4. Notes

1. **Caution:** Care should be taken when working with phenol, because it is highly corrosive. Wear gloves at all times and remove all spills immediately.
2. The vast majority of carlaviruses are present in infected tissue at very low concentrations. It is therefore recommended to use as much plant material as possible, though this will be dictated by the volume that can be handled during the first two centrifugation steps.
3. Add cold diethyl ether, which has been stored in a cold room at 4°C overnight. It is also advisable to carry out all work with diethyl ether in a well-ventilated fume hood.
4. Carlaviruses will often form aggregates with themselves and with plant material, and we have found sonicating the pellet is the best way to resuspend the virus. If a sonicator is not available, the pellet must be resuspended well with a glass rod before further cycles of low- and high-speed centrifugation.
5. Virus band can be visualized by shining a light directly at the tube, with the band being removed with a Pasteur pipet or syringe and needle.
6. The purity of virus preparation can be checked by comparing the absorbance at wavelengths of 260 and 280 nm in a spectrophotometer. Assume an A_{260} of 2.8 for a 1-mg/mL virus preparation. Alternatively, view the virus with electron microscopy.
7. We have found that the addition of bentonite to the extraction buffer increases the yield of RNA, though it is not essential.
8. Any standard RNA gel technique can be used to analyze RNA quality. However, it should be noted that it is typical for carlaviruses to have a band of genomic RNA with a substantial smear of smaller RNAs decreasing in mol wt below it. These smaller RNAs are generated from broken particles as part of the virus purification and also as part of the RNA extraction procedure.

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