Rapid Diagnostic Methods for Influenza Virus in Clinical Specimens: A Comparative Study

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A comparison of five rapid viral diagnostic techniques for identifying influenza virus in nasopharyngeal aspirates has been made on patients with influenza-like illnesses. Initial results with immune electron microscopy were positive in only one of 11 specimens from which virus was isolated and further work abandoned. Four other rapid tests were carried out on 39 specimens from which influenza virus had been isolated in tissue culture in 28. Of these 28 specimens yielding virus, 24 (85.7 percent) were positive by an indirect fluorescent antibody test (IFAT) on nasopharyngeal cells; 18 (64.3 percent) by enzyme-linked immunosorbent assay (ELISA), 19 (67.8 percent) by enzyme-linked fluorescent assay (ELFA), and 26 (92.8 percent) by a rapid tissue culture amplification method (TCA) in a continuous Rhesus monkey kidney line (LLC-MK) with identification of virus by fluorescent antibody. In terms of sensitivity, simplicity, and rapidity, a combination of the IFAT and TCA methods seems to be very useful.

The techniques for rapid diagnosis of viral infections have created much interest, including the attention of the World Health Organization [1,2]. The methods explored for the rapid detection of influenza antigen in nasal secretions have included electron microscopy [3], immunofluorescence [4–10], enzyme-linked immunoassay (ELISA) [12–16], and tissue culture amplification [15]. A fluorometric assay has also been used to identify influenza neuraminidase in nasal washings [14]. Most of these studies have compared one or two of these procedures with a standard technique such as isolation of the virus [16]. In the current investigation we have compared five rapid techniques with standard isolation methods for detection of influenza antigen in nasopharyngeal aspirates obtained from students with an influenza-like syndrome.

MATERIALS AND METHODS

Study Patients

The 39 patients were primarily students from the University of Vermont or from Yale University who came to the outpatient department, or were hospitalized in the University Health Service units with influenza-like illnesses occurring during periods of known influenza activity from 1978 to 1981. In a few of these patients the exact age was not known. Informed consent was obtained from each.
Collection of Materials

Nasopharyngeal aspirates were obtained by the principal investigator, or under his supervision, using an infant nasal suction set with a collection trap (BP Becton Dickenson and Co., Rutherford, NJ) attached to a suction pump (model LR-22132, Doerr Electric Co., Cedarburg, WI) at 26 lb/sq inch vacuum pressure following the method of Gardner and McQuilllin [8]. The day of illness on which the 39 samples were collected varied from 1 to 8 and were distributed as follows: 1, day 1; 8, day 2; 11, day 3; 10, day 4; and 3 each on days 5, 6, and 8. Approximately 2 ml of transport media (Hank's balanced salt solution, HEPES buffer, and 0.5 percent gelatin) was used to rinse the tubing of the suction set. They were immediately placed in ice for transport to the laboratory. On arrival in the laboratory the cells were gently centrifuged out at 1500 rpm for 10 minutes at 4°C and smears prepared for the indirect fluorescent antibody test; the supernatant fluid was frozen at −70°C for other tests. A cell smear was considered satisfactory when it contained numerous undamaged respiratory epithelial cells per 250× field. Satisfactory smears were obtained in 34 of the 39 aspirates (87.1 percent). This usually consisted of four to five undamaged cells per high-power field.

Standard Isolation

This term is used to indicate the routine isolation of influenza viruses carried out in the Connecticut State Laboratory using primary Rhesus monkey kidney cells and hemadsorption of red cells to indicate the presence of virus at five and 14 days after inoculation.

Serologic Tests

Standard CDC hemagglutination-inhibition and complement fixation tests on microtiter plates [16] were carried out in our laboratory or through the cooperation of the Virology Laboratory, Connecticut Department of Health Services, on acute and convalescent sera. Acute-phase sera were obtained in all, but paired sera were obtained in only 12 patients, as the remaining students failed to appear for follow-up bleedings.

Immune Electron Microscopy (IEM)

The two immune electron microscopy methods (IEM) used consisted of (1) mixing the specimen with immune sera as described by Edwards et al. [3] and of (2) antigen concentration by the agar-diffusion-filtration technique of Kelen et al. [17]. Slides covered with 0.8 percent Ionagar were used. The specimens suspected of containing virus, or a known influenza strain, were mixed with specific antisera for 30 minutes at 37°C and placed on a slide, and a formvar carbon-coated grid was laid upside down on the drop. When diffusion was complete, the grid was removed and stained with 2.0 percent phosphotungstic acid solution, pH 6.5, for a few seconds. Initially the specimens were spun at 10,000 rpm/30 minutes and resuspended in one-tenth the volume before mixing with immune serum, but later the "grid on drop" method was employed [17]. A Phillips Model EM 201 was used to examine the specimens. Specimens were usually examined independently by two observers.

Indirect Immunofluorescence Antibody Test (IFAT)

The methods closely followed those described by Gardner and associates [8,9]. Great care was taken to keep the nasopharyngeal washings iced until they were pro-
cessed and to handle the cells gently. The sediment (SD) from light centrifugation was gently resuspended in phosphate-buffered saline (PBS), washed twice, and resuspended. Pre-cleaned and acetone-stored slides were used, and rings were marked with a Tech-pen. A drop of fluid with cells was added to the slide, which was air-dried and fixed in chilled acetone for 10 minutes at 4°C. Staining of specimens can be delayed at this point by storing at 4°C overnight or freezing at −20°C for later staining. Influenza antisera prepared in chickens against various strains (a/USSR/90/77, H1 titer 1:160; A/Texas/1/77, H1 titer 1:160; B/Hongkong/5/72, H1 titer 1:160) were provided by CDC. A fluorescein-conjugated IgG fraction of rabbit anti-chicken sera (heavy and light chains) was purchased from Cappel Laboratories (Cochranville, PA). Both reagents were absorbed with tissue culture cells as described by Gardner and McQuillin [8]. We also used bovine antisera to A influenza (H1N1 and H3N2) and fluorescein-conjugated rabbit anti-bovine immunoglobulins kindly provided by David W. Barry, M.D., Wellcome Reagents Division, Burroughs Wellcome Co. (Research Triangle Park, NC). Both the Cappel and Burroughs Wellcome antisera gave satisfactory results. The cells were covered with unconjugated sera (1:10) in PBS, pH 7.2, incubated in a moist chamber at 37°C for 30 minutes, washed three times, and air-dried. Then a drop of the labeled conjugate was added, and the cells were incubated as before, washed, and air-dried. The cell preparations were usually read in a Leitz Dialux 20 fluorescence microscope with an overhead halogen light source at 250× under a water immersion lens. The results were read independently by two observers. A positive test was one with cells showing definite intracellular fluorescence not seen in negative controls prepared at the same time. Controls included uninfected cells, antiserum to another influenza group, and known influenza-infected cells.

**ELISA and ELFA**

The enzyme-linked immunosorbent assay (ELISA) and fluorescent assay (ELFA) methods were based on those of Yolken and associates [12,14], of Voller et al. [18], of Harmon and Pawlik [19], and of Chao et al. [20]. We employed an ELISA double antibody sandwich method [18]. Monospecific goat anti-influenza A/USSR/90/77, A/Texas 1/77, or B/Hongkong/5/72 (Dynatech Diagnostics, South Windham, ME) antisera was used as capture antibodies. Monospecific mouse monoclonal antibodies directed respectively against the hemagglutinin of influenza A (A/USSR/90/77) [21], A/Texas 1/77, and influenza B (B/Oregon/5/80) [22] containing sodium azide as a preservative were used as detector antibodies. The monoclonal antibody preparations were kindly provided to us by Dr. Robert G. Webster, St. Jude's Children's Research Hospital, Memphis, TN; their preparation and use have been described by him [21,22]. We modified Voller et al.'s method [18] for the ELISA test by washing plates with saline without sodium azide instead of PBS and adding 0.5 percent gelatin in making serial dilutions [19]; Tween 20, at 0.05 percent, was employed in both solutions. Microtiter wells coated with goat anti-influenza serum diluted 1:1000 in carbonate-bicarbonate buffer, pH 9.6, were placed in a moist chamber overnight at 4°C. They were washed four times for 3 minutes each with Tween 20/saline. Then test specimens were added (nasopharyngeal aspirate supernatant or control material), and the wells were again incubated in a moist chamber overnight at 4°C. They were then washed four times for 3 minutes each, mouse monoclonal antibody was added at a 1:100 dilution, and the wells were placed for 2 hours at 37°C in a moist chamber and then washed four times for 3 minutes each. Alkaline phosphatase conjugated goat anti-mouse IgG sera (Litton Bionetics, Kens-
ingston, MD) were added at a dilution of 1:50, and the specimens were placed for 2 hours at 37°C in a moist chamber, washed five times for 3 minutes each and the substrate added (p. nitrophenyl phosphate, disodium at 1 mg/ml, Sigma Chemical, St. Louis, MO) in 10 percent diethanolamine buffer. The test was read in a Titertek Multiscan Reader (Flow Labs, McLean, VA) at 405 nm at three hours, and again after incubating overnight at 4°C. The latter gave higher readings. At least five to six controls were included in each test, consisting of normal allantoic fluid, virus-negative aspirates, and a B/HK antigen in influenza A tests. Positive controls (known influenza virus at 10^-2 and 10^-3 dilutions) were also included. A positive specimen was defined as one with a reading three or more standard deviations (SD) higher than the negative controls employing virus-negative aspirates, as recommended by Harmon et al. [19] and Chao et al. [20] for ELISA detection of respiratory antigens by the ELISA test. The use of two or more SD as positive increases sensitivity slightly at the cost of reducing specificity.

For the ELFA test, the ELISA plates were washed twice with saline/Tween 20 after reading. The reagents used were those employed by Yoklen et al. [14,23]. MUP reagent (4-methylumbelliferyl phosphate from Sigma Chemical, St. Louis, MO) was added to the specimens, and they were examined in a UV box (Block-Ray, Transilluminator, long wave C-62, peak wavelength approximately 390 nm) 45 to 60 minutes later. The test was read visually by two observers independently using Contrast Control UV Safety goggles (Ultra Violet Products, Inc., San Gabriel, CA) to protect the eyes. A positive test was one showing "significantly" more UV color than the controls.

**Tissue Culture Amplification (TCA)**

Because of the low concentrations of virus that may be present in nasopharyngeal aspirates, short-term tissue culture amplification was carried out in a continuous line of Rhesus monkey kidney cells (LLC-MK2) from Flow Labs (McLean, VA). The presence of virus was then sought by immunofluorescence at 24-, 48-, and 72-hour periods in cells scraped off and fixed on a glass slide. We also examined for cytopathic effect (CPE) and for hemadsorption of guinea pig red cells (Krutulis Labs, Bridgeport, NY) at the same time [16]. We also compared the Madin-Darby canine kidney line (MDCK) (Flow Labs) and the LLC-MK2 line. Prior to inoculation both tissue culture cell lines were grown in medium 199-E containing 10 percent fetal calf serum, glutamine, and penicillin-streptomycin; after inoculation the same medium was used without serum. In a number of experiments the effect of incorporating trypsin (crystalline, bovine pancreas, Calluchem, La Jolla, CA) at 5 μg/ml in the growth media to enhance viral entry into the cell was tested, as suggested by Davies et al. [15].

**Test Comparisons**

The results of the IFAT, ELISA, ELFA, and TCA were based on the 28 specimens that yielded influenza virus in tissue culture. Of 12 paired acute and convalescent sera available from the 28 virus-positive cases all showed a fourfold or greater rise in antibody titer.

**RESULTS**

**Immune Electron Microscopy**

Eleven nasopharyngeal aspirates from patients with influenza-like illnesses, each shown to contain influenza virus by standard isolation techniques in tissue culture
Rapid tests for influenza

Carried out in the Connecticut State Laboratory, were tested by direct [3] and agar concentration IEM techniques [17]. Five grid areas were examined in duplicate preparations under a Phillips Model 201 electron microscope. Only one grid in one specimen revealed aggregated particles which resembled influenza. IEM examination carried out on serial dilutions of known laboratory strains of influenza viruses gave variable and often unpredictable results. As other investigators had also informally reported (at NIH Conference on Rapid Viral Diagnosis, Bethesda, MD, 1980) a similar insensitivity and unreliability of the IEM procedure for identifying influenza virus in clinical specimens, we abandoned the procedure.

The detailed results on 28 specimens from which influenza virus was isolated are presented in Table 1. Of 12 paired sera available from these patients all showed four-fold antibody titer rises. The individual tests are discussed below.

Indirect Immunofluorescent Antibody Test (IFAT)

Of 28 specimens yielding either influenza A or B virus in tissue culture, the IFAT was positive in 24 (85.7 percent). However, if only the 26 specimens with an adequate number of cells for IFAT examination are considered, then 24/26 or 92.3 percent were identified by IFAT. The correlation between virus isolation and the IFAT is shown in Table 2. In addition to the 28 virus-positive specimens, the IFAT was also positive in five specimens not yielding virus (Tables 2 and 3). Three of these specimens were confirmed by at least two other diagnostic tests; two were not confirmed and thus probably represent “false positives.”

ELISA and ELFA

Many different capture and detector antibodies, as well as different conjugates, diluents, and incubation periods were tested before satisfactory results were obtained. The major factor contributing to a workable system was the use of mouse monoclonal antibody, kindly provided by Dr. Robert G. Webster, which we employed as the detector antibody. The tests were read in a Titermark Multiscan Reader. A positive test was defined as one with a value of three or more standard deviations (SD) higher than the control reading. On this basis 18 of 28 (64.3 percent) specimens yielding virus in tissue culture were positive by the ELISA test. If we lowered the level of positive to two or more SD, then two more tests would be positive (20 of 28 or 71.4 percent) but one heterologous control would also fall into this range (Table 1). In the ELFA test, read visually and subjectively under an ultraviolet light, 19 (67.8 percent) of these same 28 specimens were recorded as positive by two independent observers. The correlation of these tests with tissue culture isolation in the total of 39 specimens tested is shown in Table 4. The ELISA and ELFA were both positive (or weakly positive) in six specimens from which no virus was isolated (Table 3). In three of these at least one other test was positive; the other three may be “false positives.”

Tissue Culture Amplification (TCA)

Of the 28 specimens yielding influenza virus in tissue culture 19 (67.8 percent) had been isolated in both primary Rhesus monkey kidney cell cultures (PRCC) at the Connecticut State Laboratory and in a continuous line of Rhesus monkey kidney cells (LLC-MK_2) in our laboratory; two were positive only in the former (7.17 percent) and seven were positive only in the latter (25.0 percent). The presence of virus was established by hemadsorption of red cells at five days and 14 days at the Connecticut State Laboratory and in our laboratory by fluorescent antibody tests on
| Specimen Number | Year | Age | Day of Illness | Virus Type | Virus Isol. Method | LLC-MK₂ | TC-AMPL | IFAT | ELISA | ELFA | ≥ 4-Fold Serological Rise |
|-----------------|------|-----|----------------|------------|-------------------|---------|---------|------|-------|------|--------------------------|
| 1               | 1978 | YA  | 3              | A          | +                 | +       | +       | 0    | 0     | 0    | NA                       |
| 2               | 1978 | YA  | 3              | A          | +                 | +       | +       | 0    | 0     | 0    | NA                       |
| 3               | 1978 | YA  | 4              | A          | +                 | +       | 0       | 0    | 0     | 0    | NA                       |
| 4               | 1978 | YA  | 2              | A          | +                 | +       | +       | +    | +     | +    | +                        |
| 5               | 1978 | YA  | 3              | A          | +                 | +       | 0       | +    | 0     | 0    | +                        |
| 6               | 1978 | YA  | 2              | A          | +                 | +       | 0       | 0    | 0     | 0    | +                        |
| 7               | 1978 | YA  | 3              | A          | 0                 | +       | +       | 0    | 0     | 0    | +                        |
| 8               | 1979 | YA  | 2              | A          | +                 | +       | +       | +    | +     | +    | +                        |
| 9               | 1979 | YA  | 4              | A          | +                 | +       | +       | +    | +     | +    | +                        |
| 10              | 1979 | YA  | 3              | A          | +                 | 0       | +       | 0    | 0     | 0    | +                        |
| 11              | 1979 | YA  | 3              | A          | +                 | +       | +       | 0    | 0     | 0    | +                        |
| 12              | 1980 | 20  | 6              | B          | +                 | ±?      | +       | 0    | wk    | 0    | wk                       |
| 13              | 1980 | 65  | 8              | B          | +                 | +       | wk      | 0    | 0     | 0    | +                        |
| 14              | 1980 | 20  | 5              | B          | +                 | +       | +       | +    | +     | +    | +                        |
| 15              | 1980 | 21  | 3              | B          | +                 | +       | +       | wk+  | wk+   | wk+  | +                        |
|   | Year | Gender | Age | Test | Result | Days | Year  |
|---|------|--------|-----|------|--------|------|-------|
| 16| 1980 | M      | 5   | B    | +      | +    | +     |
| 17| 1980 | M      | 4   | B    | +      | +    | +     |
| 18| 1980 | M      | 6   | B    | 0      | +    | +     |
| 19| 1980 | F      | 4   | B    | 0      | +    | +     |
| 20| 1981 | M      | 2   | A    | +      | 0    | +     |
| 21| 1981 | F      | 3   | A    | +      | +    | +     |
| 22| 1981 | M      | 3   | A    | +      | +    | +     |
| 23| 1981 | M      | 4   | A    | +      | 0    | +     |
| 24| 1981 | M      | 4   | A    | +      | +    | +     |
| 25| 1981 | M      | 3   | A    | 0      | +    | +     |
| 26| 1981 | M      | 4   | A    | 0      | +    | +     |
| 27| 1981 | M      | 2   | A    | 0      | +    | +     |
| 28| 1981 | F      | 5   | A    | 0      | +    | 0     |

| Number Positive | 21 | 26 | 24 | 18 | 19 |
|-----------------|----|----|----|----|----|
| % Positive      | 75.0 | 92.8 | 85.7 | 64.3 | 67.8 |

IFAT = Indirect immunofluorescent antibody test  
ELISA = Enzyme-linked immunosorbent assay  
ELFA = Enzyme-linked fluorescent assay  
TC-AMPL = Tissue culture amplification in LLC-MK2 cells for 72 hours with identification of virus by fluorescent antibody  
+ Day after onset nasopharyngeal aspirate was obtained  
NA = Not available  
YA = Young adult college student  
SL = State Laboratory isolation using primary Rhesus monkey kidney cells (PRMK)
cells scraped off to make smears at 24, 48, and 72 hours (rapid tissue amplification).

During the course of this work several papers reported an enhanced rate of influenza virus isolation when trypsin was added to the media and/or when the Madin-Darby Canine Kidney cell line (MDCK) was employed [15,24]. On this basis we tested the 11 nasopharyngeal virus-positive aspirates obtained in 1981 from influenza-like patients by using both cell lines, with and without trypsin in the media, and with readings made for the presence of virus at 24, 48, and 72 hours as measured by cytopathogenic effect (CPE), hemadsorption (HA), and fluorescent antibody (FA). As shown in Table 5 the best results were obtained with the use of trypsin in the media and using the FA test to identify antigen. These techniques permitted recognition of influenza virus in nine of the 11 specimens in both cell lines at 24 hours. Similar results were obtained with the MDCK line even in the absence of trypsin. In contrast, at 24 hours CPE had not occurred and only one specimen showed hemadsorption of red cells. At 48 hours after inoculation CPE and hemadsorption both appeared in MDCK cells with or without trypsin. In LLC-MK2 cells, trypsin enhanced both CPE and HA, but also caused deterioration and drop-off of cells making interpretation difficult; at 72 hours this problem was much worse.

### Table 2

Comparison of Indirect Immunofluorescence Antibody Test (IFAT) with Virus Isolation in Tissue Culture in 39 Specimens

| Virus Isolation | IFAT | + | - | Total |
|-----------------|------|---|---|------|
| +               | 24   | 5 |  5|  29  |
| -               |  4   | 6 |  6|  10  |
|                  | 28   | 11| 11|  39  |

### Table 3

Results of Rapid Diagnostic Tests on 11 Specimens Not Yielding Influenza Virus in Tissue Culture

| Specimen | Year | Age | Day of Illness | Influenza Type* | Results of Rapid Tests | Serologic Rise |
|----------|------|-----|----------------|----------------|------------------------|----------------|
|          |      |     |                |                | IFAT | ELISA | ELFA |                   |               |
| 1        | 1978 | YA  |  1             | -              | 0   |  0   |  0   | 0                  | NA            |
| 2        | 1978 | YA  |      3         | A              | +   |  0   |  0   | 0                  | NA            |
| 3        | 1980 | 19 σ |  8             | B              | wk+ |  +   | wk+ | +                  |               |
| 4        | 1980 | 19 φ |  3             | B              | wk+ |  0   |  0   | 0                  |               |
| 5        | 1980 | 19 σ |  2             | B              | 0   |  0   |  0   | +                  |               |
| 6        | 1980 | 30 σ |  4             | -              | ±   |  0   |  0   | NA                 |               |
| 7        | 1980 | 29 φ |  8             | B              | ±   |  +   |  +   | NA                 |               |
| 8        | 1980 | 19 σ |  2             | B              | +   |  +   |  +   | NA                 |               |
| 9        | 1980 | 19 σ |  4             | B              | ±   |  +   |  +   | NA                 |               |
| 10       | 1981 | YA  |  6             | A              | 0   |  +   | wk+ | 0                  |               |
| 11       | 1981 | 22 σ |  4             | A              | +   |  +   | wk+ | NA                 |               |

Total + 5 6 6 2

*As identified by the antisera employed in the test
TABLE 4
Comparison of ELISA and ELFA with Viral Isolation in Tissue Culture

|          | Virus Isolation |   |   |   |
|----------|-----------------|---|---|---|
|          | ELISA +         | - | Total |
| +        | 18              | 6 | 24  |
| -        | 10              | 5 | 15  |
|          | 28              | 11 | 39 |
| ELFA     | +               | 19 |
|          | -               | 9 |
|          | 28              | 11 | 39 |

Comparison of Tests on Stock Virus

The ability of various tests to detect stock influenza A virus (A1/FM1/47) was measured in serial tenfold dilutions. These results are summarized in Table 6. Virus could be detected in dilutions up to $10^{-3}$ by ELISA and ELFA techniques. FA examination of cells from both tissue culture systems at 24 hours also detected virus up to $10^{-3}$. The use of trypsin in the media had little effect on viral detection in LLC-MK$_2$ cells in this experiment, but did result in some enhancement of titer in MDCK cells.

DISCUSSION

This study has compared five rapid diagnostic systems for the detection of influenza antigen in nasopharyngeal aspirates from 39 patients with influenza-like illnesses. The major emphasis was on rapidity and sensitivity. Other concerns were

TABLE 5
Results of Isolation Attempts from 11 Clinical Specimens in LLC-MK$_2$ Cells and MDCK Cells with and without Trypsin in the Media and Read by Various Methods at 24, 48, and 72 Hours

| Cell Line | Trypsin in Media | Number of Positive Specimens by Method Shown |
|-----------|------------------|---------------------------------------------|
|           |                  | At 24 Hours | At 48 Hours | At 72 Hours |
|           | CPE | HA | FA | CPE | HA | FA | CPE | HA | FA | CPE | HA | FA |
| LLC-MK$_2$| 0   | 0  | 2  | 0   | 4  | 4+ 3wk | 1 | 5+ 4wk | 6+ 3wk | |
|          | +   | 0  | 9  | 3* | 9  | 9  | 3* | 9  | 7+ 3wk | |
| MDCK     | 0   | 0  | 9  | 9   | 10 | 8  | NR | 10 | NR |
|          | +   | 0  | 9  | 1+ | 9  | 10 | 11* | 11* | 10* |

* At 72 hours CPE was very difficult to read and sometimes was not readable (NR) with the LLC-MK$_2$ line in the presence of trypsin; this was also true with the MDCK line at 72 hours.
TABLE 6
Comparison of Sensitivity of Various Techniques To Measure Antigen of a
Standard Laboratory Influenza Strain (A1/FM1/47)

| Technique | Hrs. After Inoc. | Virus Dilution | Highest Titer Positive |
|-----------|------------------|----------------|------------------------|
|           |                  | $10^{-2}$      | $10^{-3}$              | $10^{-4}$ | $10^{-5}$ |
| ELISA     |                  | +              | +                      | 0        | 0         | $10^{-3}$ |
| ELFA      |                  | +              | +                      | 0        | 0         | $10^{-3}$ |
| LLC-MK$_2$| to 24 wk        | ±              | 0                      | 0        | 0         | $10^{-3}$ |
|           | 48 wk           | +              | wk +                   | 0        | 0         | $10^{-3}$ |
|           | 72 wk           | +              | wk +                   | 0        | 0         | $10^{-3}$ |
|           |                  | +              | wk +                   | 0        | 0         | $10^{-3}$ |
| MDCK*     | to 24 wk        | +              | +                      | 0        | 0         | $10^{-3}$ |
|           | 48 wk           | +              | wk +                   | 0        | 0         | $10^{-3}$ |
|           | 72 wk           | +              | wk +                   | ±        | 0         | $10^{-3}$ |
|           |                  | +              | wk +                   | 0        | 0         | $10^{-3}$ |
|           |                  | +              | wk +                   | 0        | 0         | $10^{-3}$ |

LLC-MK$_2$ = Rhesus Monkey Tissue Culture  MDCK = Canine Kidney Tissue Culture

$t+$ = 5ug/ml Trypsin In Media  to = No Trypsin In Media

*Rapid tissue culture amplification with fluorescent antibody identification of virus on cells scraped off at 24, 48, and 72 hours after inoculation

simplicity, availability of reagents, and the need for special equipment. The use of the immune electron microscopy (IEM) was abandoned early after only one of 11 specimens proved positive by virus isolation could be identified by IEM using two different concentration methods. The results for the other four rapid techniques are summarized in Table 7. The true sensitivity of the test was judged as the number positive/number from which influenza virus was isolated and identified in tissue culture (either primary Rhesus monkey kidney or a continuous Rhesus monkey cell line, LLC-MK$_2$). There were 28 such isolations from the 39 specimens tested over four years, including 20 influenza A and eight influenza B isolates. None of the rapid direct identification tests proved of sufficient sensitivity for viral diagnosis on these 28 positive specimens that it could be used alone. The day of illness on which the specimen was collected during the first five days of illness had little effect on the positivity of any of the tests, but dropped after that (Table 8). The highest rate of positivity for the IFAT test was on the third day when 10 of 11 specimens were positive. The advantages and disadvantages of the different tests were as follows. Overall, the immunofluorescent antibody test on nasopharyngeal aspirates (IFAT) provided the best combination of true sensitivity (85.7 percent), rapidity (about four hours), and simplicity. A fluorescent microscope is required for the test but is usually present in most diagnostic laboratories. The disadvantages of the IFAT are the apparent need for an aspirate using a suction apparatus, the occasional failure to obtain enough cells for examination, and the lack of a viral isolate to do further analysis of the virus strain. In addition, only a few cells may show fluorescence, and a careful search must be made for them. The need for a suction aspiration, however,
Comparison of Tests for Rapid Diagnosis of Influenza in 39 Specimens

| IFAT | ELISA | ELFA | TC Amplification |
|------|-------|------|------------------|
| 71.8 | 61.5  | 64.1 | 66.6             |

Time Required for Test in Hrs.
- TC: 4
- IFAT: 40
- ELISA: 40
- ELFA: 48-72

Simplicity
- No. of Washes: 6, 17, 19, 2
- No. of Steps: 5, 8, 10, 5

Special Reagents Needed
- Conjugate
  - 1. Conjugate
  - 2. Monoclonal AB
- Commercially Available
  - Yes
  - 1. Yes
  - 2. Not Yet
- Special Equipment Needed
  - FA Microscope
  - ELISA Reader
  - Fluorescent Box Reader
  - FA Scope or Light Scope

may not be critical, since Minnich and Ray [10] recently reported the successful identification of different respiratory viruses on cells obtained by deep nasal swabs rather than by suction aspiration. These preparations were transported to the laboratory on ice since materials for the IFAT test cannot be frozen without disrupting the cells.

In our hands the ELISA and ELFA tests were only moderately sensitive: 64.3 and 67.8 percent were positive, respectively, in 28 specimens yielding virus. The test required the use of monoclonal antibody. This antibody is not yet commercially available and its quite strain-specific. An ELISA reader is desirable to provide sensitive, objective, and reproducible readings of the ELISA test and is quite expensive. The ELFA test was read subjectively under UV illumination. At present we feel the ELISA and ELFA techniques, as we have used them, are not sufficiently sensitive nor rapid (we used an overnight incubation) for routine diagnosis. Improvements in methods may make them more useful in the future. Indeed, Berg et al. [12] and Yolken et al. [14] have reported some modifications than enhance sensitivity.

The rapid tissue culture amplification (TCA) technique has many advantages if a

| Day of Illness | Number of Specimens | Standard Isol. | TCA | IFAT | ELISA | ELFA |
|---------------|---------------------|----------------|-----|------|-------|------|
| 1             | 1                   | 0              | 0   | 0    | 0     | 0    |
| 2             | 8                   | 63             | 75  | 75   | 75    | 75   |
| 3             | 11                  | 64             | 73  | 91   | 45    | 45   |
| 4             | 10                  | 50             | 70  | 60   | 70    | 70   |
| 5             | 3                   | 66             | 100 | 100  | 66    | 66   |
| 6-8           | 6                   | 33             | 33  | 66   | 66    | 83   |
sensitive cell line is used, trypsin is added to the media, and identification of antigen is carried out by fluorescent antibody means. Most isolates can be identified within 24 hours. A solid-phase fluorescein immunoassay has also been reported to provide quantitative detection of influenza virus in tissue culture fluids [23]. The use of the ELISA test on supernatant fluid may also be a sensitive way to detect the early appearance of virus, but we do not have enough experience with it to compare it with FA. It may not detect virus earlier than FA, which detects intracellular antigen, since for detection by the ELISA test the antigen must be released from cells into the supernatant fluid. In accordance with Frank et al. [24] we found that both the LLC-MK₂ and MDCK lines give satisfactory results for influenza isolation. The former has the advantage of being sensitive to parainfluenza virus, the latter of having higher sensitivity for some influenza strains. Use of both cell lines together would be a good combination for the isolation of most respiratory viruses, excepting respiratory syncytial virus which grows best in Vero cells [25].

On the basis of our work, and the published reports of others, we feel that the combination of the IFAT on aspirated nasopharyngeal cells and of rapid tissue culture amplification on the supernatant fluid in LLC-MK₂ and MDCK cells with trypsin in the media provides a rapid and sensitive system at the current state of technology and also yields a virus for further antigenic analysis. The IFAT can identify influenza virus in about 92 percent of nasopharyngeal aspirates with adequate cells within four hours after the collection of the aspirate. The TCA provides isolation and identification by FA in 24 hours in most specimens, and occasionally a few more can be identified at 48 or 72 hours.

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