Techniques

Effect of Grid Rotation in a Magnetic Field on Virus Adsorption in Immunosorbent Electron Microscopy

L. W. Stobbs

Research scientist, Agriculture Canada, Research Station, Vineland Station, Ontario L0R 2E0.
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ABSTRACT

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The use of nickel electron microscope grids in immune electron microscopy facilitated the rotation of the grids on virus samples by an applied magnetic field. Antiserum-sensitized grids were floated on virus dilutions in microplate wells and the plate was positioned on a magnetic stirrer. Low-speed rotation was found to increase the virus-trapping capability of such grids.

In immune electron microscopy (ISEM) the number of virus particles trapped on antiserum-sensitized grids increases with incubation time (2,5). Contact between serum molecules on the grid and virus particles contained in tissue suspension is dependent on random motion within the droplet. By providing convective force within the droplet, increased exposure of the grid surface to virus particles can be accomplished, thus reducing the incubation time. This is of particular importance where extended incubation of plant homogenates is not always possible due to oxidative changes affecting virus integrity and binding. This paper reports a new procedure.

MATERIALS AND METHODS

Sources and preparation of virus. The common strain of tobacco mosaic virus (PV135, American Type Culture Collection, Beltsville, MD) was maintained on *Nicotiana tabacum* 'Harrow Velvet.' Virus used for antiserum production was prepared by polyethylene glycol precipitation (4) and purified on sucrose gradients (3). Extracts of TMV-infected plants were prepared by grinding 2 g of infected tobacco leaves in 18 ml of 0.05 M tris buffer (pH 7.2) containing 0.15 M NaCl and 0.4 M sucrose. The concentration of virus, as determined by the polyethylene glycol procedure, was ~2 mg of virus per milliliter of plant extract. Following centrifugation at 5,000 *g* for 10 min, serial twofold dilutions of the supernatant were made with tris-NaCl, pH 7.2.

Preparation of antiserum. Antiserum against TMV was prepared by injecting New Zealand white rabbits intramuscularly with virus preparations emulsified with an equal volume of Freund's complete adjuvant. Four 2-mg intramuscular injections were given at 10-day intervals and the rabbits were bled 2 wk after the last injection. The titer of the antiserum, as determined by immunoelectroosmophoresis (1), was 1/2,048.

Modified ISEM procedure. Nickel grids with 38-μm openings (400-mesh), covered with a collodion film and carbon coated in a vacuum evaporator, were floated for 30 min, membrane side down, on either TMV antiserum or normal serum diluted 1/100 with tris buffer. Unadsorbed serum proteins were removed by gently rinsing the grids with tris-NaCl buffer. Centrally located wells of microtiter plates (rows C to F, columns 5 to 8) were filled with 350 μl of virus dilutions. Serum-sensitized grids were floated on the virus suspensions and the plate was placed on a Corning Hot Plate Stirrer, model 531 (Corning Glass Works, Corning, NY) (Fig. 1). The stirrer was set at the lowest setting providing gentle rotation of the grids within the wells. Grids were maintained on the stirrer for 20 min at 22 C, rinsed in distilled water, and stained for 10 min in

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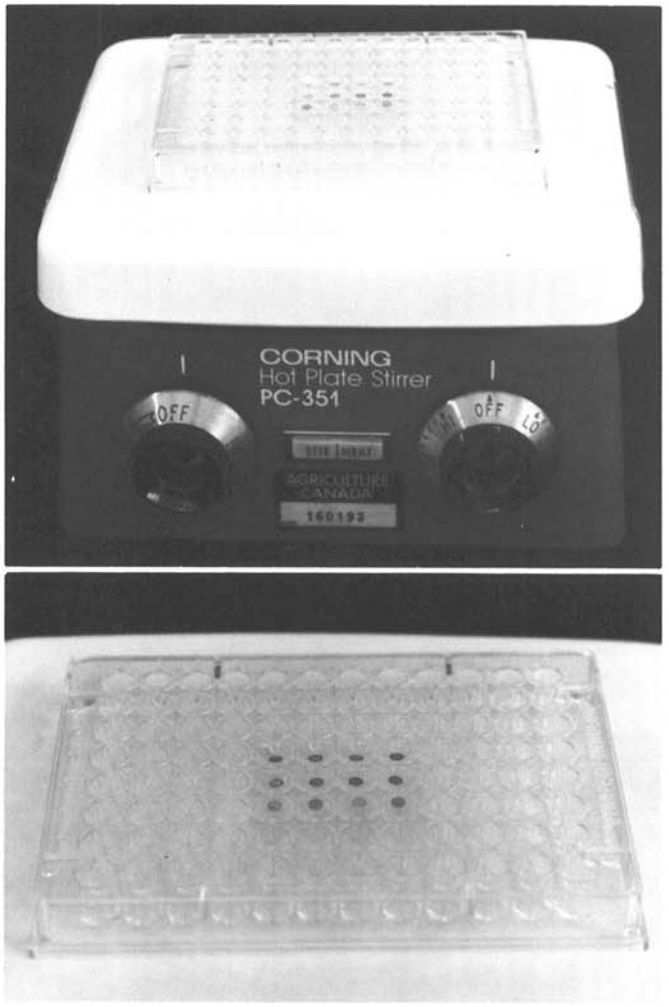

Fig. 1. Magnetic rotation of electron microscope grids. Top: ELISA microtiter plate positioned on magnetic stirrer. Bottom: Positioning of nickel grids within center wells of the plate.

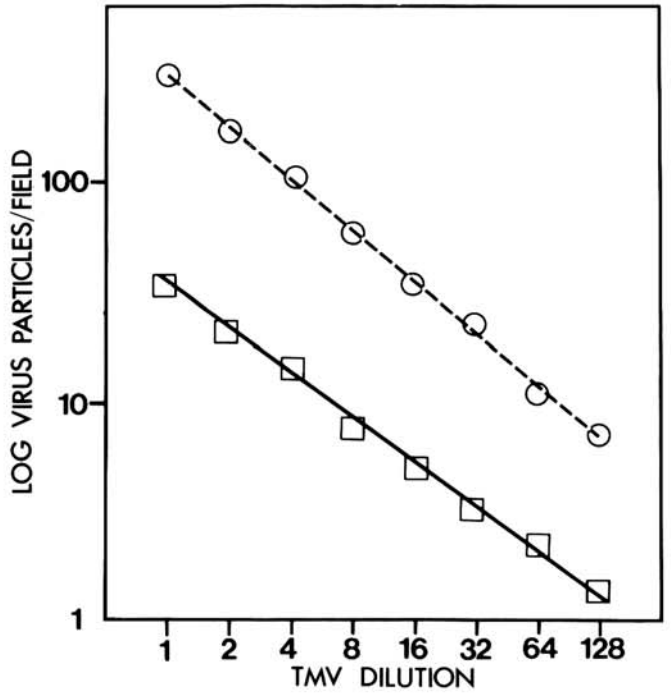

Fig. 2. Influence of the grid rotation on the virus-trapping ability of antiserum-sensitized grids. Symbols: O——O, grids magnetically rotated; and □——□, grids not rotated.

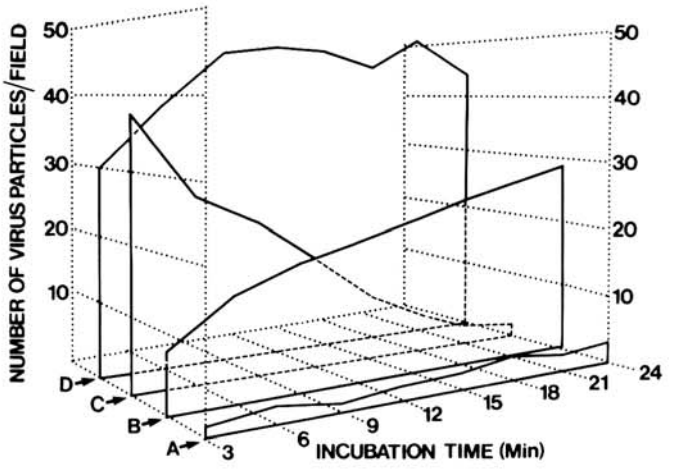

Fig. 3. Influence of speed of electron microscope grid rotation on virus-trapping ability of sensitized grids: **A,** normal serum-coated grids; **B,** antiserum-coated grids without magnetic rotation; **C,** antiserum-coated grids with high-speed rotation; **D,** antiserum-coated grids with low-speed rotation.

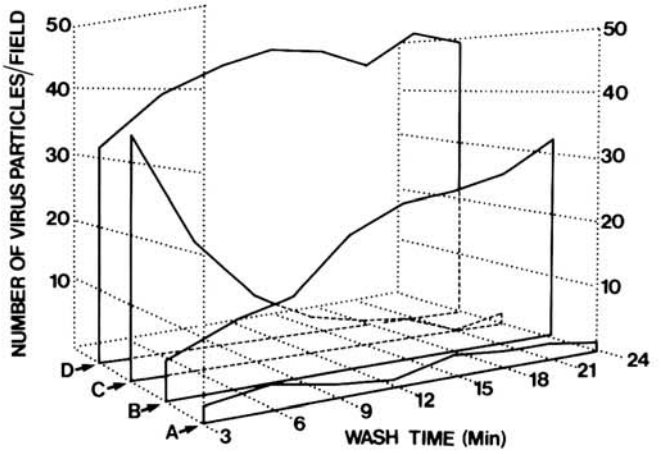

Fig. 4. Influence of the speed of electron microscope grid rotation on the antibody retention by the grids: **A,** normal antiserum-coated grids; **B,** antiserum-coated grids without rotation; **C,** antiserum-coated grids with high-speed rotation; **D,** antiserum-coated grids with low-speed rotation.

2% phosphotungstic acid, pH 6.5. After rinsing in distilled water, the grids were examined with a Philips EM 201 electron microscope. Ten fields at ×24,500 magnification were randomly sampled from different parts of five identically prepared grids of each sample and virus counts expressed as the mean log number of particles per field (Fig. 2).

To examine the effect of rotational speed on the virus-trapping ability of the grid, a group of five grids were each rotated at a low speed (~180 rpm) and a second set was rotated at a higher speed (~1,800 rpm) on 1/16 dilutions of plant sap. A strobe tachometer was used to adjust the rotational speed of the grids. Grids were sampled at 3-min intervals over a 24-min period and stained as previously described.

RESULTS AND DISCUSSION

Antiserum-sensitized grids that were magnetically rotated on the virus dilutions consistently had higher virus particle counts than grids that were not rotated (Fig. 2). Slow-speed rotation (~180 rpm) produced the most consistent binding with no visible damage to the collodion films (Fig. 3). Grids rotated in this manner acquired as much virus in 3 min as those floated without rotation on droplets for 24 min. Negligible amounts of virus were trapped on grids treated with normal serum. Increasing the speed of rotation to

1,800 rpm resulted in a significant drop in the amount of trapped virus over the test period accompanied by the separation of the collodion membranes from several of the grids.

To examine whether the reduction in virus binding at the higher rotational speed was a result of antibody stripping from the collodion membrane, antiserum-treated grids were floated on tris-NaCl-sucrose buffer in the microplate wells and rotated at 180 or 1,800 rpm. Grids were removed at 3-min intervals, floated on 1/16 dilutions of plant sap for 20 min without rotation, and stained as previously described. Grids washed in buffer at 1,800 rpm before exposure to virus exhibited a similar decline in virus trapping ability whereas those rotated at 180 rpm exhibited no significant loss in virus acquisition (Fig. 4). By using low-speed grid rotation, significant improvement was made in virus acquisition in the immune electron microscopy procedure.

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