

Rapid Detection of Influenza Virus by Shell Vial Assay with Monoclonal Antibodies

MARK J. ESPY,¹ THOMAS F. SMITH,^{1*} MAURICE W. HARMON,² AND ALAN P. KENDAL²

Section of Clinical Microbiology, Mayo Clinic and Foundation, Rochester, Minnesota 55905,¹ and Influenza Branch, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333²

Received 24 March 1986/Accepted 8 July 1986

Of 45 influenza virus strains (43 type A and 2 type B) detected in conventional tube cell cultures (average time, 4 days), 25 (56%) were detected by immunofluorescence in the shell vial assay 24 h postinoculation. The specific fluorescence produced should allow this procedure to be readily adapted by laboratories with various degrees of experience with immunofluorescence methodology.

In temperate countries, influenza virus infections occur every winter and epidemics associated with excess mortality have occurred at least once every 3 years since 1957 (1, 9). These infections occur especially in individuals at high risk for lower respiratory tract complications, such as persons with chronic disorders of the cardiovascular or pulmonary system and older persons without known underlying chronic diseases (2). Rapid techniques for the detection of cytomegalovirus and herpes simplex virus in shell vial cell cultures 16 h postinoculation by using monoclonal antibodies and the indirect fluorescent antibody procedure have yielded results superior to those obtained with conventional tube cell cultures (5, 6). In this communication, we report the use of monoclonal antibodies specific for influenza virus types A and B and immunofluorescence microscopy for the rapid detection of these viruses in a shell vial cell culture assay and compare the results with recovery of the virus in conventional tube cell cultures.

Throat swabs ($n = 250$; Culturettes; Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.) from patients with upper respiratory tract infections, obtained during the period December 1984 through March 1985, were rotated vigorously and thoroughly extracted in 2 ml of serum-free medium. Specimens (0.2 ml) were inoculated into two tube cell cultures (16 by 125 mm; Viomed Laboratories, Inc., Minneapolis, Minn., and M.A. Bioproducts, Inc., Walkersville, Md.) and into each of four 1-dram (ca. 3.7-ml) shell vials that contained round cover slips (diameter, 12 mm) seeded with primary rhesus monkey kidney (RMK) cells, as previously described (5). The tube cell cultures were incubated at 36°C on a roller drum and examined for cytopathic effects and for hemadsorption with guinea pig erythrocytes (11). The inoculated shell vials were centrifuged ($700 \times g$ for 1 h at 25°C), incubated at 36°C for 24 or 48 h, and stained with monoclonal antibodies by the indirect immunofluorescence technique. Monoclonal antibodies (A1, A3, B1, and B2) in mouse ascites fluid were directed to the nucleoprotein antigen of influenza virus type A and to the nucleoprotein and hemagglutination antigens of influenza virus type B and were prepared at the Centers for Disease Control, Atlanta, Ga. (7, 12). Goat anti-mouse immunoglobulin G labeled with fluorescein isothiocyanate was obtained from Cooper Biomedical, Inc., West Chester, Pa. (catalog no. 1211-0081).

Staining of the cover slips was performed by aspirating the medium from the vials and adding 0.2 ml of the ascitic fluid pools containing the monoclonal antibodies at a dilution of 1:300. The vials were incubated in a moist chamber at 36°C for 30 min and washed with phosphate-buffered saline (PBS). The PBS was aspirated immediately, and the shell vials were washed two more times with PBS (5 min each). After the PBS wash solution was aspirated, the cover slips were stained with 0.2 ml of conjugate at a dilution of 1:100 and then incubated at 36°C for 30 min in a moist chamber. The cover slips (in the shell vials) were washed as described above, mounted (cells face down) on glass slides, and examined at a magnification of $\times 200$ with a microscope (American Optical Corp., Buffalo, N.Y.) equipped with a mercury fluorescence light source. The results were recorded independently of the conventional tube cell culture results.

The effect of centrifugation on the infectivity of influenza virus type A (H3N2) was studied with RMK cell monolayers on cover slips in shell vials. Of 40 vials inoculated in each experiment, 10 each were centrifuged for 30, 45, and 60 min at $700 \times g$. The 10 vials that were not centrifuged were held at room temperature for 1 h. After centrifugation, serum-free medium was added to each vial, and they were incubated at 36°C for 24 h. The number of fluorescent foci on each cover slip was determined microscopically after the cover slips were stained with monoclonal antibodies as described above. The results were then analyzed statistically by two-way analysis of variance.

In the 250 specimens inoculated into conventional tube and shell vial cell cultures, 45 influenza virus strains were detected (43 type A and 2 type B). All of the strains were recovered in conventional tube cell cultures (mean time, 4 days); 25 (56%) of the isolates, including both type B strains, were detected in shell vials 24 h postinoculation. Two additional influenza virus type A strains were detected 48 h postinoculation, yielding a sensitivity of 60% (27/45) for the shell vial assay when compared with the conventional tube cell cultures. The shell vial assay was never positive in the absence of the isolation of influenza virus in conventional tube cell cultures (specificity, 100%). Positive specimens produced distinct homogeneous fluorescence throughout the infected cells that was easily and specifically recognized against the dark background of the uninfected cells of the monolayer (Fig. 1A and B). Although the patterns of fluorescence were identical for influenza virus types A and B,

* Corresponding author.

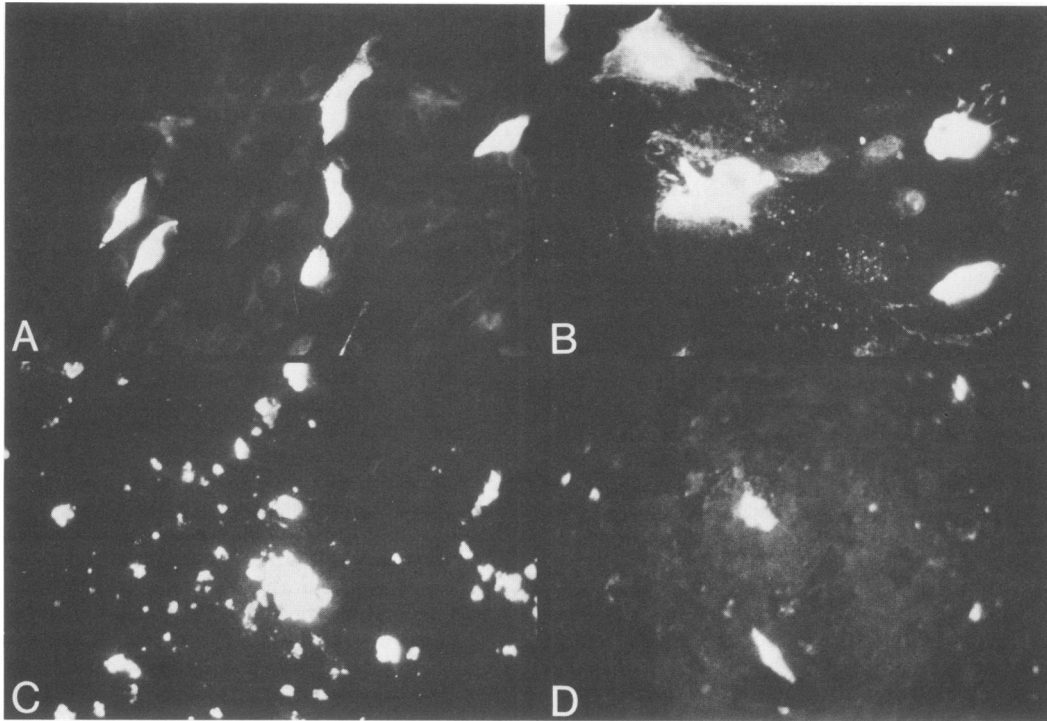


FIG. 1. Immunofluorescence of influenza virus-infected cells in shell vials 24 h postinoculation and staining of cells, obtained directly from patient, on glass slides. Shown are shell vials with influenza virus types A (A) and B (B) and glass slides with influenza virus types A (C) and B (D). Magnification, $\times 250$.

the monoclonal antibodies reacted specifically with the homotypic reference strains of the virus.

Centrifuged specimens (30, 45, and 60 min) produced more fluorescent foci in the shell vial assay system than did the noncentrifuged specimens; 30 min was as effective as the longer centrifugation times ($P < 0.05$) (Fig. 2).

The rapid laboratory diagnosis of influenza virus infections has usually been based on the detection of virus-infected cells in respiratory tract secretions by immunofluorescence techniques or alternatively by immunoassay procedures (3, 8). Recently, Shalit et al. (10) demonstrated that monoclonal antibodies to influenza virus types A and B developed by the Centers for Disease Control produced more sensitive (69 versus 46%) and accurate (86 versus 75%) results than did polyclonal antisera when they were com-

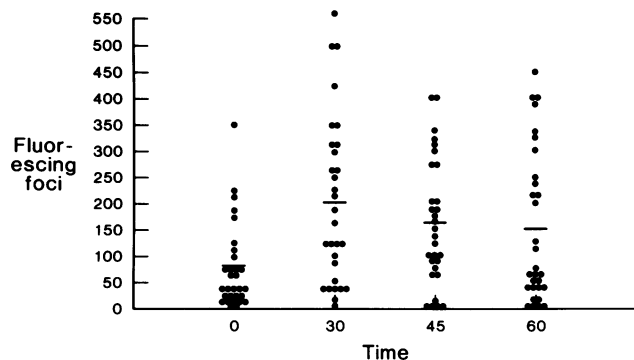


FIG. 2. Effect of time of centrifugation at $700 \times g$ on infectivity of influenza virus type A in shell vial assay.

pared for the recovery of 13 isolates from nasopharyngeal specimens inoculated into cell cultures. In their study, slides stained with monoclonal antibodies had reduced nonspecific fluorescence due to cellular debris compared with slides stained with polyclonal antisera. Although their test results were 100% specific, three (25%) of the potential positive specimens were interpreted as equivocal. In our laboratory, examination of washed cells from upper respiratory tract specimens that were placed on slides and stained by immunofluorescence revealed much cell debris. The fluorescence observed with monoclonal antibodies to influenza virus types A and B was frequently difficult to interpret as a specific reaction because many cells were disrupted and commonly clumped together (Fig. 1C and D). Subjective interpretation of specific fluorescence in these specimens is difficult even for laboratories with substantial experience, and the problem is usually increased due to a lack of intact cells on the slide.

In contrast to the predominance of influenza virus type A strains detected in 1985, influenza virus type B was the most prevalent during the last outbreak. Of 134 specimens submitted to our laboratory in January and February 1986, 36 (27%) were positive for influenza type B virus in shell vials; 22 (67%) were detected 24 h postinoculation. Thus, the shell vial assay was applicable for the early detection of clinical isolates of both the A and B influenza virus serotypes.

All of the procedures described for the rapid detection of influenza virus infections with these monoclonal antibodies developed by the Centers for Disease Control (direct detection by fluorescence in nasopharyngeal specimens [sensitivity, 69%] [10], enzyme immunoassay [sensitivity, 6 to 12% at 24 h and 79 to 94% at 48 h] [12], shell vial assay [sensitivity, 56% at 24 h]) lack the ultimate sensitivity obtained by isolation of the influenza virus serotypes in conventional cell

cultures. Because of the potential severity of these infections, rapid diagnostic results as obtained with the shell vial assay can hasten proper medical management and chemotherapy; however, conventional tube cell cultures are required in addition to inoculation of shell vials to assure the maximum sensitivity for the laboratory diagnosis of influenza virus infections (2, 4).

Compared with conventional tube cell cultures, advantages of the shell vial assay include the inoculation of cells on cover slips, thus eliminating the need to remove cells from the cultures and prepare smears for the fluorescent antibody stain. In addition, the fluorescence produced in influenza virus-infected shell vial cell cultures occurred in individual cells or in clusters of intact cells that were frequently present in small plaques that allowed specific identification of the virus. Because of the common practice of batching specimens in routine medical laboratory practice, the shell vial assay probably produces results that are reported to the attending physician as quickly as those obtained with the direct immunofluorescence test with cells obtained directly from the patient. Since specific immunofluorescence is easily recognized and has sensitivity comparable to that of other assays, we believe that the shell vial assay using these recently developed monoclonal antibodies is readily adaptable to laboratories that wish to use immunofluorescence for the diagnosis of influenza virus infections.

LITERATURE CITED

1. **Barker, W. H., and J. P. Mullooly.** 1980. Impact of epidemic type A influenza in a defined adult population. *Am. J. Epidemiol.* **112**:798-813.
2. **Centers for Disease Control.** 1985. Prevention and control of influenza. Recommendation of the Immunization Practices Advisory Committee. *Ann. Intern. Med.* **103**:560-565.
3. **Daisy, J. A., F. S. Lief, and H. M. Friedman.** 1979. Rapid diagnosis of influenza A infection by direct immunofluorescence of nasopharyngeal aspirates in adults. *J. Clin. Microbiol.* **9**:688-692.
4. **Dolin, R., R. C. Reichman, H. P. Madore, R. Maynard, P. N. Linton, and J. Weber-Jones.** 1982. A controlled trial of amantadine and rimantadine in the prophylaxis of influenza A infection. *N. Engl. J. Med.* **307**:580-583.
5. **Gleaves, C. A., T. F. Smith, E. A. Shuster, and G. R. Pearson.** 1985. Comparison of standard tube and shell vial cell culture techniques for the detection of cytomegalovirus in clinical specimens. *J. Clin. Microbiol.* **21**:217-221.
6. **Gleaves, C. A., D. J. Wilson, A. D. Wold, and T. F. Smith.** 1985. Detection and serotyping of herpes simplex virus in MRC-5 cells by use of centrifugation and monoclonal antibodies 16 h post-inoculation. *J. Clin. Microbiol.* **21**:29-32.
7. **McQuillin, J., C. R. Madeley, and A. P. Kendal.** 1985. Monoclonal antibodies for the rapid diagnosis of influenza A and B virus infections by immunofluorescence. *Lancet* **ii**:911-914.
8. **Minnich, L., and C. G. Ray.** 1980. Comparison of direct immunofluorescent staining of clinical specimens for respiratory virus antigens with conventional isolation techniques. *J. Clin. Microbiol.* **12**:391-394.
9. **Palese, P., and J. F. Young.** 1982. Variation of influenza A, B, and C viruses. *Science* **215**:1468-1474.
10. **Shalit, I., P. A. McKee, H. Beauchamp, and J. L. Waner.** 1985. Comparison of polyclonal antiserum versus monoclonal antibodies for the rapid diagnosis of influenza A virus infections by immunofluorescence in clinical specimens. *J. Clin. Microbiol.* **22**:877-879.
11. **Smith, T. F.** 1985. Viruses, p. 537-624. *In* J. A. Washington II (ed.), *Laboratory procedures in clinical microbiology*, 2nd ed. Springer-Verlag, New York.
12. **Walls, H. H., M. W. Harmon, J. J. Slagle, C. Stocksdales, and A. P. Kendal.** 1986. Characterization and evaluation of monoclonal antibodies for typing influenza A and influenza B viruses. *J. Clin. Microbiol.* **23**:240-245.