2. Centrifuge at 10,000*g* for 20 min at 4°C.
3. Filter aqueous phase through large kimwipes supported by cheesecloth. Make sure that no chloroform or carbon tetrachloride remains in this initial extract (*see* **Note 3**).
4. Add PEG (mol wt 8000) to 4% (w/v) and NaCl to 1.75% (w/v). Stir on ice for 1 h (*see* **Note 4**).
5. Centrifuge at 10,000*g* for 15 min at 4°C. Carefully discard supernatant and retain the pellets.
6. Resuspend pellets in ca. 0.1 vol of initial extract, using cold 0.5*M* borate, pH 8.0 (*see* **Note 5**).
7. Just before low-speed centrifugation, add Triton X-100 to 0.25% (v/v) and stir until thoroughly dissolved. Low-speed centrifuge at 8000*g* for 15 min at 4°C. Retain the supernatant.
8. Ultracentrifuge the retained supernatant at 70,000*g* for 1.5 h at 4°C (*see* **Note 6**).
9. Resuspend pellets in buffer C, using about one-thirtieth vol of the initial extract (*see* **Note 5**).
10. Repeat **steps 7–9**, but resuspend pellets in 5 mL buffer C. Centrifuge briefly at low speed (8000–10,000*g*) for 1–2 min.
11. Layer supernatant on 10–40% sucrose density gradients made in buffer C, and centrifuge 2 h at 95,000*g* in a SW 28 swing bucket rotor (*see* **Notes 7–9**).
12. If desired, dilute virus-containing fractions with at least 3 vol of buffer C and mix thoroughly. Ultracentrifuge as in **step 8**, above, to concentrate virus-containing fractions.
13. Resuspend pellet in 1 or 2 mL of buffer C (*see* **Note 10**).

3.2. Purification of Maize Dwarf Mosaic Virus

This method, originally developed by W. Langenberg, has been used by a number of laboratories for purification of potyviruses infecting Gramineae, particularly maize dwarf mosaic (MDMV) and the related sugarcane mosaic virus (SCMV), sorghum mosaic virus (SrMV), and johnsongrass mosaic virus (JGMV). Langenberg reports that addition of 2*M* guanidine HCl, at **steps 1–6**, will significantly increase virus yield (personal communication).

1. Collect 500–1500 g infected tissue and grind in a large, prechilled blender with a minimum of 1 L buffer B. Strain the contents through several layers of cheesecloth, squeezing out as much sap as possible (*see* **Notes 11–13**).
2. Add carbon tetrachloride to 5% (v/v) and mix in a blender for about 5 s.
3. Centrifuge for 10 min at 10,000–15,000*g* at 4°C and reserve the supernatant (*see* **Note 14**).
4. While stirring, add Triton X-100 to 0.25% final concentration. Then, add solid PEG to 6% final concentration. Stir until dissolved (about 0.5 h) (*see* **Notes 15** and **16**).
5. Centrifuge solution for 20 min at 10,000–15,000*g* at 4°C and discard supernatant. Resuspend pellet in ca. 100 mL buffer C and clarify by centrifuging for 10 min at 10,000*g* at 4°C, reserving the supernatant.