



Tikrit University
College of Veterinary Medicine

Lect.8 Practical Virology

Subject name: Isolation and Purification
Viruses

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Lecturers link

Isolation Virus

to study of virus structure , reproduction of virus, vaccine production of virus , requires virus isolation and purification.

Methods of Virus isolation includes:

1-Embryonated eggs

2-Lab experimental animals

3-Cell culture

* Sample collection

1-Symptoms as criteria .

2-Animal viruses: Nasal swabs, stool samples....

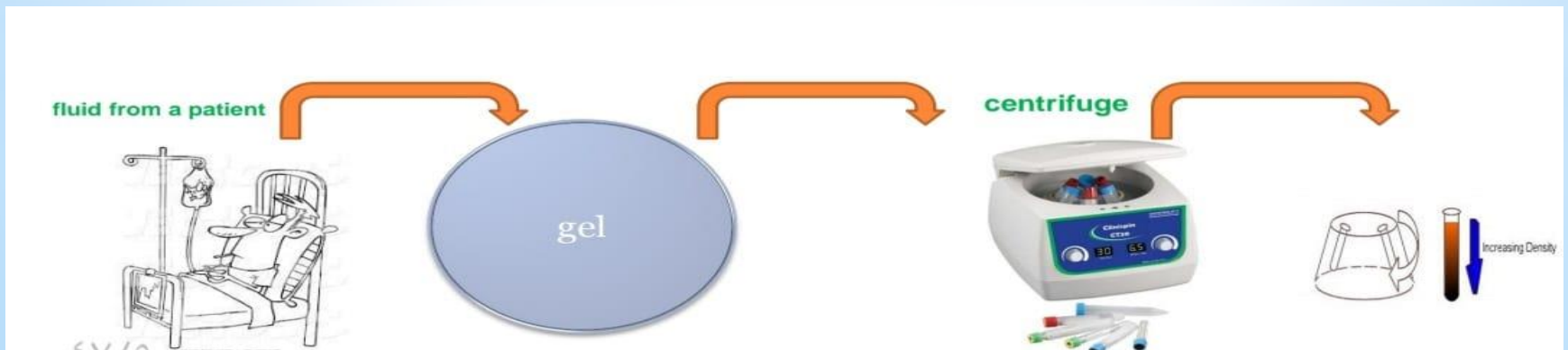
3- Usage of transport medium: eg Phosphate broth with 0.5% gelatin .

4-Concentration of viruses.

To isolate the virus, a heterogeneous sample (fluids from the patient or a culture) is taken and added to the agar.

Then spin in the centrifuge.

The sample contents settle into separate piles, or bundles, at different depths according to their density



Because all microorganisms have a distinct density, it is possible to obtain pure samples that contain only

Contains specific viruses and does not contain any other material.

There is only one way to confirm this: scanning with an electron microscope

If the microscopic image reveals contamination, this means that the sample contains some substances that have

For the same virus density, in this case, steps must be added to the isolation process until we obtain a sample

It contains only the virus.

Such as size and electrical affinity

* Isolating viral constituents

To isolate virus contents, viruses must be broken down into their component parts by addition

Special detergents to isolate the virus.

These molecules include the proteins that decorate and line the outer membrane and the contents of the inner nucleus:

DNA or RNA

* Separating the virus components

To separate these molecules from each other (Proteins, enzymes and nucleic acids)

We use an electrical separation device (electrophoresis)

As the electric field pulls the particles from during the gel so that the separation according to their weights (rather than density)

Some packages contain proteins others contain genetic material, either RNA or DNA.

* Virus purification

First of all, why is virus purification is needed?. The main reason behind isolation or purification of virus is to study the virus structure, reproduction and other aspects of their biology. There are four most widely used techniques.

Differential and Density Gradient

Centrifugation

An aqueous suspension of cell components and viruses is made by disrupting the infected cells in a buffer. Viruses can then be isolated by differential centrifugation; to separate the particles of different sizes, suspension is centrifuged at various speeds. With the help of gradient centrifugation, viruses can be purified based on their size and density.

* **Precipitation of Viruses**

Many proteins are purified through precipitation with concentrated ammonium sulfate. As the capsid of virus is a protein coat, viruses can also be purified with the same method. Viruses require specific concentration of ammonium sulfate to be precipitated. Initially the concentration of ammonium sulfate is set just below the precipitation concentration of viruses to remove the precipitated contaminants. After removing the precipitated contaminants more ammonium sulfate is added and the precipitated viruses are collected by centrifugation

Denaturation of contaminants

Generally, viruses are less easily denatured than many normal cell constituents. In this method contaminants are denatured and precipitated with heat or a change in pH to purify viruses. Solvent treatment with organic solvents like butanol and chloroform is also used to purify some viruses.

* **Enzymatic digestion of cell constituents**

Cellular proteins and nucleic acids can be removed from many virus preparations through enzymatic degradation because viruses are more resistant from the attack of nucleases and proteases than free nucleic acids and proteins

* Thank you all