a ratio of 0.28:0.72 (DIG-UTP:UTP) vs the one recommended by the manufacturer (0.35:0.65). However, since no direct correlation between a specific sequence pattern and transcription efficiency has been observed, the optimal DIG-UTP:UTP ratio must be determined experimentally for a given template, if synthesis of the probe continues to be a problem.

3. If sensitivity is poor when using clarified sap extracts to detect RNA viruses, samples can be denatured by heating at 60°C for 15 min in the presence of formaldehyde, since this treatment helps to increase the hybridization signal slightly ***(12)***. Additionally, samples can be phenol-extracted and concentrated by ethanol precipitation. However, this last step is not recommended for routine diagnosis, because the procedure is obviously more laborious. In addition, solutions containing phenol are highly toxic and special safety precautions must be taken.
4. When possible, chemiluminescent detection should be the method of choice because of its higher sensitivity over the colorimetric one. In addition, when clarified sap extracts are used, the remainder of the natural green-brownish color of leaves on the membranes interferes directly with the colorimetric detection, probably because of the reduction of the nitroblue tetrazolium by components of the plant sap; the light emission is not altered by the presence of these components.
5. If nylon membranes are used, the sensitivity of the chemiluminescent detection method can be increased by UV crosslinking of RNA-containing plant samples, which results in a 5- to 10-fold increase over the standard baking methods. When no UV source is available, the samples can be fixed to the membrane by baking for 15–30 min at 120°C or for 2 h at 80°C.
6. In some applications (for instance, to diagnose several viruses on the same membrane), it would be desirable to have the possibility of reprobing. Unfortunately, digoxigenin-labeled cRNA probes tend to remain bound to RNA dot blots following stripping treatments that remove ^{32}P-labeled cRNA probes, thus making reprobing not recommended when using this kind of probes. Then, it is advisable to duplicate membranes with equivalent samples and probe them with the different probes.
7. To make the nonisotopic dot-blot hybridization technique more accessible to nonspecialized laboratories, an alternative procedure can be applied to prepare and clarify field samples ***(13)***. Sap extracts are obtained by homogenizing the tissue with 1 vol 50 m*M* sodium citrate in a resistant plastic bag with a hand model homogenizer. Using sap-impregnated cotton buds, the samples are then applied to nylon membranes by uniformly pressing them until a lateral diffusion is observed. The nucleic acids are then bound to the membrane and hybridized, as previously described. This procedure avoids the need for centrifugation to clarify the samples and the use of micropipets to load them. Although the material directly applied on the central area is not accessible to the luminescent substrate, the diffused material gives a very specific and reliable doughnut-like hybridization signal, which is just as sensitive as clarifying the samples by centrifugation.