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Comparison of Directigen FLU-A with Viral Isolation and Direct Immunofluorescence for the Rapid Detection and Identification of Influenza A Virus

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Directigen FLU-A, an enzyme immunoassay membrane test, was compared prospectively to isolation in cell culture and direct immunofluorescence (IF) for the detection of influenza A virus. One hundred ninety specimens were evaluated by Directigen FLU-A and cell culture; 184 of these specimens were also tested by direct IF. The sensitivity of Directigen FLU-A compared to isolation in cell culture and direct IF was 100%. The specificities of Directigen FLU-A compared to isolation and direct IF were identical, 91.6%. Fourteen specimens that were positive by Directigen FLU-A did not yield virus in culture; two of the specimens, however, were positive by direct IF, and four other specimens were not specimens of choice for the test. A positive Directigen result had positive predictive values of 62.6 and 75.0% compared to isolation and direct IF, respectively; a positive Directigen result with an intensity reading of 2+ or greater, however, had positive predictive values of 85 and 100% compared to isolation and direct IF, respectively. In all comparisons, the negative predictive value was 100%. There was no evidence that cross-reactivity occurred with non-influenza A antigens. Directigen FLU-A should serve as a convenient screening test for influenza A and as a rapid test supported by isolation in cell culture during an influenza outbreak.

Disease resulting from influenza infection ranges from mild respiratory illness to fatal viral pneumonia; secondary bacterial pneumonia may be a serious complication. Influenza typically occurs in epidemics which may result in an average of approximately 30,000 excess deaths in the United States (13). The elderly and individuals with underlying health problems are particularly at risk.

Serious disease is generally associated with influenza types A and B; type C infection may result in a mild respiratory infection, usually in children (11). Influenza virus may be isolated in embryonated eggs or cell cultures (5, 16), although a cytopathic effect may not always be evident. Infection is usually detected in inoculated cultures by hemadsorption, which may identify most isolates within 3 days (15). The isolates are identified specifically by the hemagglutination inhibition test (9), immunofluorescence (IF) (18), or enzyme-linked immunosorbent assay (22). Isolation and definitive identification of the isolate invariably require several days. Application of centrifugation enhancement, however, to identification of influenza virus in clinical specimens via inoculation of cell cultures reduces the time for identification to 24 h (4, 20).

IF (2, 6, 12, 14, 19) and enzyme-linked immunosorbent assay (1, 6, 10, 17) have been most commonly applied to the rapid detection of influenza antigen in clinical specimens. Rapid detection is particularly important because of the availability of amantadine, an antiviral agent shown to be beneficial in the treatment and prophylaxis of influenza A infections (3, 8, 12, 21).

In this study we evaluated (7) and compared a newly developed assay that required less than 15 min to complete with isolation in cell culture and direct IF for detection of influenza A virus in clinical specimens.

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MATERIALS AND METHODS

Specimen collection. One hundred ninety specimens were obtained from 190 individual patients with a presumptive diagnosis of influenza infection as part of routine diagnostic protocols. Nasopharyngeal washes were collected from 173 patients as previously described (25); throat swabs were obtained from 7 patients; one nasopharyngeal swab was taken from a single patient. Four tracheal aspirates, two sputum specimens, two bronchial washes, and one gargle were also obtained from individual patients; these specimens are not considered specimens of choice by the manufacturer. One hundred forty patients were less than 2 years old; 31 patients were between 2 and 10 years old, and the remainder of the patients were greater than 10 years old. All specimens except the sputum specimens, bronchial washes, and gargle were placed in transport medium consisting of tryptic soy broth, 0.5% gelatin, and antibiotics. Specimens were transported to the laboratory on wet ice and stored at 4°C until processing, usually within 4 h but never later than 24 h. Approximately 0.4 to 0.5 ml of each specimen was stored at −70°C for further analysis.

Virus isolation. Specimens were inoculated onto monolayers of primary rhesus monkey kidney (PRMK), MRC-5, and HEp-2 cell cultures. The maintenance of the inoculated cultures and the identification of viral isolates were described previously (24). In brief, inoculated cultures were viewed daily for 2 weeks for cytopathic effect. Hemadsorption tests employing guinea pig erythrocytes were performed at 4 and 22°C on PRMK cultures every 2 to 4 days. Virus isolates were identified by indirect IF, using monoclonal antibodies applied to cells scraped from culture tubes (23).

Direct IF for detection of influenza A in cells obtained from clinical specimens. Following the inoculation of cell cultures, the cells from approximately 0.5 ml of each specimen were obtained by centrifugation at 500 × *g* for 10 min. The cells

TABLE 1. Comparison of Directigen FLU-A with isolation of influenza A virus in cell culture

Result by Directigen	No. of specimens	No. of results by isolation in cell culture	
		Positive	Negative
Positive	37	23	14
Negative	153	0	153

were prepared and stained by direct IF for the detection of influenza virus A; influenza virus B; parainfluenza viruses 1, 2, and 3; and respiratory syncytial virus antigens as described elsewhere (25). The reagents used in the indirect fluorescent antibody procedure were monoclonal antibodies to the viruses prepared in this laboratory (23, 25) and fluorescein-conjugated anti-mouse immunoglobulin G.

Directigen FLU-A. The Directigen FLU-A antigen test was performed according to the directions of the manufacturer (Becton Dickenson Microbiology Systems, Cockeysville, Md.) on unmodified samples of clinical specimens at the same time that the inoculation and IF procedures were conducted. The test is an enzyme immunomembrane filter assay. Influenza A antigen in 125 μl of specimen is bound to the surface of a membrane. The antigen is detected by an enzyme-linked immunosorbent assay procedure utilizing enzyme-conjugated monoclonal antibodies that are specific for a conserved epitope of the nucleoprotein. A result was available in less than 15 min. A complete purple triangle indicated a positive result; a purple dot in the center of the triangle was evidence of a valid test, although the dot could be obscured by a strong positive reaction. Reactive tests were graded as weak and 1+ to 4+ on the basis of the intensity of the purple triangle. A test was judged negative if a purple triangle was not visible and the control dot was apparent. An uninterpretable reading resulted when neither a triangle nor control dot appeared. Following an uninterpretable test result, the specimen was diluted in viral transport medium and the test was repeated.

Blocking assays were performed by the manufacturer on the aliquots of specimens held at −70°C. The basic test procedure was utilized but included the addition of an incubation step with blocking antibody prior to the addition of the detection reagents.

RESULTS

Comparison of Directigen FLU-A with isolation of influenza A virus in cell cultures. All of the 190 specimens collected were inoculated into cell cultures. Thirty-seven specimens produced positive reactions by Directigen FLU-A; 23 of these specimens yielded influenza A virus in cell cultures. All of the specimens read as negative by Directigen FLU-A were also negative in cell culture. The sensitivity and specificity of Directigen FLU-A compared with isolation in cell culture were 100 and 91.6%, respectively; the positive predictive value was 62.6%, and the negative predictive value was 100% (Table 1).

Of the 190 specimens tested, 10 were initially uninterpretable because of difficulties in filtration. The specimens were diluted 1:2 to 1:5 and retested. Of two culture-positive specimens, one reacted positive and one was negative in the Directigen FLU-A test. One of eight remaining specimens that were culture negative was reactive in the Directigen FLU-A test.

TABLE 2. Intensity of the Directigen FLU-A reactions relative to the time required for identification of virus in cell culture

No. of days to identification of virus in cell culture	No. of Directigen results at grading intensity of:			
	1+	2+	3+	4+
2–3	2	3	1	7
4–5				3
6–7	4	1		2

The intensity of the Directigen FLU-A reactions relative to the number of days required for the identification of virus in cell culture is shown in Table 2. Overall, there was a tendency for specimens yielding virus in culture in less than 5 days to also show stronger intensity readings by Directigen FLU-A.

Sufficient quantities of 13 of the 14 specimens giving false-positive results were available for blocking assays. Five specimens were negative in blocking assays, although two of the five specimens were positive by direct IF. The remaining eight specimens, however, were not positive in the Directigen FLU-A test after freeze-thawing, indicating possible lability of the antigen identified by the test. Five of the eight specimens that did not repeat after freeze-thawing had given 1+ intensity readings, two were weak, and one was 3+. Weak intensity readings were therefore associated with false-positive results and antigen lability. The absence of a positive reaction with a specimen following freeze-thawing prevented the performance of blocking assays.

Comparison of Directigen FLU-A with identification of influenza A antigen by IF. Some laboratories use direct IF exclusively to detect influenza A or only as a rapid test without inoculation of cell cultures. Similarly, in this study, not all of the specimens examined were inoculated into cell cultures and also tested by direct IF. The 184 specimens tested for influenza antigen by Directigen FLU-A and direct IF were therefore compared. Twenty-four specimens were reactive by both tests, and 152 specimens were unreactive by both tests. There were eight specimens that were reactive by Directigen FLU-A and unreactive by direct IF (Table 3). Compared to direct IF, Directigen FLU-A had a specificity of 91.6% and a sensitivity of 100%; the positive and negative predictive values were 75.0 and 100%, respectively.

A comparison of direct IF versus isolation in cell culture was made to better evaluate Directigen FLU-A versus IF under the conditions of this study. Direct IF was 100% sensitive and 98.1% specific compared to isolation in cell cultures; 3 of 23 specimens tested by direct IF and isolation were reactive by IF but did not yield virus in cell culture (false-positive); the positive predictive value was 86.9%. One hundred fifty-eight specimens were negative in both tests; there were no false-negative direct IF tests. Two specimens that were culture negative but direct IF positive were reactive in the Directigen FLU-A test.

TABLE 3. Comparison of Directigen FLU-A with direct IF

Result by Directigen	No. of specimens	No. of results with direct IF	
		Positive	Negative
Positive	32	24	8
Negative	152	0	152

TABLE 4. Comparison of Directigen FLU-A with isolation in cell culture and/or direct IF

Result by Directigen	No. of specimens	No. of results by isolation in cell culture and/or direct IF	
		Positive	Negative
Positive	29	23	6
Negative	155	0	155

Comparison of Directigen FLU-A with isolation in cell culture and/or detection of influenza A antigen by direct IF. One hundred eighty-four specimens were tested by all three procedures. Compared to isolation in cell culture and/or direct IF, Directigen FLU-A was 100% sensitive and 96.3% specific; the positive and negative predictive values were 79.3 and 100%, respectively (Table 4).

The intensity of the Directigen FLU-A results in relation to the number of specimens yielding or not yielding virus in cell culture and the number of specimens reactive or nonreactive by direct IF is shown in Table 5. Of the 14 specimens reactive by Directigen but negative in cell culture, 11 had weak (5 specimens) or 1+ (6 specimens) Directigen readings. All eight of the specimens negative by direct IF gave weak (three specimens) or 1+ (five specimens) readings of intensity by Directigen FLU-A. Low-intensity readings are therefore more likely to be associated with false-positive results.

Identification of viruses other than influenza A. For the 190 specimens examined, 57 viruses other than influenza A were isolated in cell cultures and/or identified by direct IF; these included 51 identifications of respiratory syncytial virus, 4 of adenovirus, 1 of cytomegalovirus, and 1 of parainfluenza virus type 3. There were two dual infections of influenza A and respiratory syncytial virus; in both instances Directigen FLU-A identified influenza antigen. In one instance, Directigen FLU-A reacted weakly with a specimen that yielded adenovirus in cell culture. The direct IF was not reactive for influenza A, and the adenovirus destroyed the PRMK culture in 3 days; hemadsorption was not performed, and the culture could not be finalized as negative for influenza A. The Directigen FLU-A reaction was, nevertheless, considered to be a false-positive, although the criteria were stringent. There was no indication from the study that cross-reactivity or interference occurred in Directigen FLU-A tests because of the presence of non-influenza A antigens.

DISCUSSION

Directigen FLU-A compared favorably in sensitivity (100%) and specificity (91.6%) with isolation in cell culture, generally considered as the standard by which other diagnostic tests are compared. There were, however, 14 false-positive Directigen results, which produced a false-positive rate of 8.4% and a positive predictive value of 62.2%. Of the 14 specimens giving false-positive results, 11 gave Directigen intensity readings of weak or 1+, indicating that the greater the intensity reading of the test, the more likely the result is to be a true-positive. Low intensity readings may not be neglected, however, because 10 of the 23 true-positive readings were 1+ or 2+.

TABLE 5. Comparison of the intensity of Directigen FLU-A results with isolation in cell culture and direct IF

Intensity of Directigen test	No. of specimens	No. of results by:			
		Culture		Direct IF	
		Positive	Negative	Positive	Negative
4+	12	12	0	12	0
3+	2	1	1	1	0
2+	5	4	2	4	0
1+	12	6	6	5	5
Weak	6	0	5	2	3

Of the specimens tested in this study, only the nasopharyngeal washes, nasopharyngeal swab, and throat swabs are designated as specimens of choice by the manufacturer. The two sputum specimens and two of the four tracheal aspirates tested gave false-positive results with Directigen FLU-A. If the nine specimens not considered to be specimens of choice were eliminated from the evaluation, the specificity of Directigen FLU-A versus isolation in cell culture would be 93.6% and the positive predictive value would be 69.7%; in comparison with isolation in cell culture and/or direct IF, the specificity was 98.7% and the positive predictive value was 92.0%. Although the number of nonchoice specimens evaluated was small, adherence to the manufacturer's recommendations for specimen collection seems prudent.

Blocking results were not available for 8 of the 14 specimens giving false-positive results because reactivity in the Directigen FLU-A test was not recovered after freeze-thawing. Some number of these specimens, therefore, may have been true-positives. Alternatively, freeze-thawing may remove the factor(s) responsible for some false-positive results. Until the latter point is investigated, however, specimens should not be frozen prior to testing by the Directigen test.

Directigen FLU-A showed the same specificity and sensitivity compared with direct IF as it did compared with isolation in cell culture. The positive predictive value versus direct IF was 75.0%, however, reflecting the smaller number of false-positive results. Compared with cell culture and/or direct IF, the specificity increased to 96.3% and the positive predictive value was 79.3%. The fewer false-positives (six) seen in the latter comparison reflect the increased number of reactive specimens encountered when multiple tests are utilized. In all comparisons, the negative predictive value was 100% and the false-negative rate was 0. A negative Directigen FLU-A result, therefore, was a reliable indicator that influenza A was not present. A positive result with an intensity reading of 2+ or greater had positive predictive values of 85.0 and 100% versus cell culture and direct IF, respectively; the false-positive rates were 1.9 and 0%, respectively. Directigen readings with intensities greater than 2+, therefore, may be accepted with a high degree of reliability.

The Directigen FLU-A test is easy to use and produces results in less than 15 min. Availability of the test to non-medical center environments during an influenza outbreak should be an important adjunct to the clinical diagnosis in making recommendations regarding antiviral treatment. In the diagnostic virology laboratory the test may be a useful addition to or substitute for direct IF as a rapid procedure, particularly during hours when direct IF service is not available.

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