product significantly different in size from the predicted viral product. For RT-PCR, it does require that the cDNA synthesis uses a nonspecific primer, i.e., oligo(dT) or random primers, rather than specific primers targeted to the viral sequence. Such a control confers several advantages, because it acts as a control for both the cDNA synthesis and the PCR, and distinguishes between true and false negatives, which is important in epidemiological work. Before using such a control, it is vital to check all the primers both singly and in combination with each other, on infected and uninfected samples, to ensure that there is no interference between the primer pairs, i.e., that the control primers do not amplify the target sequence, or that one test and one control primer do not amplify some other, unknown sequence (*see* **Note 21**).

4. Notes

1. Plant samples can alternatively be ground in microcentrifuge tubes using plastic grinders or wooden applicator sticks (Fischer Scientific, Pittsburgh, PA), or in a pestle and mortar. The latter must be cleaned extensively between use, and, for DNA viruses, treated with a DNA-degrading compound, such as 10% sodium hypochlorite, to avoid crosscontamination of samples.
2. Interphase material may contain RNases and RT-PCR inhibitory substances, and hence care should be taken to avoid pipeting off any of this material. Should the interface not form a tight layer, it is recommended to carry out another phenol:chloroform extraction on the supernatant. To aid separation of the supernatant from the interface material, a physical barrier like high-vacuum silicon grease can be included, as described in **Subheading 3.1.5**.
3. For extraction of RNA, it is advisable to break up precipitated RNA during the 70% ethanol wash, and perform more than one wash, if necessary, because material with RNase activity is often trapped within the precipitated RNA.
4. If a RNA–DNA precipitate is particularly difficult to dissolve in sterile ddH_2O, this is generally either caused by overdrying of the pellet, or by pelleting through centrifugation being excessive.
5. SDS releases both RNA and DNA from protein complexes and partially inhibits RNase action. Extraction with phenol-SDS buffers at 60ºC will be fairly effective at removing DNA contamination from RNA extractions.
6. Guanidinium methods: These RNA extraction methods are particularly effective for tissues high in RNases, because both guanidinium thiocyanate and β-mercaptoethanol inactivate RNases irreversibly.
7. A variety of technical modifications have been proposed and tested by many labs around the world: RNA isolation from plants has always been a slightly tricky problem. The technical approaches need to overcome two major problems: degradation of RNA, hence the use of powerful RNase inhibitors; and inhibitory contaminants, which are a special problem for plant tissue. In many cases, PCR inhibitors can be ignored by simply diluting the sample and using the extreme sensitivity of PCR to amplify desired fragments. When target molecules are rare