which heavy losses caused by aggregation would occur during centrifugation. These viruses are purified from pellets obtained during the first sap centrifugation, using Triton X-100 and/or urea as detergent.

4. Large cellular components are eliminated at this stage, but there is a risk of also losing aggregated viral particles. So it is necessary to let the virus resuspend slowly at 4°C with gentle stirring. Some viral particles may be damaged, but the losses will be less important during centrifugation. (Ethylenedinitrolo)tetra-acetic acid (EDTA), which is employed in many virus purification protocols, and which is thought to favor virus disaggregation, is, curiously, not used in most furovirus purification protocols.
5. The virus can be further purified by ultracentrifugation through a Nycodenz (Nycomed, Oslo) gradient ***(28)***. In this case, the virus is adjusted to a volume of 8.4 mL, with 10-times-diluted extraction buffer, and 5.85 g of Nycodenz are added. The volume is then adjusted to 11.5 mL with the same buffer, followed by centrifugation at 300,000*g* overnight at 15°C in a vertical rotor. The opalescent band is collected and diluted with the 10-times-diluted extraction buffer. Virus is then concentrated by ultracentrifugation.

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