Diagnostic Updates

Immunosorbent Electron Microscopy (ISEM) Using Crude Plant Extract for Detection of Plant Viruses

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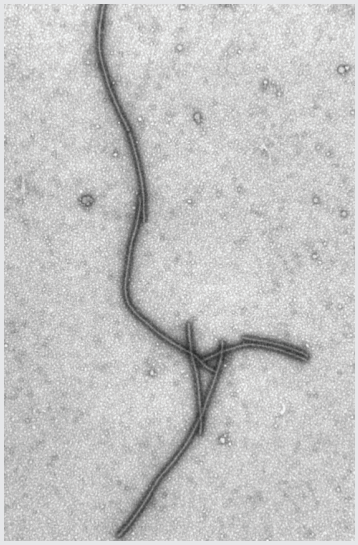
The University of Minnesota Plant Virology Laboratory and Plant Disease Clinic would normally utilize ISEM using partially purified extract versus the described crude extract below. The crude extract can be used in place of partially purified virus extract when diagnosticians do not have access to an ultracentrifuge.

1. Sample preparation: Grind 0.1 g tissue (using mortar and pestle or sample mesh bag) in 1-2 ml phosphate buffer (100 mM, pH 7.0). Mix 500 µl sample extract and 500 µl $CHCl_3$ in 1.5 ml tube and vortex vigorously. Centrifuge for 5 minutes at full speed. Use supernatant for examination.
2. Coating: Dilute whole antiserum (AS) 1/500 in ELISA carbonate coating buffer; Incubate TEM grid, film side down on 10 µl diluted AS in a moist chamber for 15-20 minutes.
3. Rinsing: Transfer the grid briefly to 10 µl 100 mM phosphate buffer pH 7.0.
4. Trapping: Transfer the AS coated grid to 10 µl clarified sample extract. Incubate 15 minutes (at room temperature) to overnight (at 4°C). For low virus concentration shake gently in platform shaker at room temperature.
5. Decoration: Transfer grid without rinsing to 10 µl AS diluted 1/250 in PBS.
6. Staining for TEM: Rinse grid with 4-6 drops (10 µl) Phosphotungstic acid - 2% (PTA) NaOH pH 7.0 or 4-6 drops dH_2O and 2 drops Uranyl acetate - 1% (UA).
7. Examination: Use the grid for TEM.



Bean Yellow Mosaic Virus, decorated with antibody.

Note that even though there are broken pieces the decoration indicates that they are BYMV. Photo courtesy of Dimitre Mollov.

Phosphate Buffer:
100 mM $NaPO_4$ (mono and dibasic) pH 7.0

Carbonate Coating Buffer:
60 mM $(Na)2CO_3$ pH 9.6.