

8. For small samples, or if the first pellets are very small, volumes can be reduced and the ultracentrifugation done in 10-mL tubes. Underlay 10-mL tubes with 2 mL of cushion.
9. The time and speed for ultracentrifugation may vary depending on the type of cushion used (20% sucrose, 10% sucrose, or no cushion). In general, centrifugation should be done at speeds ranging from 150,000g for 3.5 h to 212,000g for 1.5 h. If problems arise with centrifuge tubes cracking, lower the speed and lengthen the time used.
10. For very pure virus (e.g., for rabbit injection for antibodies), a 5–25% linear sucrose gradient in 20 mM sodium phosphate, pH 7.0, may be used. Centrifuge in a swinging-bucket rotor at 100,000g for 3 h. The viral band can be visualized and collected by its light-scattering property. A maximum of ca. 5 mg of virus can be loaded onto one 34-mL gradient. The pooled virus bands are diluted fourfold in 20 mM sodium phosphate, pH 7.0, and pelleted by ultracentrifugation at 212,000g at 4°C for 2 h. For antisera, the virions must be stabilized with formaldehyde treatment (25).

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