Virus purification and concentration

Aims of purification

- 1-for studying physical properites of the virus
- 2-for studying chemical properites of the virus
- 3-for vaccine production
- 4-for removal of the impurities from the virus suspension

Problems involved in virus purification

- 1-inactivation of virus infectivity during purification procedures
- 2-un stability of some viruses
- 3-high titres of the virus is required for purification
- N.B the inveloped viruses is difficult to be purified due to their protective envelop which contain lipids derived from host cell

- The first step in purification is virus release from infected cell by disrupting the cell membrane through:
- 1-break down the infected cell membrane in homogenizer
- 2-disrubting the cell with ultra sonic vibration
- 3-alternative freezing and thawing
- This step is followed by different methods for purification

Methods of virus purification

- 1- Physical methods
- 2- Chemical methods
- 3-biological methods

Physical methods

- 1- Ultra filtration
- 2- Ultra centrifugation
- 3- Dialysis against PEG
- 4- Chromatography
- 5-Gel filtration
- 6- Electrophoresis
- 7- Electrofocusing

1- Ultra filtration

- The virus suspension may be purified by filtration through cellulose acetate membrane (CAM).
- The virus can be separated,concentrated as aresult of retention of large molecule that can not be penetrate the filter

2- Ultra centrifugation

- It’s the common method used for purification in which the sedimentation of the particles depending on their size , density and the viscosity of suspending fluid

Types of ultracentrifugation

- 1- Rat zonal UC
- 2- Equilibrum density UC
- 3- Differential UC
- 4- Analytical UC

1- Rat zonal UC

- Different sucrose gradient as 50%-40%-30%-20%-10% is formed in centrifugation tube
- The virus suspension is putted over it and during the centrifugation the particles is separated according to(the sedimentation co-efficient)
- At the end of centrifugation, the virus particles is obtained through a small whole puncture at the bottom of centrifugation tube

2- Equilibrum density UC

- In this method asolution of high salt density as calcium chloride or cesium chloride is used
- During the centrifugation, the virus particles are separated according to (the buoyant density)
- At the end of centrifugation each virus particle make zone according to its size
- At the end of centrifugation ,the tube is punctured from the bottom and the zones are collected

3- Differential UC

- By alternative low and high speed centrifugation
- Beginning by one cycle of low speed at 3000rpm which resulting in sedimentation of large non viral debris
- Then the supernatant is exposed high speed centrifugation at 30000rpm for 1hrs in which the virus suspension will be sedimented in form of pellet which could be resuspended in PBS 1:25

- Disadvantages :
- 1- obtaining of partially purified viruses
- 2- inactivation of some viruses
- 3- some viruses stick and aggregate together giving false result

4- Analytical UC

- Few mls of virus suspension is exposed to very high speed 60000rpm in which the virus particles will be banded at a zone corresponding to their size
- This zone is called boundary zone

3-Dialysis against poly ethylene glycol

- This method is used for concentration of smaller volumes of virus suspension by using sterile dialysis bag
- The bag is tied from one end then Filled with the virus suspension and then tied from the other end
- The closed bag is putted in abeaker and covered with PEG in which the water and other small molecules will pass through the dialysis bag into PEG leaving the virus and other non dialyzable molecules in the bag
- The concentration of the virus by this method reach 1:10 or 1:100.

4- Chromatography

- 1- Ion exchange chromatography:
- Components of different net charge are passed through gel matrix to which positive group as DEAE-sephadex or negative charge as DEAE cellulose are bound
- 2- Adsorption chromatography:
- Sample componenta are separated according to their different polarites
- 3-Affinity chromatography:
- The sample components are separated according to the biological specificity as Ag,Ab

5- Gel filtration

- The gel is used for separating molecules acc to their molecular size and weight

6- Electrophoresis

- Sample components are separated acc to differences in mws
- Sample is applied in the top of the gel and when electric field is applied the sample components will migrate into the matrix of the gel to distance corresponging to their size

7- Electrofocusing

- 1- Column electrofocusing
- 2- Flat-bet electrofocusing

Chemical methods

- 1-Precipitation
- A) Precipitation with ammonium sulphate
- B) Precipitation with poly ethylene glycol
- C) Alcohol precipitation
- 2- Extraction with solvents and detergants
- 3- Two phase aqueous polymer method
- 4- Formation of complex compound

Precipitation with ammonium sulphate

- Ammonium sulphate is used with the virus suspension by 50% concentration
- Then incubate at 4c for 2hr
- Then centrifugation at 3000rpm
- The sediment is resuspended in saline

Precipitation with poly ethylene glycol

- It give concentration ranged from(4-10%)
- Used for concentration of FMD(10%)
- Used for concentration of Influenza virus(8%)

Alcohol precipitation

- This method give efficient concentration but the purification is limited due to precipitation of the protein with the virus

2- Extraction with solvents and detergants

- Its used with nacked viruses by using organic solvents as ether, chloroform, sodium deoxy cholate and sodium dodecyl sulphate (SDS) and recently Arcton 113
- Arcton 113 used for purification of NDV and Viscular stomatitis

Two phase aqueous polymer

- The virus suspension is mixed with two polymer:
- 1- Poly ethylene glycol
- 2- Na dextran sulphate
- At 4c for 48hr leading to formation of 2layers of different volumes
- The virus present in the layer of small volume and can be obtained by precipitation with potassium chloride

4- Formation of complex compound

3-Biological method

- 1- Haemagglutination and elution
- 2- Serum neutralization
- 3- Digestion with enzymes