

## Comparison of Polyclonal Antiserum Versus Monoclonal Antibodies for the Rapid Diagnosis of Influenza A Virus Infections by Immunofluorescence in Clinical Specimens

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**A pool of monoclonal antibodies was compared with polyclonal antiserum for the rapid detection of influenza A virus in 28 clinical specimens by immunofluorescence. Monoclonal antibodies showed higher sensitivity (69 versus 46%) and accuracy (86 versus 75%) and easier slide interpretation than did polyclonal antiserum. The procedure proved useful for rapid detection of a community outbreak of influenza A virus infection.**

The diagnosis of influenza A virus infections is currently dependent on isolation of the virus in cell cultures or embryonated eggs, procedures usually requiring between 5 and 10 days (2). The mobilization of public health resources such as a community vaccination program and the effective use of drugs such as amantadine for prophylaxis depend on a rapid method of virus diagnosis. Detection by direct immunofluorescence (IF) of antigens of influenza A virus in nasopharyngeal cells from patients has been reported (1, 4, 5), but is not commonly used because of the lack of consistently reliable reagents. Monoclonal antibodies were used to identify influenza A isolates in cell culture (6), but have not been used for IF of nasopharyngeal cells. We report here the results of a comparative study involving the use of a polyclonal antiserum versus a pool of monoclonal antibodies for the rapid diagnosis of influenza A virus infections by the IF procedure on cells obtained from clinical specimens from patients with suspected influenza A virus infection.

Clinical specimens were collected from 28 patients (aged 1 to 46 years) with influenzalike symptoms during an outbreak of influenza A virus infection in the Oklahoma City area during January and February 1985. Throat swabs (Calgiswab Type III; Spectrum Diagnostics, Inc., Glenwood, Ill.) were obtained from 15 patients and placed in transport medium containing Eagle minimal essential medium with 5% fetal bovine serum and gentamicin. Nasopharyngeal washes were obtained from an additional 13 patients and placed in transport medium containing tryptic soy broth, 1% gelatin, and gentamicin plus amphotericin B (Fungizone). Specimens were inoculated into duplicate cultures of primary rhesus monkey kidney cells, Hep-2 cells, and human fibroblasts for isolation of viruses (2). The specimens were processed concurrently to obtain cells for analysis by IF (3). Each specimen was vortexed for 1 min, centrifuged at 1,200 rpm for 5 min, and washed three times in 0.01 M phosphate-buffered saline (PBS; pH 7.0). The cells were resuspended in 0.5 ml of PBS, and 20  $\mu$ l was applied to circumscribed areas of slides; the slides were air dried and fixed in acetone for 10 min.

Polyclonal antiserum (chicken) against influenza A virus, a negative serum control (chicken), and fluorescein-

conjugated sheep anti-chicken antibody were obtained from Wellcome Reagents, Burroughs Wellcome Co., Greenville, N.C. A pool of three monoclonal antibodies against epitopes present on viral matrix or nucleoprotein and reactive with influenza type A (H<sub>1</sub>N<sub>1</sub>) and (H<sub>3</sub>N<sub>2</sub>) viruses was obtained from the World Health Organization Collaborating Center for Influenza, Centers for Disease Control, Atlanta, Ga. The antibodies were in ascites fluid and did not inhibit hemagglutination. Goat anti-mouse immunoglobulin M-immunoglobulin G antibodies (fluorescein conjugated) were obtained from Tago Immunodiagnostics, Burlingame, Calif. Optimum dilutions of reagents were determined by block titrations with cell cultures infected with influenza type A (H<sub>1</sub>N<sub>1</sub>) and (H<sub>3</sub>N<sub>2</sub>) viruses.

Immunofluorescence (IF) tests with polyclonal antiserum were performed as follows: 20  $\mu$ l of a 1:5 dilution of antiserum in PBS was applied to each antigen well; control chicken serum was applied to a comparable well. Slides were incubated for 30 min in a humid chamber at 37°C, washed in PBS for 10 min and then in distilled water for 1 min, and air dried; 20  $\mu$ l of a 1:40 dilution of fluorescein-conjugated sheep anti-chicken antiserum in PBS-0.02% Evans blue was applied to each well. Incubation, washing, and drying were repeated as above. The same procedure was used for the monoclonal antibody pool, except that a 1:50 dilution of monoclonal pool and a 1:40 dilution of fluorescein-conjugated goat anti-mouse immunoglobulin M-immunoglobulin G were used. Each slide was viewed by fluores-

TABLE 1. Comparison of isolation of influenza A viruses in cell culture with immunofluorescence of cells obtained from the same clinical specimens

Isolation in cell cultures	Immunofluorescence test with:			
	Polyclonal antiserum		Monoclonal antibodies	
	No. positive	No. negative	No. positive	No. negative
Positive ( <i>n</i> = 13)	6 (10) <sup>a</sup>	7 (3)	9 (12)	4 (1)
Negative ( <i>n</i> = 15)	0	15	0	15
Total	6 (10)	22 (18)	9 (12)	19 (16)

<sup>a</sup> Numbers in parentheses are the numbers obtained if equivocal results were considered positive.

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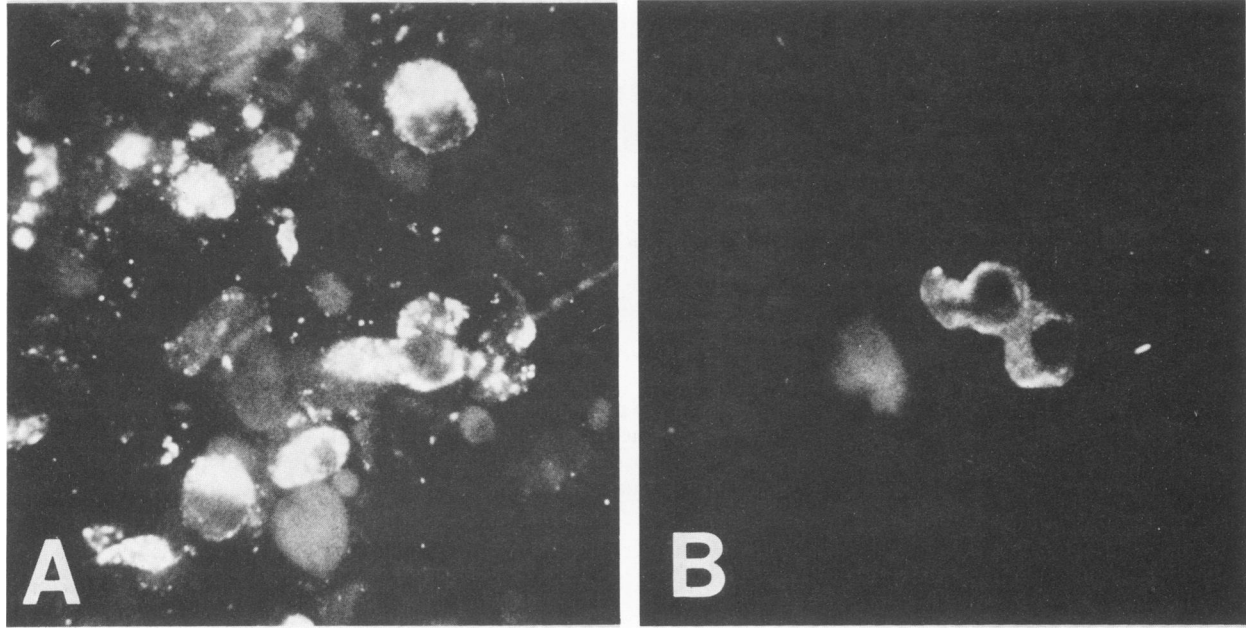


FIG. 1. Immunofluorescence of cells from a nasopharyngeal wash of a patient with culture-proven influenza A (H<sub>3</sub>N<sub>2</sub>) virus infection. (A) Polyclonal antiserum; (B) monoclonal antibodies.

cence microscopy by two independent readers ignorant of the viral culture results. Slides were read as positive or negative when both readers were in agreement and as equivocal when the readers disagreed.

Of the 28 clinical specimens, 13 yielded influenza A virus, 3 yielded respiratory syncytial virus, and 1 yielded parainfluenza 3 virus in cell cultures. Eleven specimens did not yield a virus.

Table 1 presents the correlation between isolation of virus and IF results with polyclonal and monoclonal antibodies. Analysis of the IF results are presented in Table 2. Positive IF reactions with polyclonal or monoclonal antibodies on cells obtained from clinical specimens are shown in Fig. 1.

The average time for slide preparation, immunofluorescent staining, and reading was approximately 4 h. No significant difference was found between IF results of slides prepared from throat swab specimens and those from nasopharyngeal washes. Specimens tested by polyclonal antiserum were, however, more difficult to interpret than those tested by monoclonal antibodies owing to increased, nonspecific fluorescence of cellular debris and of bacteria adherent to epithelial cells; these phenomena were not apparent when monoclonal antibodies were used. A typical positive reaction with either antibody preparation produced a bright fluorescence in the cytoplasm and a dark nucleus.

The immunofluorescence test with polyclonal antiserum or monoclonal antibodies rapidly detected influenza A virus antigens directly in cells obtained from clinical specimens. Monoclonal antibodies showed greater sensitivity and accuracy than did polyclonal antiserum, and the monoclonal preparation was easier to interpret. No false-positive results were found with either antibody preparation. All equivocal IF results were culture positive (Table 1). A lack of experience with the procedure, in particular the evaluation of fluorescent patterns, may have led to the equivocal readings. Additional experience is likely to produce results indicating increased sensitivity, as reflected in Table 2.

Pooled monoclonal antibodies against virus antigens common to influenza A virus strains may be useful for rapid detection of outbreaks of influenza A virus infections owing to the high specificity and positive predictive value of the test. Despite the relatively low sensitivity, in the context of rapidly identifying a community outbreak of influenza there are multiple specimens available for analysis, and any number of positive tests will verify an outbreak of influenza A virus infection within a few hours. In an individual patient, however, the low sensitivity of the test is a limitation; a positive test is, nevertheless, useful owing to its high positive predictive value.

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TABLE 2. Analysis of immunofluorescence results obtained with polyclonal antiserum and monoclonal antibodies

Type of antibody	Sensitivity (%)	Specificity (%)	Predictive values (%)		Accuracy (%)
			Positive	Negative	
Polyclonal	46 (77) <sup>a</sup>	100	100	68 (83)	75 (89)
Monoclonal	69 (92)	100	100	79 (94)	86 (46)

<sup>a</sup> Percentage if equivocal results were considered positive.

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