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Commentary

Multiple Stages of Purifying Viral Components

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DESCRIPTION

The essential components of infectious viral particles are nucleic acid (the genome) and protein. In addition, all enveloped viruses contain lipid in the envelope and carbohydrate in their glycoprotein peplomers (as well as that in the nucleic acid). Virus purification techniques involve filtration, dialysis, precipitation, ultracentrifugation, chromatographic and flow field-flow fractionation methods that are optimized and used together and sequentially in different combinations.

Purifying viruses and their components

Laboratory-grown viruses must be purified to remove impurities from the host cells in order to be used for future research. The techniques frequently offer the benefit of concentrating the viruses, making it simpler to study them.

Centrifugation

To cleanse viruses, centrifuges are frequently employed. Ultracentrifuges, which have a high speed of about 100,000 rpm, are powerful enough to concentrate viruses, in contrast to low speed centrifuges, which have a top speed of just 10,000 rpm. Differential centrifugation makes use of this power disparity. This technique involves using low speed centrifugation to separate a viral combination from the larger, heavier impurities. High speed centrifugation is then used to concentrate the viruses, which are tiny and light and are first left in suspension. After differential centrifugation, virus suspensions frequently contain contaminants from the procedure's inability to remove debris with the same sedimentation coefficient in these circumstances, buoyant density centrifugation-a variation of centrifugation is performed. Using this technique, dense solutions of sugars or salts that form a density gradient, from low to high, in the tube during the centrifugation are once again centrifuged at extremely high speed for several hours with the viruses collected from differential centrifugation. Preformed gradients, which meticulously layer solutions of progressively lower density on top of one another, are sometimes utilized. The viral particles produce distinct layers of, frequently visible, concentrated viruses in the tube because they are unable to sink into solutions that are denser than they are, much like an object in the Dead Sea. Since it is relatively innocuous but readily self-forms a gradient when centrifuged at high speed in an ultracentrifuge, caesium chloride is frequently employed for these solutions. A technique known as buoyant density centrifugation can also be used to separate the proteins and nucleic acids that make up viruses.

Electrophoresis

Rotavirus proteins electrophoresed on polyacrylamide gels as well as stained with Coomassie blue. Electrophoresis is the process of separating molecules according to their electric charges. This technique can be used to separate and purify viruses and all of their constituent parts. Typically, a supportive medium like agarose or polyacrylamide gels is used for this. Using stains like coomasie blue for proteins or ethidium bromide for nucleic acids, the separated molecules are made visible. Occasionally, the viral components are made radioactive prior to electrophoresis, and they are then visualized on photographic film in a procedure called autoradiography.

Sequencing of viral genomes

Sequencing is one of the primary virology methods because most viruses are too small to be seen under a light microscope, making it difficult to identify and analyse them. In scientific and clinical research, as well as for the detection of newly emerging viral illnesses, molecular epidemiology of viral pathogens, and drug-resistance testing, viruses are sequenced using conventional Sanger sequencing and Next-Generation Sequencing (NGS). In GenBank, there are more than 2.3 million distinct viral sequences. The most used method for producing viral genomes is now NGS, which has superseded conventional Sanger. A key technique in viral epidemiology and viral categorization is viral genome sequencing.

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Cloning

Cloning can be employed in place of virus growth when pure viruses or viral components are required for diagnostic procedures or vaccinations. The severe acute respiratory syndrome coronavirus 2 RNA sequence made it possible to produce tests promptly during the beginning of the COVID-19 pandemic. Cloning viruses and the parts of viruses can be done using a number of tested techniques. The most often used cloning vectors are plasmids that have undergone laboratory modification (small circular molecules of DNA produced by bacteria). A small portion of the viral nucleic acid is incorporated into the plasmid, which is then repeatedly replicated by bacteria. Without the need for native viruses, viral components can then be created using this recombinant DNA.