

Influenza virus purification using the Thermo Scientific Sorvall TCF-32 and CC 40 large-scale ultracentrifuge systems

Key words: *Virus, density gradient technique, continuous flow ultracentrifugation*

Introduction

To produce viral vaccines, it is necessary to purify the virus or antigen from the culture media. This purification allows for the removal of contaminating particles and proteins which are not desired in the final product. Density gradient centrifugation and ultracentrifugation have been used for many years to isolate viruses.

One centrifugation technique that is used in purifying and concentrating viral particles is isopycnic density gradient separation. Using this technique, viral particles are “banded” in the gradient solution that has the same density as the particle. This is also referred to as equilibrium centrifugation.

Purification of the viral material is achieved by choosing the proper density gradient material and concentration. The density gradient solutions are selected based on the density of the viral particles and contaminants, and the chemical compatibility of the gradient solution. These techniques are widely used in standard tube/bottle centrifugation.

However, commercial viral vaccine production demands high quantities of virus to be processed. Incorporating continuous flow centrifugation with density gradient techniques, it is possible to purify large amounts of virus while minimizing labor involved in the process.

The Thermo Scientific™ Sorvall™ CC 40 continuous flow ultracentrifuge system can purify and harvest research, pilot and production volumes of culture fluid. The Thermo Scientific TCF-32 rotor system, used for research or pilot production, can process up to 15 L, and the Sorvall CC 40 can process production volumes up to 100 L.



Figure 1. The Thermo Scientific Sorvall CC 40 continuous flow ultracentrifuge system.



Figure 2. The Thermo Scientific TCF-32 continuous flow rotor.

These case studies discuss the use of isopycnic density gradient separation using the TCF-32 and CC 40 systems for the purification and concentration of influenza viral particles from eggs.

Annually, millions of chicken egg embryos are used for vaccine production. An isopycnic separation is performed using a sucrose gradient to remove egg protein and debris from viral particles.

Research scale

The TCF-32 system has a top speed of 32,000 rpm (102,000 x g), a maximum flow rate of 9 L/hr with a sediment capacity of 430 mL (or 940 mL with the accessory core). It is ideal for viral separations in vaccine production. The capabilities of the system include simple pelleting; concentrating samples on a cushion gradient and density gradient separations.

The system includes an autoclavable titanium rotor body, sealing attachment and lubricating unit.

Production scale

The Sorvall CC 40 system has a top speed of 40,000 rpm (118,000 x g), and a maximum flow rate of 45 L/hr. There are several cores available that offer flexible sedimentation capacity and application options. Capabilities also include simple pelleting, concentrating samples of a cushion gradient, and density gradient separations.

The control unit includes a microprocessor, refrigeration unit and the vacuum pump. The main unit consists of the high-frequency induction drive motor, a temperature controlled rotor chamber and the rotor lift assembly. Several rotors are available.

Methods

Sucrose is used as the gradient medium. Sucrose is readily soluble in water and forms solutions with densities from 1.0–1.32 g/mL. Sucrose is economical to use in large volumes and is readily available. The density range must cover the range of the particle of interest and the contaminants. Generally, the density gradients are selected so that the particle of interest is in the middle of the range. In this case study 20%, 35%, 50%, and 60% sucrose were chosen for the TCF-32 study. The influenza particles banded in the 36-45% sucrose area. Sedimentation coefficient is used to determine how “fast” the particles will sediment from solution. The larger the number the “faster” it will sediment. This helps determine the flow rate of the

system. The sedimentation coefficient of the influenza virus is 700s. Other sedimentation coefficients can be found in most centrifugation text books, or they can be calculated.

As with most influenza cultures, the culture and growth of the virus is performed in chicken eggs. The eggs provide culture media for incubation of the virus. After sufficient growth, the eggs are harvested. The fluid is pre-clarified using centrifugation or filtration and is ready for isolation and/or purification.

Procedures

Purification is achieved using the TCF-32 continuous flow rotor system. In this procedure, the use of the continuous flow rotor allows 10 L of viral culture to be purified in one run. This in turn decreases the labor-intensive task involved in routine loading and unloading of batch style rotors. To start the procedure, the rotor system is assembled. Once assembled, the centrifuge is put in zonal mode and accelerated to 3,000 rpm. At 3,000 rpm the rotor is filled with a buffer solution. After the buffer solution is injected, the density gradient solutions are added. In this specific case, 20%, 35%, 50%, and 60% sucrose is injected into the rotor from lowest to highest density. After injection of the sucrose gradient, the buffer solution is circulated through while the centrifuge is accelerated to 32,000 rpm.

At 32,000 rpm, the clarified egg culture is injected into the rotor at 5L/hour. After the culture is added, buffer solution is once again circulated through the rotor for the while the influenza virus “band” for one hour. This allows all the viral particles and contaminants to reach the density gradient point where they reach equilibrium and effectively purify the virus. After viral banding, the centrifuge is decelerated to 3,000 rpm. Fraction collection occurs after the deceleration phase while the centrifuge is spinning at 3,000 rpm. A high-density sucrose solution is used to extrude out all buffer and gradient from the rotor. Fractions are collected in 20 mL aliquots, except for the first 100 mL, which is collected as a pre-fraction. The refractive index of each fraction is read using a refractometer. From those readings, it can be determined which fraction(s) contains influenza particles.

In the CC 40 system, fractions are collected after the rotor is completely decelerated, and no extrusion fluid is necessary since the inlet and outlet are separate connections. The fractions are removed from the rotor using a peristaltic pump.

Virus purification results

The data in Figure 3 shows the relative linearity of the fractions collected after completion of the TCF-32 run. A smooth gradient curve indicates a smooth loading and unloading of the gradient materials and an efficient separation. Fraction 23, the last fraction collected, contained 65% sucrose, showing that the extrusion solution had pushed all the gradient solutions out of the rotor.

The fractions were all tested for the presence of virus, and it was found that the virus was contained in fractions 15, 16 and 17. A graph would be plotted showing the presence influenza viral particle in the specific fractions, similar to Figure 3. The influenza virus was efficiently concentrated and purified from 10 L to 40–60 mL.

Figure 4 illustrates a sample of a CC 40 separation of the influenza virus; the absorbance (optical density ~280/650 nm) and the chicken cell agglutination (CGA) assay are both plotted. Also shown is the % sucrose curve.

The viral material is concentrated in two to three 100 mL fractions, allowing for concentration from approximately 60 L to a few hundred milliliters of the material needed for the vaccine. Purification and concentration of the viral material in one step greatly enhances the efficiency of vaccine production. The separation above was performed at 30,000 rpm at a flow rate of 15 L/hr.

Conclusion

Viruses have been successfully isolated and purified using both Thermo Scientific continuous flow ultracentrifuge systems. The TCF-32 rotor is used for research and pilot scale production, and the CC 40 system can be integrated into commercial-scale production. Other viruses that have been purified and isolated using these systems include Hepatitis B (recombinant), HVJ (Sendai), New Castle Disease, Rabies, Influenza, and HIV.

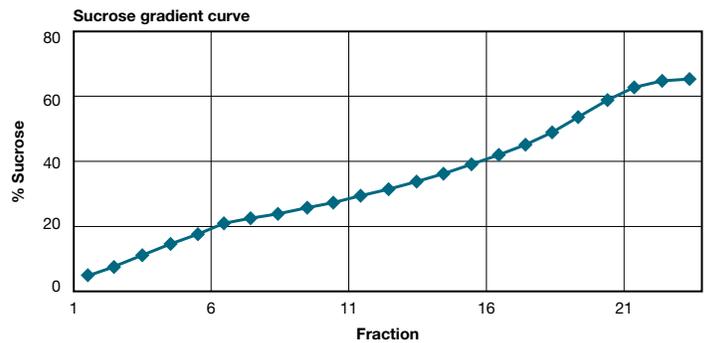


Figure 3. Relative linearity of the fractions collected after completion of the TCF-32 run. A smooth gradient curve indicates a smooth loading and unloading of the gradient materials and an efficient separation.

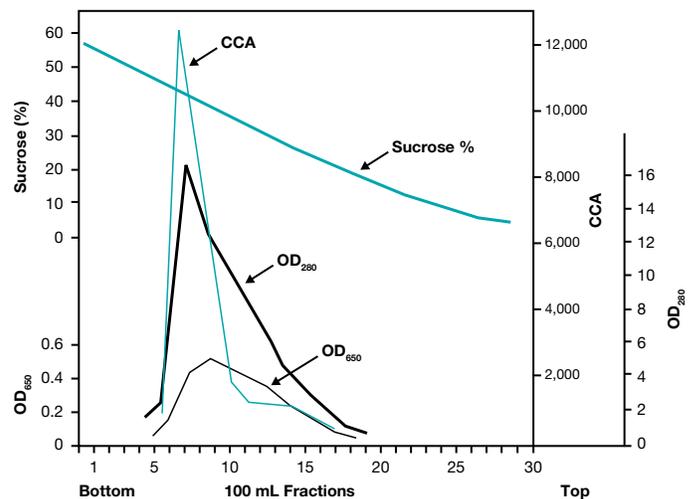


Figure 4. Infection virus production using the Sorvall CC 40 system.

Note: This note has been modified from its original version as authored by Carrie Bracco, Kendro Laboratory Products, Newtown, CT 06470. It has been formatted to fit this format.

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