Economical Laboratory Support System for Influenza Virus Surveillance

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When increases in influenzalike illnesses were first detected during the 1984 to 1985 and 1985 to 1986 influenza seasons, throat swab specimens were mailed by physicians across the United States to one hospital laboratory, and each specimen was inoculated into a single tube of cell culture. Of 165 specimens, 52 (32%) were positive for influenza A or B virus within 3 days of receipt when tested by fluorescence microscopy, permitting positive results to be received by the physicians within 1 week on the average. Approximate direct cost was $16 per specimen.

Influenza virus epidemics in the United States occur on a regular basis, causing a yearly average of about 20,000 deaths (K. J. Lui and A. P. Kendal, Am. J. Public Health, in press), about 250,000 hospitalizations, and increases in medical costs of about $300 million for hospitalization alone (2). With increased emphasis on the use of antiviral agents for control of influenza A virus infection (1), a rapid, economical diagnostic system that is available to physicians regardless of their geographical location in relation to virus laboratories is needed.

A previous report showed that 80% or more of all cell cultures which were ultimately positive for influenza virus could be detected within 3 days by fluorescence microscopy (6). Our objective was to determine whether this approach could be used in a central laboratory to diagnose influenza for family physicians enrolled in the Centers for Disease Control sentinel practice influenza surveillance program. The physicians were requested to send throat swab specimens collected from their patients to a central laboratory (located in Las Vegas, Nev.) when they first observed increases in the number of influenzalike illnesses among their patients.

Detailed instructions for the collection, processing, and mailing of specimens were provided to participating physicians along with a kit that included the following: (i) one Styrofoam shipping container with a cardboard sleeve; (ii) three 2-gram (3.5-g) specimen-collection vials containing 2 ml of tryptose-phosphate broth supplemented with 5% gelatin, antibiotics, and antmycotics (MicroBio Associates, Tempe, Ariz.); and (iii) a virus isolation request form, a preaddressed, prepaid label for first-class U.S. mail, and a biohazard label. (Vials were stored frozen before shipping, and the physicians were instructed to refreeze them and to keep them frozen until use.)

Physicians were asked to obtain throat specimens, with their own dacron swabs, from patients who had an influenzalike illness (fever, ≥100°F [37.8°C], and a cough or sore throat) for less than 48 h. The need to submit specimens when an outbreak of influenza was first suspected was emphasized. If the vial was to be stored before mailing, it was to be refrigerated. To minimize costs and simplify the procedure for the physician, all vials were to be shipped without ice packs by first-class mail within 24 h. Specimen kits were replaced as used.

When received, each specimen was inoculated (0.2-ml samples) into a single tube of primary monkey kidney cells (Bartells Laboratories, Seattle, Wash.) which had been washed with phosphate-buffered saline. The culture was incubated for 1 h at 34°C to allow absorption of the virus, and then 2 ml of serum-free minimal essential medium was added. Tubes were incubated stationary at 34°C for 72 h. Monoclonal A and B antibodies were used to identify the influenza viruses by indirect fluorescent-antibody assay (6), usually on day 3 after specimen inoculation. However, cell cultures were examined on days 1 and 2, and any that showed cytopathic effect and hemadsorption were tested by indirect fluorescent-antibody assay at that time.

Rapid turnaround was essential to meeting physician needs and to providing early surveillance results. Therefore, laboratory testing was performed 7 days a week, and all positive results were reported by telephone. Final reports on all specimens were sent by mail.

During the influenza season, laboratory support was offered to physicians throughout the country who submitted weekly reports to the Centers for Disease Control indicating the numbers of patients seen with influenzalike illness. During the 1984 to 1985 season, 19 physicians in 14 states submitted specimens. The participation increased during the next season (1985 to 1986) to 36 physicians in 21 states.

Three times as many specimens were submitted during the 1985 to 1986 season as during the previous one, largely reflecting the increase in physician base. During both seasons, approximately 65% of physicians submitted two or three specimens when they first identified outbreaks of influenzalike illness among their patients, 25% submitted only one specimen, and 10% sent five or more specimens over a 3-month period. There was no correlation between the number of positive test results and the number of specimens submitted.

A total of 52 influenza viruses were isolated from the 165 specimens tested, for an overall isolation rate of 32%. The maximum isolation rate was 46%, during January 1986. During the 1984 to 1985 season, all viruses from positive specimens were identified as influenza type A (H3N2). During the 1985 to 1986 season, viruses from positive specimens consisted of 39 type B and 4 type A (H3N2).
strains. The viruses isolated were the prevalent strains circulating in the nation at the time of specimen collection (3, 4). During the 1985 to 1986 season, when sufficient numbers of isolates were obtained for analysis, laboratory diagnosis confirmed the presence of influenza B virus at or before the time of peak morbidity (Fig. 1).

The time in transit of specimens from physicians' offices to the central laboratory ranged from 1 to 18 days according to records supplied by the physicians. The average time in transit was 4.1 days for positive specimens and 5.2 days for negative specimens, suggesting that delays in shipment may have reduced isolation rates. There was no consistent relationship between transit time and either the distance traveled or the day of the week specimens were sent, but the longest transit times were from states on the East Coast.

On the average, results were reported 3 days after receipt. Of the specimens received, 5% were contaminated or were toxic to the cell cultures used and were retrenched and re inoculated, requiring an extra 1 to 2 days of handling. However, an equal percentage (5%) of specimens were positive within 48 h of inoculation.

The entire direct cost per specimen ranged from $12.00 to $16.00. These costs included specimen collection materials and shipping charges of $4.00, isolation and identification materials costs of $3.00, and charges for 20 to 30 min of technical time of about $5.00 to $9.00.

Rapid diagnosis of influenza is desirable at the onset of an outbreak to facilitate both vaccination and specific chemotherapy, particularly for inadequately protected persons at high risk (1). The influenza virus should be typed because antiviral therapy is directed solely against influenza A virus. Recently developed type-specific monoclonal antibodies have provided reagents suitable for identification (6). These reagents have been used to detect influenza virus antigens in nasal aspirates and sputum samples by indirect fluorescent-antibody assay and in nasal aspirates by a time-resolved fluoroimmunoassay. Such tests can yield results in a matter of hours (5, 7). Influenza virus isolation may be necessary or advantageous, however, to study the properties of the virus or when collection of nasal aspirates is not feasible.

In this study, the average isolation rate of 32% obtained with culture confirmation by antigen detection within 3 days of inoculation was much higher than that usually reported to the Centers for Disease Control by laboratories when traditional methods are used (4; unpublished data). High isolation rates possibly resulted from a combination of good judgment and specimen collection by physicians and the high stability of the virus in the collection and mailing kit, which was designed to prevent desiccation, reduce contamination and toxicity, and protect against temperature fluctuation.

This study successfully demonstrated that an economical laboratory system can be established to support influenza surveillance. Even with the relatively lengthy transit times for specimens sent across the country by mail, the methodology described here proved to be efficient in documenting the spread of influenza viruses to geographical locations where physicians would otherwise have lacked laboratory confirmation in time for such information to be of use in community control activities. Overnight shipment of specimens would reduce delays in transit, and rapid shipment, coupled with more sensitive immunoassay detection methods, should be capable of providing results within 3 days. We believe that this approach, which could be applied equally by regional, state, or local laboratories, will dispel the commonly held view that laboratory diagnosis of influenza is too slow or too expensive to be beneficial for use in influenza control by physicians practicing in the general community.

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LITERATURE CITED