will allow clones secreting antibodies of undesired specificity to be discarded. Until the specificity of an antibody is established, it is of little use for differential diagnosis.

Dilutions of purified antibody (1 mg/mL) in the range of 1:250–1:4000 should be tested against dilutions of purified virus or sap from infected plants diluted 1:10–1:1000 in buffer. Selection of working dilutions should be chosen by the ratio of reaction of known positive material relative to the negative control (*see* **Notes 2** and **3**). The combination of antibody, virus, and sap dilution showing the highest signal to background should be chosen.

4. Notes

1. Mice should be permanently marked to allow identification at a later date. This is usually done by ear-punching or tattooing, but electronic tagging devices can be used.
2. It is important that the assay system used at this stage should be the same as the test system for which the antibody is intended. Antibodies assayed by ELISA, for example, do not always perform well in western blots or immunofluorescence assays. This is because some epitopes, when blotted or electrophoresed, lose the three-dimensional structure required for antibody recognition, and this is particularly important when using MAbs.
3. It is vital that whenever antibodies are used, appropriate controls (infected and healthy plant tissue) are included in the assay, to reduce the risk of false-positive or false-negative results. The negative control should be of the same type, preferably the same variety, of plant as used in the assay.
4. Some workers use feeder layers of peritoneal macrophages to provide the necessary cytokines to support hybridoma survival after cell fusion or cloning. MTM ***(6)*** performs the functions of feeder cells and supports the growth of primary hybridomas and cloned cells. It is easy to use, can be stored frozen for long periods of time, and can be assessed for sterility when first made.
5. Batches of FBS ***(5)*** vary in their ability to support cell growth and should be assessed by cell-cloning assays prior to purchase. Most suppliers will provide small quantities for assessment and reserve the required amount until cell growth studies have been performed.
6. Other enzyme-deficient cell lines have been developed for hybridoma production, and some workers report that they are better than HAT-sensitive lines.
7. It is not important for antibody production if, at this stage, the virus particles are a little aggregated or broken.
8. For the purpose of antibody production, do not add sodium azide to the purified virus. If mercaptoethanol or other noxious substance has been used in the antigen purification, this can be removed by dialysis or by using Centricon ultracentrifugation tubes.
9. Viable cell counts can be carried out either by trypan blue exclusion in visible light or by using acridine orange–ethidium-bromide in UV light. The acridine