7. pH adjustments: When adjusting the pH, slowly drop the acidic or basic solution into the extract and stir with a magnetic stirrer to avoid extremely low or high pH and drastic pH changes.
8. Other clarification procedures: The method described to clarify the extract is acidification, because the virus can withstand the acidity for the time required to remove the precipitate by centrifugation. If the purification fails, check especially this step, preferably by electron microscopy, to make sure the virus is not lost here. Precipitation of plant material starts around pH 5.2, so maybe a slightly higher pH, as in **Subheading 3.2.**, can help. Otherwise the clarification with hydrated calcium phosphate (HCP) has been used to efficiently adsorb host material at the buffer concentration of 0.03*M* ***(16–18)***. The HCP is prepared by adding slightly less than an equal volume of 0.1*M* $CaCl_2$ to a 0.1*M* solution of Na_2HPO_4. The white precipitate (HCP) should be washed in distilled water 15–20 times by repeated decantation and resuspension of the precipitate, to remove soluble salts. A volume of HCP equivalent to 0.9 of the original weight of tissue is normally needed to clarify the extracts. The buffer concentration of 0.03*M* should be obeyed, since the virus is also adsorbed at lower ionic strength and the host material is not efficiently adsorbed in higher concentrations. Finally, ilarviruses can also be concentrated by 10% polyethylene glycol (PEG) 8000 and 1% NaCl (Wang, personal communication). In addition, this would allow the processing of more tissue and larger volumes when the virus titers are low and/or only small-volume ultracentrifuge rotors are available.
9. Alternative method to remove host contaminants: Host material may also be efficiently removed from virus preparations by precipitation with an antiserum prepared against host protein. The procedure is described in detail by Gold ***(19)***.
10. Choice of final purification steps: Usually the sucrose gradient purification is sufficient, however, equilibrium density gradient centrifugation has also been applied, using either CsCl (only after fixation of the purified virus with aldehyde) or Cs_2SO_4.
11. **Caution:** The general precautions for work with single-stranded RNA, such as sterile buffers, glassware, and use of examination gloves to prevent contamination with RNases, should be strictly obeyed. Work at low temperature or on ice is recommended, until otherwise stated.
12. When extracting viral RNA with phenol and chloroform, three phases normally appear after centrifugation. The upper aqueous phase, containing RNA, should be very carefully removed. The intermediate and lower phases, composed of denatured proteins and organic solvent, should be strictly avoided. Any trace of them may interfere with the suitability of the RNA preparation for cloning work.
13. During the wash steps, it is important to note that the RNA pellet should not be strongly shaken and thereby released from the tube wall; otherwise, the RNA may be easily lost when decanting the solution. Gently overturning the centrifuge tube several times may be sufficient.
14. Choice of RNA extraction methods: The described method is normally successful and yields RNA with little degradation and is well-suited for cloning steps. In