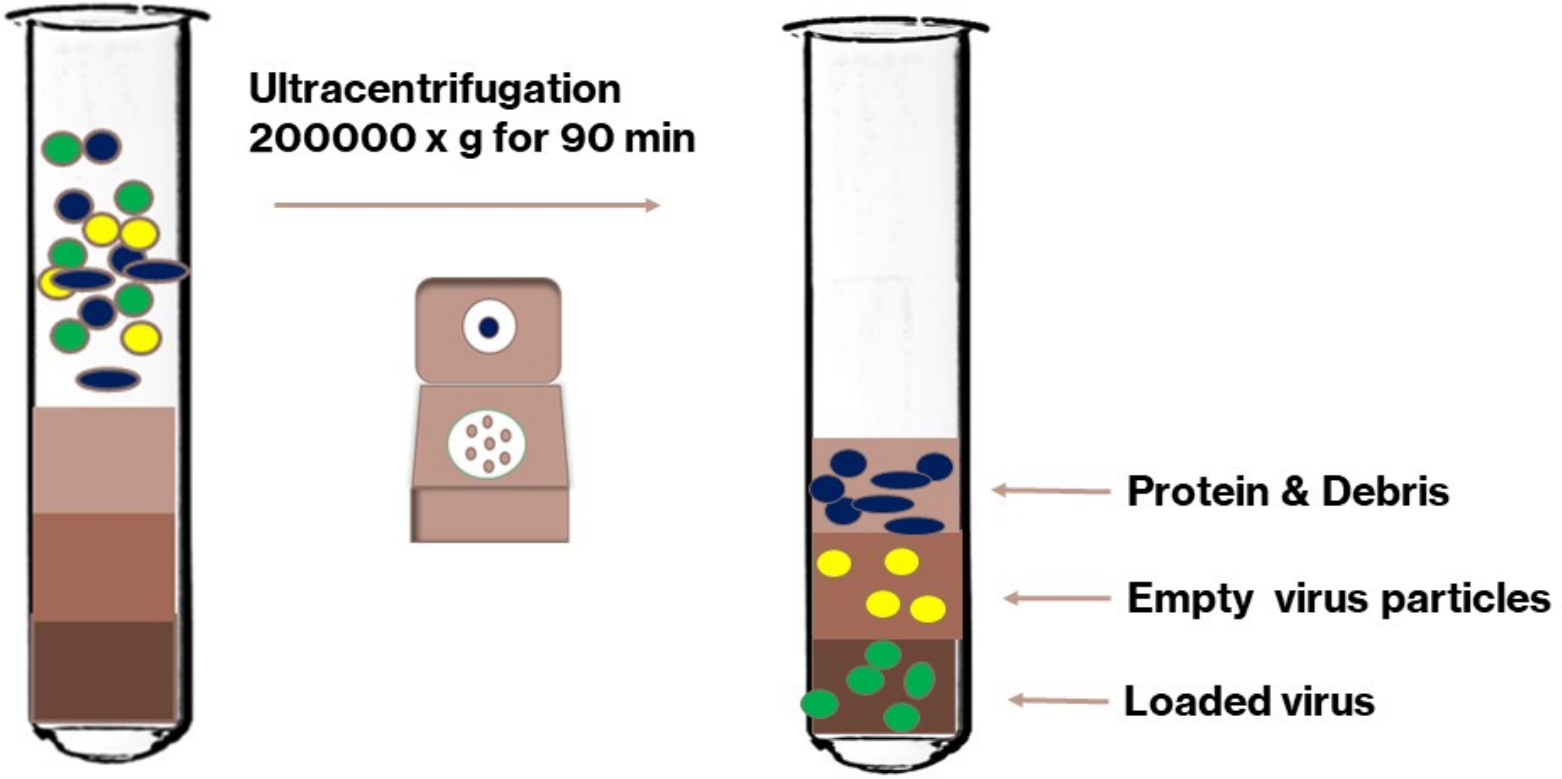

Harvesting
Centrifugaton
5000 x g for 2 min
Wash with
sod.
benzoate
Centrifugaton
5000 x g for 2 min
Virus
Debris

Density gradient centrifugation

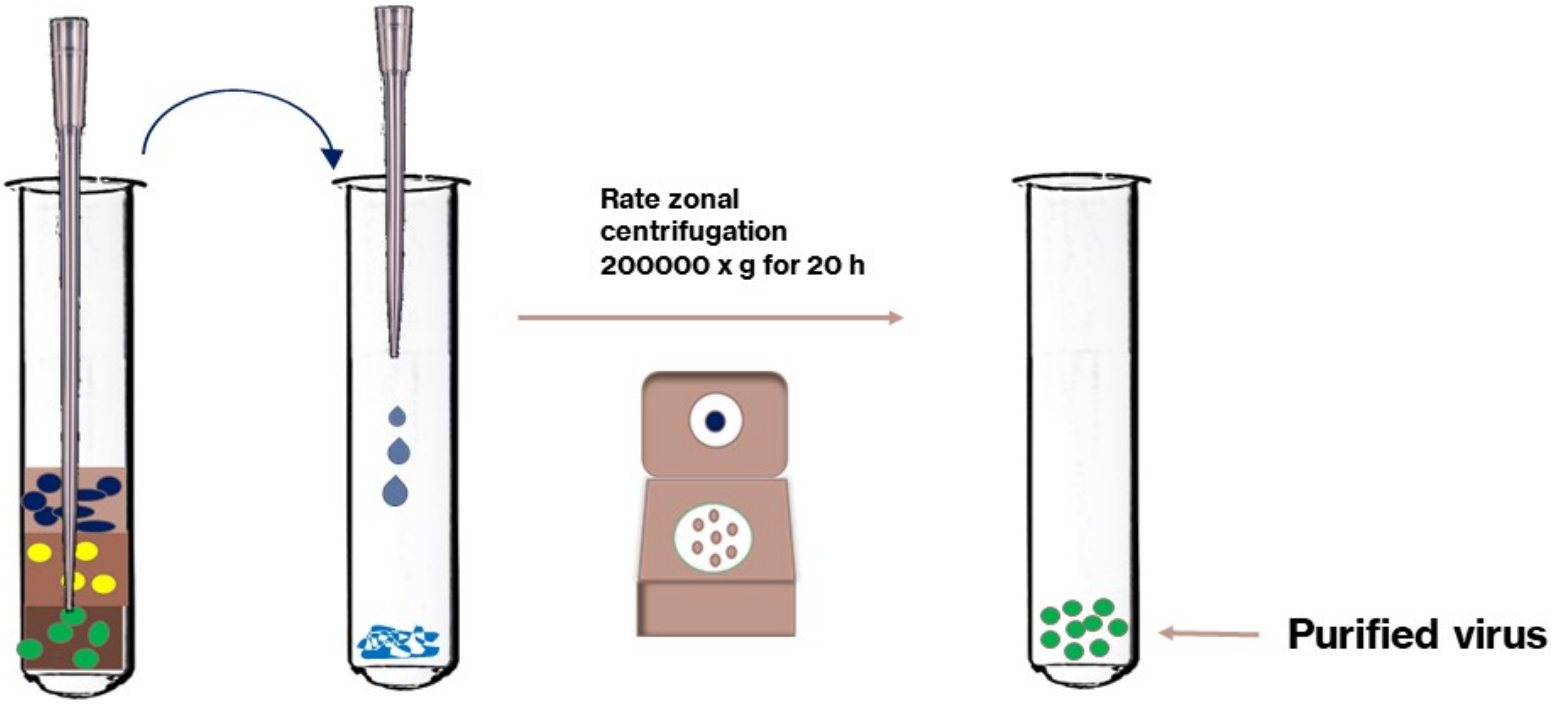
- Based on size and density of virus particle

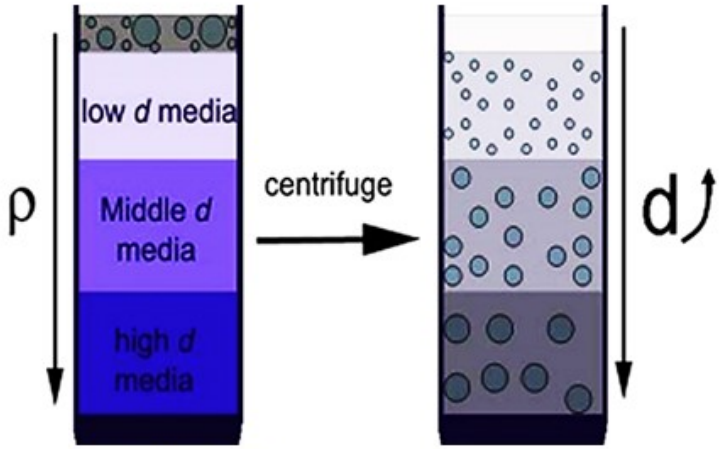


Density gradient centrifugation



Zonal centrifugation





Source: YunKuang

Protocol

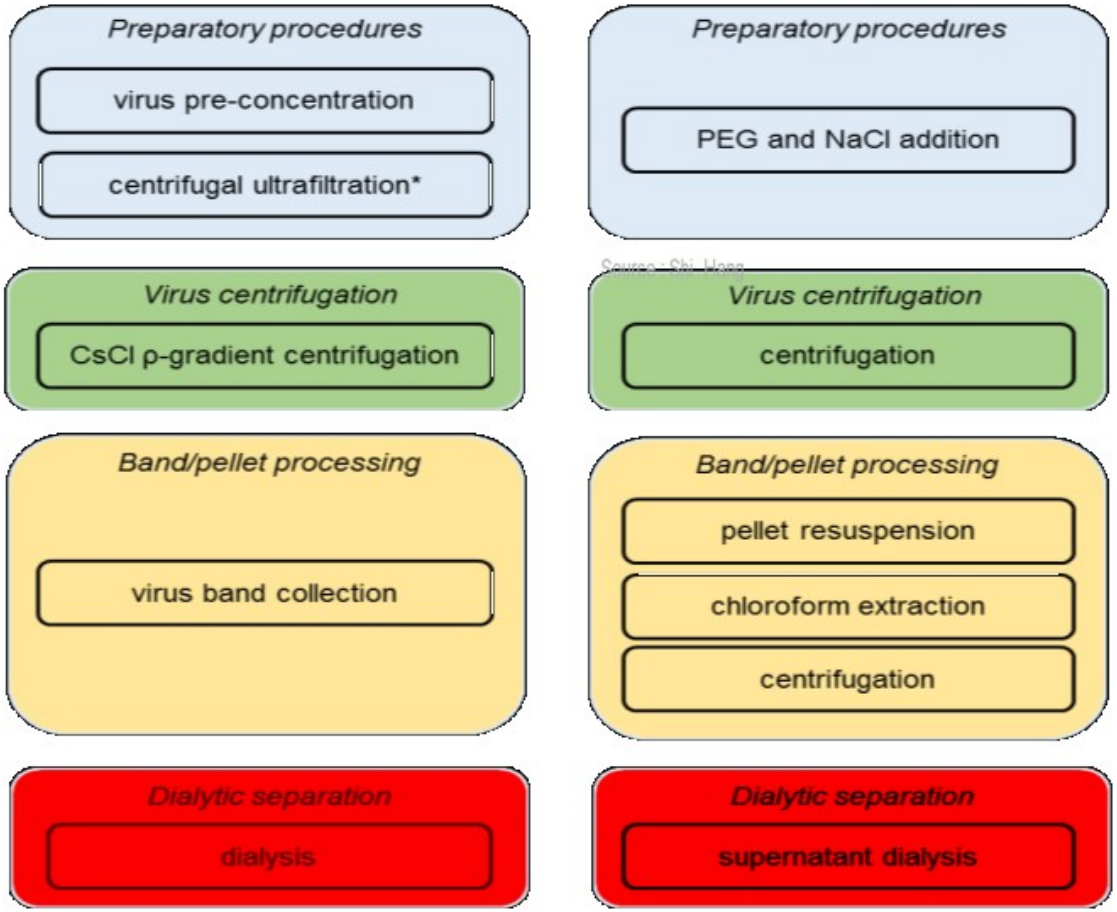
- Pre-concentration of virus prior to purification
- Pelleting via centrifugation at 28,000 g for 90 min at 4°C
- Resuspension of pellet in pH 7.6 buffer (10 mM Tris, 10 mM MgCl2; later in the text the buffer is denoted Tris-MgCl2) by overnight nutation at 4°C
- Preparation of CsCl solutions with different densities
- Loading of CsCl solution layer-by-layer into a tube with the highest density layer at the bottom and the lowest density layer at the top
- Capping of CsCl solutions with a 25% sucrose solution as a cushion.

Contd..

Protocol

- Resuspension of virus onto sucrose cushion
- ultracentrifugation at 30,000 rpm at 18°C for 3 h
- virus band will obtained.
- Collection of virus band with a syringe by puncturing the tube.
- Storage of purified virus at 4°C

Schematic diagram of purification with CsCl density gradient and polyethylene glycol precipitation



CsCl density gradient centrifugation

PEG precipitation

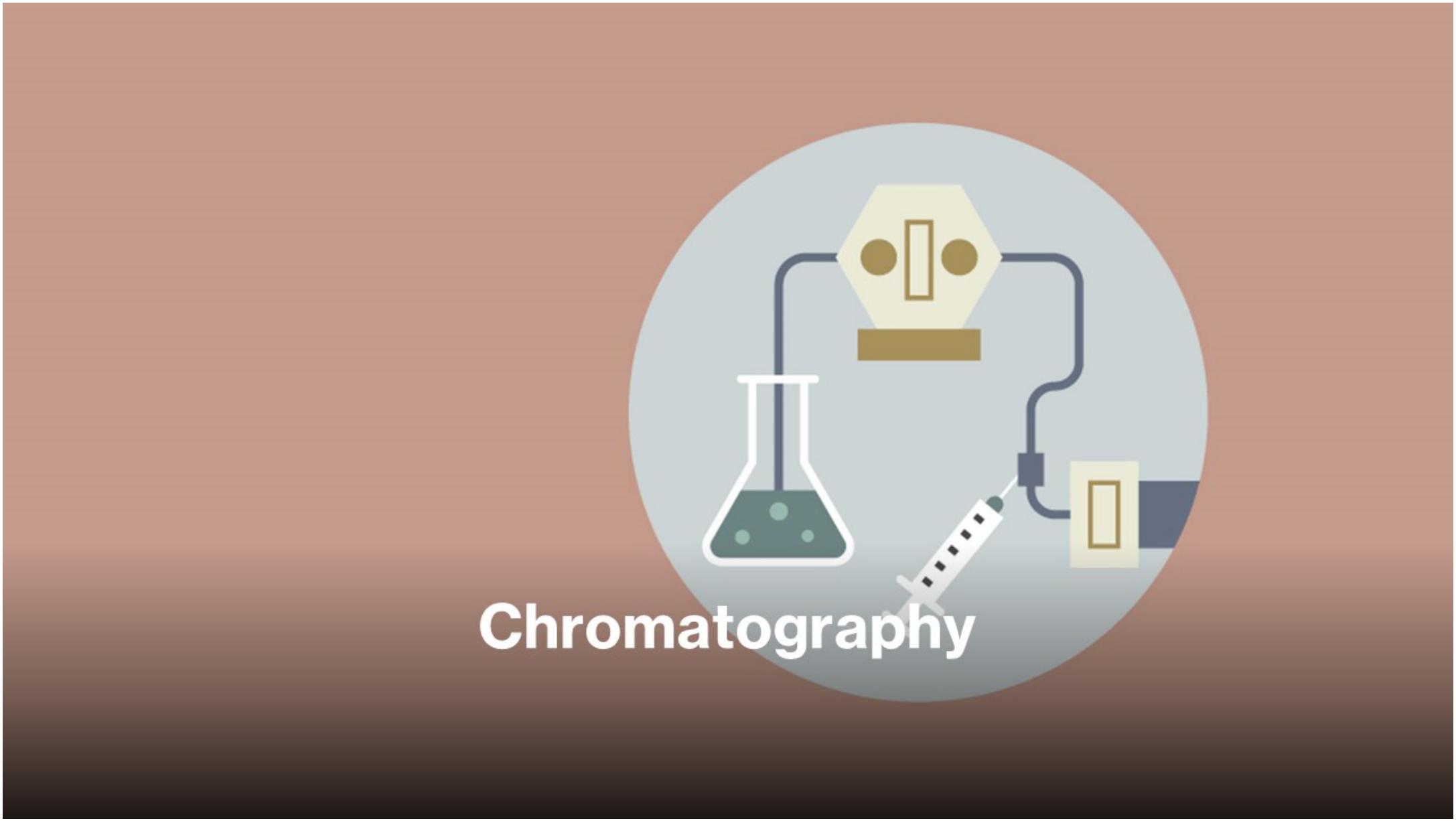

Pause (k)

Centrifugation

Viral harvesting

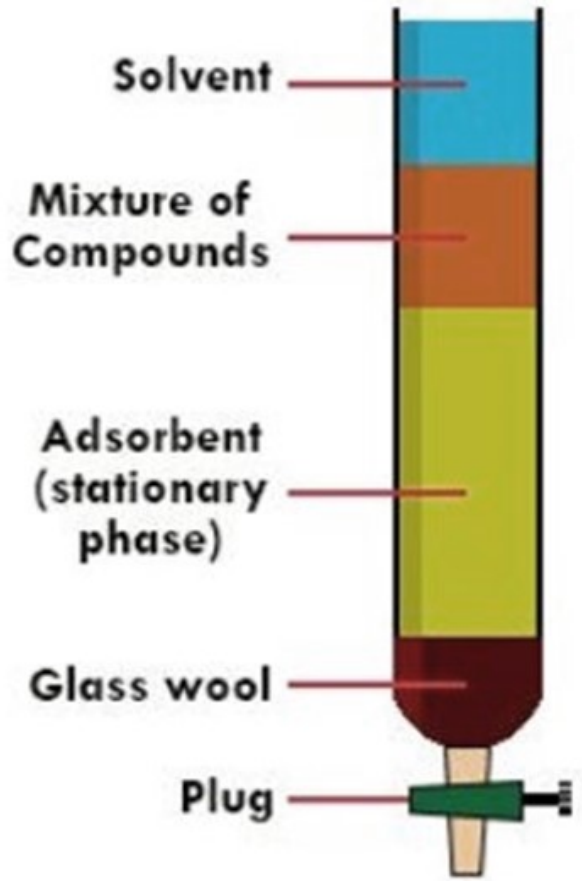
Isolation

Purification


Chromatography

Purification by Chromatography

- Chromatographic technique separates the target virus based on its interactions with chromatographic resin.
- Interactions depend on the choice of the resin and virus properties such as size, charge, hydrophobicity and ligand specificity
- All determine separation efficiency.
- Drawbacks - possible virus degradation due to harsh desorption condition and virus aggregation



Chemistry@TutorVista 2019

Thank you