Note 2). The solution is gently stirred until the PEG is completely dissolved, then held without stirring for 1 h at 4°C. After centrifugation for 30 min at 12,000*g*, the pellets are resuspended in a maximum of 3 mL of buffer C (*see* **Note 3**).

3.2.4. Separation on Sucrose Gradient

Sucrose gradients 10–40% are prepared from two stock sucrose solutions (*see* **Subheading 2.1., items 4** and **5**). Viral pellets, resuspended in buffer C, are loaded directly onto six gradients (500 µL per gradient) and ultracentrifuged for 2.5 h at 300,000*g*, in a Beckman SW 41 rotor. Gradient tubes are scanned at 254 nm with a ISCO ultraviolet absorption monitor and the UV absorbing regions corresponding to virus fractions are collected in ultracentrifuge tubes.

3.2.5. Concentration

To concentrate the virus, the virus-containing fractions are ultracentrifuged 16 h at 95,000*g* in a Beckman 60 Ti rotor. The final virus pellet is then resuspended in 200 µL of buffer C. The virus concentration can be estimated by measuring the absorption at 254 nm. Concentration is then calculated based on the formula: 2.4 U of OD_{254nm} correspond to a concentration of 1 mg/mL of virus.

3.3. Viral RNA Isolation

3.3.1. RNA Extraction

The virus suspension is incubated in the presence of proteinase K (200 ng/µL) and 0.5% SDS for 15 min at 50°C. This suspension is then extracted with an equal volume of Tris-HCl saturated-phenol:chloroform:isoamyl alcohol (25:24:1, [v/v/v]), and then with an equal volume of chloroform:isoamyl alcohol (24:1, [v/v]). The aqueous phase is ethanol-precipitated in the presence of 0.1 vol of 3*M* sodium acetate, pH 5.3, and 2.5 vol of 96% ethanol at –20°C, for at least 1 h. Viral RNA is recovered by centrifugation for 20 min at 4°C at 15,000*g*. The RNA pellet is finally resuspended in 10–20 µL of TE buffer or water.

3.3.2. Analysis of Viral Nucleic Acid

RNA is denatured with formaldehyde and electrophoresed in a 0.8% agarose formaldehyde gel, as described by Miller ***(21)***, with few modifications (*see* **Note 4**). The RNA volume should be reduced to 1 µL, to which 2.2 µL of buffer D and 5.8 µL of buffer E (*see* **Subheading 2.3., item 3**) are added. After denaturation by heating at 70°C for 10 min, 1 µL of gel-loading buffer is added and the sample is loaded on the gel (*see* **Subheading 2.3., items 5–7**). After electrophoresis, the gel is stained for 15 min and destained in water (*see* **Subheading 2.3., item 8**).