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NOTES

Procedure for Fluorescent-Antibody Staining of Virus-Infected Cell Cultures in Plastic Plates

ALFRED R. PURSELL* AND JOHN R. COLE, JR.
Veterinary Diagnostic and Investigational Laboratory, Tifton, Georgia 31794

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Acetone fixation and fluorescent-antibody staining of virus-infected cell cultures were performed in plastic plates. Proper addition of acetone as a fixative did not alter the plastic.

The fluorescent-antibody (FA) staining technique is extensively used to stain viruses growing in tissue culture cells. Most procedures for FA staining use acetone as the fixative for the cells (2-4). Plastic plates would be very useful for the isolation and identification of viruses if FA staining of the viral isolates could be done directly in the plates; however, this has been considered impractical because of the effect of acetone on the plastic.

A procedure for the acetone fixation and FA staining of virus-infected cells in plastic plates is reported.

Cell lines of bovine kidney, porcine kidney (PK-15), and monkey kidney (Vero) were grown in either the 96-well, flat-bottom microtiter plate or the 24-well (multiwell) tissue culture plate (Falcon Plastics, Oxnard, Calif., and Linbro Chemical Co., New Haven, Conn.). Cell cultures were inoculated with test viruses including infectious bovine rhinotracheitis, parainfluenza-3, and pseudorabies, and incubated at 37 C in an atmosphere of 5% CO_2 and 95% air until ready to stain. Specific conjugated hyperimmune sera obtained from the Diagnostic Reagents Section, National Animal Disease Center, Ames, Iowa, were used undiluted for staining.

Cells were fixed and stained as follows. (i) Aspirate medium using pipette on vacuum line or rubber bulb. (ii) Rinse with phosphate-buffered saline (PBS), pH 7.0; aspirate PBS. (iii) Add PBS to each well to be stained (0.2 ml per 24-well plate; 0.05 to 0.075 ml [2 drops from Pasteur pipette] per microtiter plate). (iv) Gently add acetone to PBS in the wells using Pasteur pipette and fill wells about three-fourths full. (Final concentration of acetone is about 80%.) It is essential that the PBS be added to the plate before adding the acetone. Acetone alone or as an 80% solution in PBS will etch the plate, making it impossible to read (Fig. 1). (v) Fix cells for 10 min; aspirate acetone-PBS. (vi) Add conjugate to cover bottom of well and incubate at 37 C in a moist chamber for 30 min. (vii) After incubation, add PBS to each well. (viii) Aspirate PBS-conjugate and rinse with PBS for 5 min. (ix) Aspirate PBS and rinse with distilled water for 5 min. (x) Aspirate distilled water, and invert plates on absorbent surface to drain excess fluid.

Plates were examined microscopically by inverting them on the stage of a Leitz Orthoplan microscope equipped with a Ploem illuminator and 150-W Xenon lamp, a long-working-distance 16× objective (no. 519-229, E. Leitz, Inc., Rockleigh, N.J.), and 6.3× eyepieces.

Because of possible safety hazards resulting from incomplete inactivation of the virus, studies were performed on acetone fixation of ether-sensitive (pseudorabies) and ether-resistant viruses. PBS-acetone was used in the plastic plate and acetone alone with cell cultures growing on glass cover slips. Cells were fixed for 10, 20, 30, 60, and 120 min in each of the fixation procedures. At the end of each fixation time, the PBS-acetone was aspirated from the cells in the plate and 1.5 ml of medium (Hanks balanced salt solution plus 3% normal calf serum) was added to the well. At the same time one cover slip was removed from the acetone and placed in a tube containing 1.5 ml of medium. The cell sheets were rinsed for a minimum of 10 min. The medium was inoculated onto cell cultures which were observed for 7 days.

After acetone fixation by either procedure, growth of the ether-sensitive virus was not detected during the observation period. However, cytopathic effect was observed at 24 h in all cells inoculated with the medium containing

the ether-resistant virus fixed with PBS-acetone or acetone alone.

Since acetone does not inactivate ether-resistant virus even after 2 h, additional safety precautions are needed. All fluids aspirated from the wells should be collected in a closed container and autoclaved. If a vacuum system is used, two collecting vessels containing disinfectant should be used between the siphon tube and the vacuum source. After aspirating fluid from the wells, the siphon system should be decontaminated by autoclaving.

The absorbent surface used to drain the plate should be incinerated. Before reading, the plate should be sealed with tape to protect the microscope stage from virus contamination.

Figures 2, 3, and 4 are photomicrographs of infectious bovine rhinotracheitis-, parainflu-

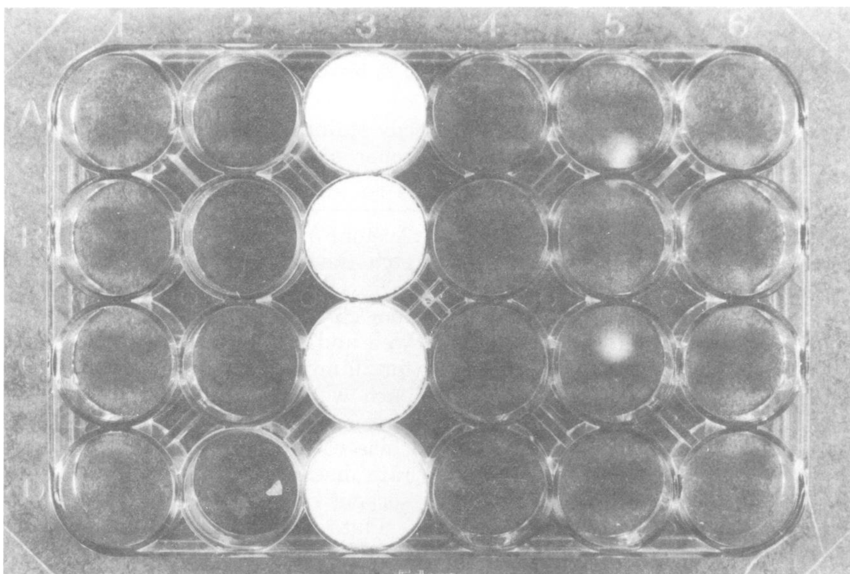

FIG. 1. *Multiwell plate showing reaction of acetone (row 3) and PBS and acetone treatment (row 2).*

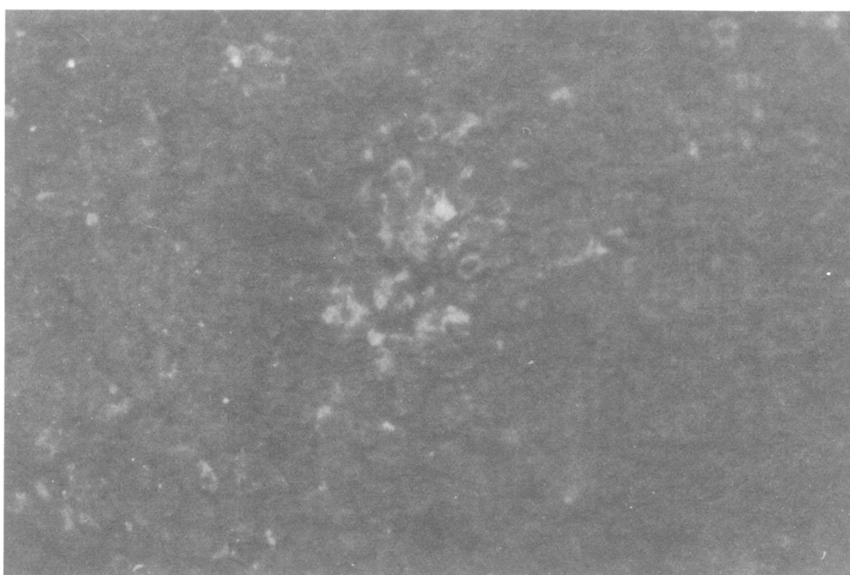

FIG. 2. *Bovine kidney cell monolayer infected with parainfluenza-3 virus and stained with parainfluenza-3 conjugate. ×100.*

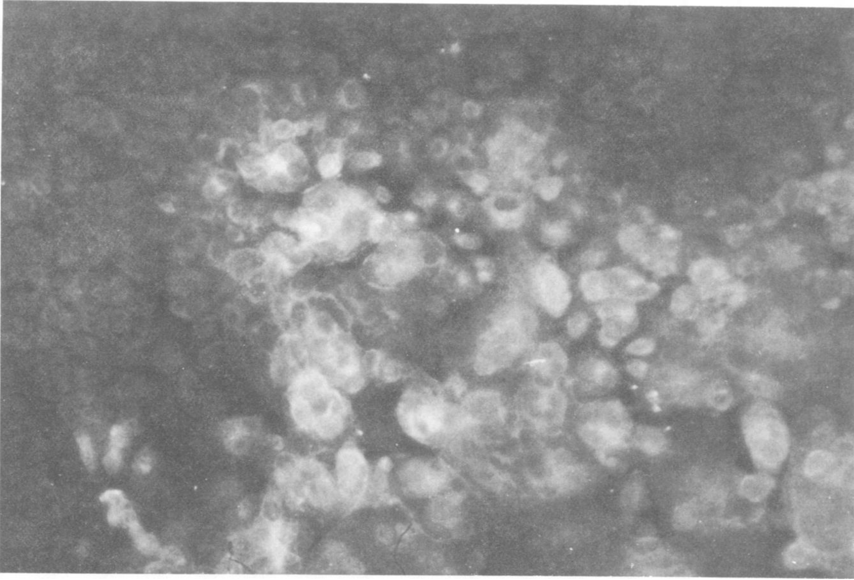

FIG. 3. *Bovine kidney cell monolayer infected with infectious bovine rhinotracheitis virus and stained with infectious bovine rhinotracheitis conjugate. ×100.*

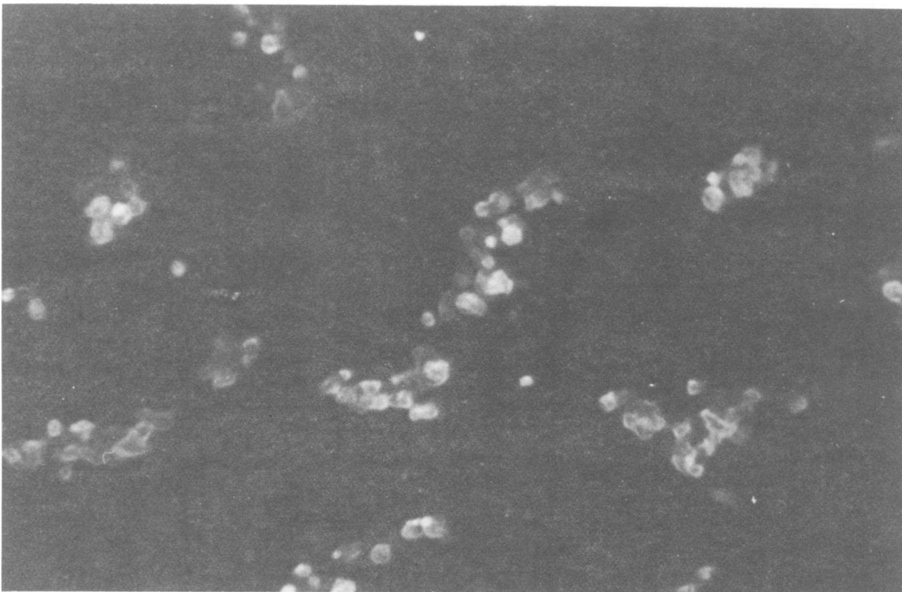

FIG. 4. *Porcine kidney cell monolayer infected with pseudorabies virus and stained with pseudorabies conjugate. ×100.*

enza-3-, and pseudorabies virus-infected cell cultures stained by this procedure. No interfering autofluorescence was observed in the noninfected control wells.

This technique is very useful for the FA identification of viral isolates, for testing viral isolates against a large number of FA conjugates, or for procedures using a FA serum-virus neutralization technique (1). It is as simple to perform as any standard FA tissue culture technique. The specific fluorescing cells are readily observed in the wells. Autofluorescence from the cells or plastic was not detectable.

Because of the limitation of the working dis-

tance of the objectives and diminished light intensity with 10× eyepieces, higher magnification for observing fine detail could not be obtained. Also, a red suppression filter (BG-38, E. Leitz, Inc., Rockleigh, N.J.) is recommended for photography with this system to eliminate a reddish background which is barely detectable to the eye.

Selected cell cultures in the plates can be maintained for further tests, such as observing cytopathic effect or plaque formation, by sealing the unstained wells with tape. If the seal is not complete, cross contamination within the plates or leakage from the plate may occur. This procedure can be made as safe as the standard FA technique now in use if the additional safety precautions mentioned above are used. Since the ether resistance of viruses used may not be known, the precautions should be used when performing all FA procedures. Sealing and inverting the plates are not necessary if an inverted microscope equipped for fluorescence microscopy is available in the laboratory.

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