

these purposes, 1 ml is estimated as 32 or 33 drops, and the starting sample weight is taken as its liquid equivalent (1 g = 1 ml).

Step 7. The final virus-containing solution was obtained at this step by centrifuging the slurry ($12,200 \times g$ for 5 min) and carefully removing and retaining the supernatant.

The standard procedure was used for CpMV, PMV, TMV, and TRSV.

Variations on the Standard CVC Procedure. The variations differ from the standard procedure *only* at the specific steps indicated. Consult the standard procedure description above for processing details.

Variation A. Extraction of tissue at Step 2 was made with SP buffer containing 6.5% *n*-butanol at the rate of 1 ml extracting solution to 1 g tissue. No other solvents were used. This variation was used for CpMV and TNV.

Variation B. At Step 1, the tissue was macerated in 2 ml of 0.5 M citrate buffer, pH 6.5, containing 5 mM ethylenediaminetetraacetic acid (EDTA), and 0.5% thioglycolic acid. No solvents were used. At Step 6, the pellet was resuspended in the same solution as was used in Step 1. Step 2 was omitted. This variation was used for CMV.

Variation C. This was the same as Variation B except that, at Step 2, 1 volume of chloroform was stirred into each volume of exudate with the aid of a Vortex Mixer. This variation was used for CMV.

Variation D. At Step 1, 0.1% sodium sulfite and 0.02 M EDTA were added to the SP buffer. At Step 6, the pellet was resuspended in 0.05 M Tris buffer, pH 8.2, containing 0.02 M EDTA. This variation was used for lettuce virus.

Variation E. At Step 1, the tissue was triturated in liquid nitrogen with a mortar and pestle. It was then extracted with SP buffer that contained 1.5% sodium sulfite, at the rate of 2 ml/g tissue. At Step 6, the pellet was resuspended in this same buffer solution. No solvents were used, and Step 2 was omitted. This variation was used for NSPV.

Variation F. All steps were the same as those in Variation E except that Step 7 was followed by further treatment. The supernatant from Step 7 was mixed vol:vol with 2% aqueous uranyl acetate and then centrifuged for 5 min at $12,200 \times g$. The supernatant was retained. This variation was used for NSPV. Note: electron microscope grids that were prepared from this fraction were rinsed and stained in the usual fashion, which is described in the following section. The uranyl acetate added at this step was only added to clarify the virus solution and not for its staining qualities. In fact, most of the uranyl acetate is lost to the pellet during the subsequent centrifugation.