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VMC 609:Techniques in microbiology and immunology

Topic : Concentration and purification of animal viruses by chemical agents

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Concentration of virus

- Concentrations of virus in wild samples → too low to be detected → especially when molecular biological detection methods are employed

- Concentration methods are:
- useful to recover the low number of viruses from large volumes of samples
  - important in the virological analysis

Virus purification

- Viruses need to be purified for many studies of properties or structure of the virus requires to be distinguished from those of the host cells or culture medium

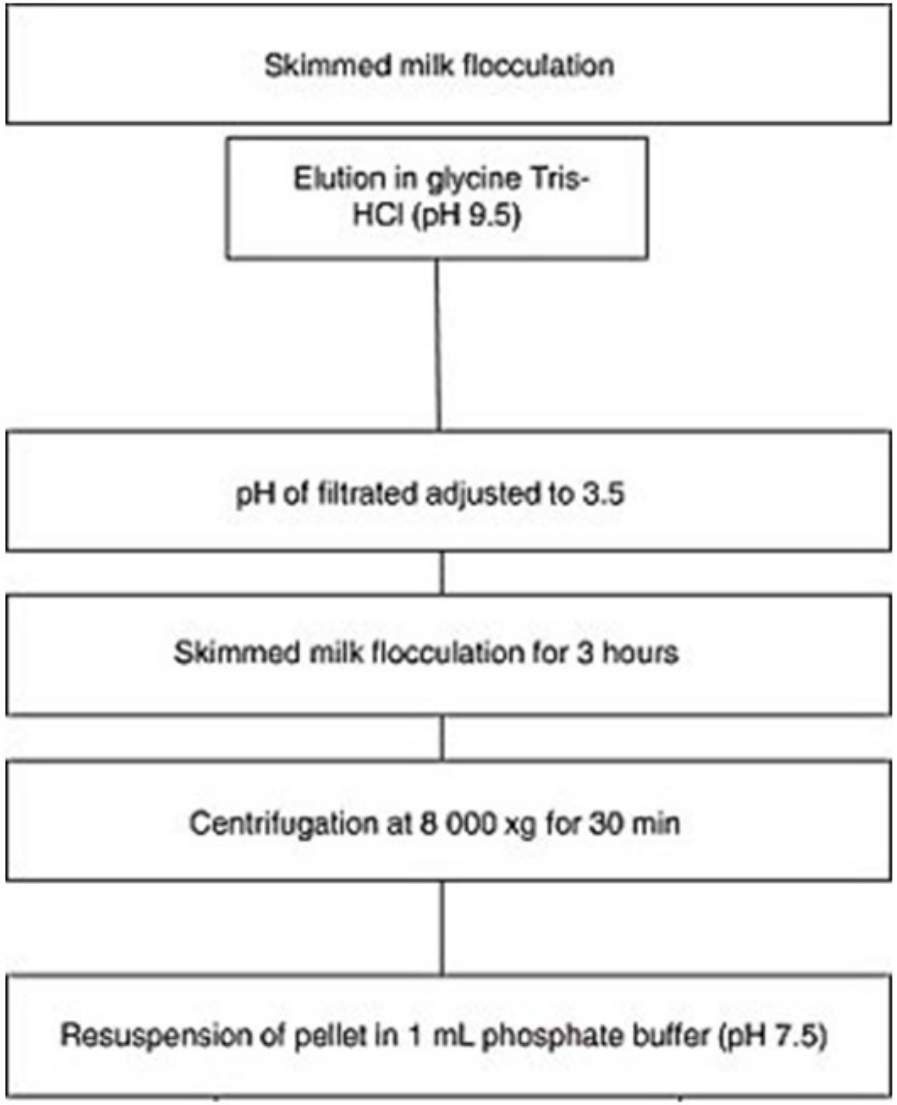
- physical separation of virus in a concentrated form → from the host cell milieu in which it has grown

- Purpose:
  - For analyses of structure of viral polypeptides
  - For study of function of membrane glycoproteins,

Concentration methods

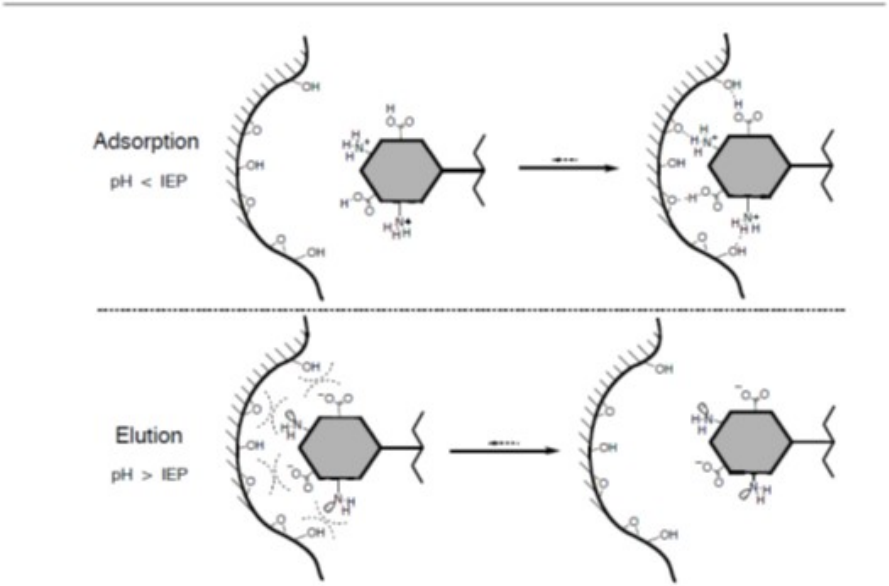
- Four different methods were used to concentrate virions from the clinical samples:
  - protein precipitation with PEG
  - organic flocculation with SMF(Skimmed-milk flocculation)
  - filtration with positively charged filters - MAF(Monolithic adsorption filtration), GW (glass wool filters).

Skimmed-milk flocculation



Monolithic adsorption filtration

- A flow-through concentration technique, called monolithic adsorption filtration (MAF)
- A macroporous epoxy-based monolithic column was prepared by direct polymerization in a small glass column to achieve a covalent binding to the glass surface.



Glasswool

- Glass wool is a cost-effective choice for concentrating viruses.
- Oiled sodocalcic glass wool packed into housings and used as columns
- Viruses adsorb to the surface of glass wool at neutral pH due to the positive charges and hydrophobic binding sites on the surface

PEG precipitation

- Concentration of viral suspensions by precipitation techniques is a useful starting point for virus purification
- **Advantages over other concentration methods**
  - Precipitation of viruses by high molecular weight polyethylene glycol-6000 (PEG) is an effective concentration method because :
    i. viruses are slowly precipitated in a cold
    ii. high-salt environment which protects them from chemical and physical denaturation
    iii. PEG precipitation is more gentle than physical concentration by ultracentrifugation or molecular sieve filtration

- PEG based concentration are also done in the cold, but ulltracentrifugation often packs the virions so tightly, even atop sucrose cushions, that they cannot be resuspended without significant loss of virus, and ultrafiltration requires magnetic mixing to keep the filter cleared and loses a great deal of virus trapped in the filter itself.

Protocol

1. Process should be started with a large-volume virus culture in which calf serum and other protein additives have been withheld from the maintenance medium.
2. At complete CPE, the cells and medium are harvested by scraping with a scrapper.
3. Pooled harvest is clarified by large volume, low-speed centrifugation at 12,000 rpm (15,000 g), for 20 rain at 3ºC)
4. Transfer the supernatant to a large beaker in an ice bath on a magnetic stirrer.
5. Slowly add NaCl to a final concentration of 2.3%, with constant but gentle stirring.

6. Slowly add PEG-6000 to a final concentration of 7.0%, also with constant and gentle stirring.
7. Cover the beaker and stir for about 1 h more to ensure complete solubilization of the PEG.
8. Transfer the beaker and ice bath to a refrigerator, and allow the virus (and other proteins) to precipitate overnight at 4°C.
9. Collect the precipitate by the same centrifugation method used for clarification (step 3).
10. Aspirate or drain the centrifuge bottles thoroughly to remove as much PEG as possible.

- Resuspend the precipitate in a small volume of TES buffer (0.01 M Tris-HCl, pH 7.2, 0.002 M EDTA, 0.15 M NaCl).

  ☛The buffer should be added at about 2 ml per centrifuge bottle and aspirated thoroughly with a syringe and 22-gauge needle.

- The suspension is then transferred to a clean tube, and each bottle is rinsed with an additional 1 ml of buffer which is added to the pooled suspension

- Finally, the PEG is removed (pelleted) by centrifugation of this pooled suspension at 13,000 g for 4 min at 23°C in a Microfuge.
- The supernatant now contains approximately 100-fold concentrated virus in isotonic TES buffer; the virus preparation may be considered enriched, but not purified.

Ultrafiltration-based method

- Millipore Ultrafree-15 Centrifugal Filters 100,000-MW cutoff (Millipore, Milford, MA, USA) were washed with 10 mL of bidistilled sterile water (four filters per sample), centrifuged at 2000 × g for 10 min and the filtered water was discarded.
- A 45- mL sample of sewage was transferred to three pre-washed filters (15 mL each), centrifuged at 2000 × g for 1 h at RT and the filtered volume was discarded.
- The viruses were eluted from each filter by using 4 mL of glycine buffer 0.25 N and pH 9.5.
- The eluted viruses (approximately 12 mL) were transferred to a sterile 50-mL tube, incubated for 30 min at 4 ◦C (vortexed every 10 min) and centrifuged at 3000 × g for 30 min at 4 ◦C.
- The supernatant was then recovered and transferred to a pre-washed filter.
- The filter was centrifuged at 2000 × g for 1 h at RT and subsequently mixed by vortex and centrifuged at 2000 × g for 2 min at RT. Finally, the volume retained by the filter was collected in 100 L.

Purification

- To obtain virus particles free from debris

- **Purification process :**
  i. Preparation of harvest
  ii. Methods of purification
  iii. Criteria for purity
  iv. Maintenance of harvest

Methods of purification

Methods of purification	Filtration
	Centrifugation
	Adsorption
	Precipitation
	Solvent extraction
	Chromatography

Thank you