Clinical and Laboratory Diagnosis of Influenza Virus Infections

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Abstract

Influenza epidemics account for more than 20,000 deaths in the United States each year, as well as substantial morbidity, medical costs, and time away from work and school. Since the 1950s, the principal weapon against these seasonal epidemics has been killed virus vaccine formulations. Despite massive efforts to immunize at-risk individuals against influenza, not everyone receives the vaccine. In addition, use of some drugs, such as amantadine and rimantadine, can lead to the development of drug resistant viruses in infected individuals and to transmission of these viruses to susceptible individuals. The many factors that contribute to the high annual incidence of influenza virus infections mandate prompt clinical recognition and appropriate patient management. Rapid diagnostic tests have been developed that may make it possible to avoid the use of antibacterial drugs, quickly decide whether isolation of infected patients is needed, and discharge hospitalized patients sooner.

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Influenza virus is unique among respiratory viruses in its ability to cause seasonal increases in mortality in adults. It is estimated that this number exceeds 20,000 in the United States and is particularly prevalent in populations that have compromised immunity (pregnant women or the elderly) or underlying medical conditions (cardiac or pulmonary disease). Fortunately, most cases of influenza are not so severe. Nevertheless, the societal consequences and the healthcare costs generated by annual influenza epidemics are substantial. A number of studies have shown that during influenza season there are significant increases in visits to physicians' offices and emergency departments, as well as school and work absenteeism.[1-4] Since the 1950s, the major weapon against these seasonal epidemics has been killed virus vaccine formulations. Initially, vaccine use was limited to selected populations, and it was not until 1976 that a more expanded vaccination policy was adopted. Concomitantly, amantadine was approved for use in the treatment of influenza virus infections, initially for treatment of H2N2 influenza viruses and later for treatment of all influenza A viruses.[5] Current recommendations for targeted populations are discussed elsewhere.[6]

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Despite the widespread use of vaccine before and during each influenza season, not all at-risk individuals are immunized. However,

numerous studies have also shown that the protective efficacy of inactivated vaccine is not 100%; in healthy adults it ranges between 70% and 90% and it can be as low as 30% to 50% in the elderly.[6] Furthermore, use of amantadine and the related drug rimantadine can quickly result in the development of viruses in infected individuals who are resistant to these agents. These viruses can be transmitted to susceptible individuals. Because these factors can contribute to the high annual incidence of influenza virus infections, prompt clinical recognition is important in selecting appropriate choices for the management of the patient.

Clinical Diagnosis

Development of symptoms in infected persons is typically characterized by the sudden appearance of systemic, respiratory, and, less frequently, ocular manifestations. Patients can often pinpoint the hour of onset.[7] The most important systemic symptom is fever within 12 hours of onset; maximum threshold of 100°F to 106°F can be reached. This is often accompanied by chills (occasionally, frank shaking chills); headache; pain in the muscles of the back and legs; fatigue; and weight loss. A cough and clear nasal discharge may be present at the onset of illness, but they are usually overshadowed by the systemic symptoms. In addition, severe eye pain on lateral gaze, tearing, burning, and photophobia may be present. Classically, the febrile period is 3 days, with the fever typically being lower on the second and third days of illness. As the fever subsides, an accompanying diminution of systemic signs is observed. This often results in respiratory symptoms becoming more perceptible; symptoms can last 3 to 4 days after the patient is afebrile. A full recovery can require a convalescent period of 1 or more additional weeks, during which the most frequent complaints are cough, lethargy, and malaise.

Treatment of uncomplicated illness can involve symptomatic therapy for reduction of systemic signs combined with specific agents designed to inhibit the virus. Relief of fever and body aches is usually attempted with acetaminophen and ibuprofen, because the use of salicylates and salicylate-containing medications in children with influenza is associated with Reye's syndrome.[8] Amantadine and rimantadine are chemically related antiviral drugs that bind to the viral M2 protein, blocking its ion-channel activity and preventing viral uncoating in an infected cell. However, the M2 protein is only present in influenza A viruses, thereby rendering these drugs ineffective against influenza B. Two members of a new class of antivirals have recently been approved for use in treatment of influenza virus infections. Zanamivir (Relenza®, Glaxo Wellcome)[9] and oseltamivir (Tamiflu®, Roche Laboratories Inc)[10] are inhibitors of viral neuraminidase, an enzyme expressed on the surface of both influenza A and B. Functionally, the enzyme mediates release of virus from infected cells. As a result of exposure to a neuraminidase inhibitor (NI), the cell-to-cell spread of virus is blocked. Because neuraminidase is present in all influenza types, NIs are appropriate for treating infections caused by either influenza A or B. To be most effective in reduction of symptoms resulting from infection, treatment with any of the antivirals must begin within the first 48 hours after onset of the illness.[9-11]

In addition to the acute respiratory illness seen in most patients, certain subgroups can develop complications as a result of their infections. The major complication of influenza virus infection is pneumonia, which develops as a consequence of primary viral infection of the lungs or secondarily as a bacterial pneumonia in the recovering patient. Primary viral pneumonia is rare and occurs predominantly in people with underlying cardiovas-

cular disease or pregnant women, but it also has been seen in otherwise healthy adults. It begins as a dry cough within 24 hours of onset of the initial fever and later becomes productive of bloody sputum. This rapidly progresses to dyspnea, hypoxemia, and cyanosis, with bilateral radiological findings of interstitial infiltrates without evidence of consolidation. Bacteriological examination of the sputum is unrevealing, and patients do not respond to antibiotic therapy. A rapid deterioration is often seen, and mortality is high despite supportive care and efforts to ventilate the patient artificially.

Bacterial pneumonia secondary to influenza virus infection is a more common occurrence, and is most often seen in patients with underlying pulmonary disease. It often begins during the early stages of convalescence. In these patients, fever and cough can be accompanied by the production of sputum, as well as physical and radiological evidence of consolidation. Bacterial pathogens usually associated with this disease include *Streptococcus pneumoniae, Haemophilus influenzae*, and *Staphylococcus aureus*. Rare cases of secondary pneumonia caused by *Neisseria meningitidis* or *Streptococcus pyogenes* have also been reported. Patients will respond when they are treated with appropriate antibiotics that can be selected based on sputum Gram stain and culture results. Mixed viral and bacterial pneumonia can present with any combination of symptoms and responds variably to antibiotic therapy.

Laboratory Diagnosis

The diagnosis of influenza infection can be made on clinical grounds alone in the appropriate setting. Several studies have shown that during influenza season, clinical diagnosis of influenza in people with fever, cough, and myalgias can be as high as 85% sensitive.[12-18] However, laboratory diagnosis of influenza infection plays an important role in understanding and characterizing the epidemiology of influenza, controlling institutional outbreaks, and therapeutic management of patients. Furthermore, the availability of specific treatment for influenza A and B recently has focused a great deal of attention on the development of diagnostic techniques that facilitate making a rapid and accurate diagnosis. From the perspective of both the physician's office laboratory (POL) and the hospital laboratory, an ideal test could provide results quickly and accurately, and be performed by staff that possesses minimal technical expertise. The most dependable and best-performing tests are those that are used often and for which well-established quality assurance measures are available.[19] A test with these characteristics could be easily incorporated into the normal flow of laboratory operations and be performed by staff that has very little technical skill.

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Culture. The confirmation of a clinical diagnosis of influenza virus infection can be accomplished using a variety of methods in the laboratory. Detection of an infectious virus or viral antigens ultimately requires obtaining appropriate patient specimens. Virus can be isolated from nasal and pharyngeal swabs, as well as from nasal washes and sputum. Specimens should be placed in containers of viral transport medium supplied by the laboratory. The gold

standard for laboratory detection of this virus continues to be isolation by culture. Traditional methods have involved inoculating specimens into hen's eggs. Whereas egg inoculation is not practical in today's diagnostic laboratory, it is still used for producing current vaccines, because eggs remain the best method of quickly generating very high titers of virus. In the diagnostic laboratory, however, specimens are inoculated into cells that are susceptible to influenza infection, typically primary rhesus monkey kidney, cynomolgus monkey kidney, or Madin-Darby canine kidney cell cultures. Virus is detected by examining the cells for cytopathic effect (CPE) and/or expression of viral hemagglutinin on the surface of infected cells. Viral hemagglutinin is detected by adding a suspension of guinea pig erythrocytes to the culture and examining it for adsorption of the erythrocytes to the surface of the cells in culture (hemadsorption). Standard culture protocols suggest daily examination of cultures for the development of CPE and hemadsorption at intervals during 10 to 14 days of culture or if CPE is observed. The advantage of culture is its sensitivity, but its disadvantage is that whereas CPE or HA expression usually takes 2 to 3 days to develop, it can take as long as 7 to 10 days.[19]

Although traditional culture is the most sensitive method for the detection of virus, it often does not provide results in a time frame that is clinically relevant. Because the antiviral therapy is only effective when given in the first 48 hours after the onset of symptoms, a variety of strategies have been developed to reduce the turnaround time of results obtained by traditional cultures. Rapid culture methods effectively detect influenza with a minimal loss of sensitivity. Rather than using traditional cultures in which the cells line the inner surface of 16 x 125 mm culture tubes, rapid cultures utilize shell vials containing a 1-cm diameter round coverslip to which the culture cells adhere or 24-well culture plates with the cells growing on the bottom of the well. Specimens are then added to the culture, and the vial or plate is subjected to centrifugation. Although the benefits of centrifugation are not entirely clear, it is thought to enhance the binding of virus to the cells in culture and decrease the time required for infection to occur. After an overnight incubation, the culture medium is removed, and the cells are washed and fixed. Viral antigens are detected in the cell cultures using fluorescent- or immunoperoxidase-labeled antibodies specific for influenza virus. The rapid culture methods have been shown to be 60% to 100% sensitive in detecting influenza virus relative to traditional culture methods.[20-22] The main drawback to this method is the increased level of expertise required for performing and interpreting these types of cultures.

Serology. Detecting elevations in antibody titers can be a useful tool for diagnosing infections, but it is usually applied only when other attempts at diagnosis fail. For diagnostic purposes, it is best to use paired acute and convalescent sera (usually collected 3 to 4 weeks apart) that are tested at the same time. Traditional assays measure the level of type-specific antibody by complement fixation tests and subtype-specific antibody by hemagglutination inhibition. Today, serological assays are most widely used for evaluating the epidemiology of influenza and vaccine. Hemagglutination inhibition and neutralization tests can be used to identify the type, subtype, and strain of virus that causes infections. These tests are also useful in determining the immunogenicity of vaccines as well as the level of antibody resulting from naturally occurring infections that is present in a population. Because serological assays for diagnostic or epidemiologic purposes do

not fit into the routine of most clinical laboratories, these assays are often reserved for large reference centers or public health laboratories.

Fluorescent antibody staining of exfoliated cells. Further attempts at reducing the time to detection of influenza virus have addressed examining the original specimen directly for evidence of virus. Microscopic techniques typically involve depositing cells in the specimen on microscope slides and staining them with fluorescently labeled antibodies to the virus. Several commercially available kits containing a pool of antibodies directed against common viral respiratory pathogens (influenza, parainfluenza, adenovirus, and respiratory syncytial viruses) provide broad screening capability. If the initial screen is positive, then replicate slides can be stained with individual virus-specific reagents. Although these methods can be quite useful, they are currently less sensitive for most viruses than culture.[23-27] Improperly collected (ie, lack of cellular material) or transported specimens (ie, not placed in transport media or refrigerated in transit) also contribute to reduced sensitivity. In addition, significant technical skill is required to perform and accurately interpret direct virus detection tests.

Antigen detection. Viral antigens can also be detected in clinical specimens using commercial enzyme immunoassay devices (similar to those used for group A *Streptococcus* testing) and are now widely used for identifying influenza infections. Several devices currently available for physicians and laboratory workers include Directigen® Flu A (Becton-Dickinson, Cockeysville, Maryland), FLU OIA® (Biostar, Boulder, Colorado), and ZstatFlu® (ZymeTx, Oklahoma City, Oklahoma). These devices vary with regard to the time required for performance of the test (15 to 45 minutes), the virus types detected (influenza A only or both influenza A and B), test format structure, and the specimens recommended for testing.

Directigen® Flu A is the most widely used antigen detection device and also the most comprehensively evaluated in the literature. To perform this test, a specimen is extracted and added to a membrane in the test device. The influenza antigen present (either soluble or cell-associated) binds nonspecifically to the membrane as the specimen passes through. Antigen on the membrane is then detected by adding enzyme-conjugated antibodies specific for influenza A nucleoprotein, followed by the addition of enzyme-specific substrates that turn purple in the presence of labeled antibody.

There are 8 steps involved in the assay, and all are performed by adding reagents from dropper bottles to the test device. These steps are carried out at room temperature and include specimen extraction, adsorption to the membrane, addition of antibody conjugate, and color development, with appropriate wash steps in between. The total time needed to perform a test is approximately 15 minutes. Each kit contains external positive and negative control reagents that are run on separate devices, and each device contains an internal positive control that develops only if the test reagents were added in the appro-

Table. Performance Characteristics of Directigen® Flu A

Specimen Type	Total Number of Cells	Sensitivity	Specificity
NP aspirate	717	92% (68/74)	88% (568/643)
NP wash	175	96% (23/24)	90% (136/150)
Pharyngeal swab	183	67% (10/15)	92% (156/168)
NP swab	54	88% (21/24)	97% (29/30)

NP = nasopharyngeal.
Source: Reference 28.

priate order. Recommended specimens include nasopharyngeal washes, swabs, aspirates, and pharyngeal swabs. The Table shows the results of the validation study included in the Directigen® packet insert[28] and indicates that nasopharyngeal washes and aspirates are superior to other specimens. According to the manufacturer, the overall sensitivity and specificity were 91% and 90%, respectively, compared with culture. The manufacturer also performed a physician office evaluation and reproducibility study using 160 in vitro prepared nasal wash specimens and showed there was a 100% correlation between expected results and the results obtained at the 4 physician office sites. Published studies evaluating the performance of Directigen® report sensitivities ranging from 41% to 100% and specificities of 71% to 100%, depending on the type of specimen collected, who collected the specimen, and patient populations studied.[23,25,29-34] The Clinical Microbiology Laboratories at the University of Rochester Medical Center has recently evaluated the performance of Directigen® over the course of 2 winter seasons, without restrictions on specimen type or patient population (ie, under routine conditions) and demonstrated sensitivities of 60% to 77% and specificities of 80% to 90%. This performance was consistent between the seasons, with the variation a result of the type of culture used as the gold standard.[35] The major limitation of the current Becton-Dickinson device is that it is not designed to detect influenza B. The manufacturer is currently conducting a premarket evaluation of a next-generation Directigen® kit that detects and differentiates between influenza A and B.

Not only are epidemics of influenza B less common than epidemics of influenza A, but influenza B is also often encountered in varying proportions during influenza A outbreaks. However, the availability of drugs that are effective against both influenza A and B has made the rapid identification of both types more of a diagnostic issue. As previously mentioned, both influenza A and B possess neuraminidase on their surface. ZstatFlu® is a test that detects neuraminidase in specimens. Clinical material is mixed with a synthetic, chromogenic neuraminidase substrate. In the presence of neuraminidase the substrate is cleaved, releasing the free chromogen, which precipitates to produce a blue color. This precipitate is then collected and concentrated in a device for visualization of the color. Although other viruses (such as parainfluenza 1 to 4 and mumps viruses) possess neuraminidases, the synthetic substrate used in this test is specific for influenza A and B neuraminidase. This assay can be performed in fewer steps than Directigen®, but they are somewhat more labor intensive. The specimen is extracted in a detergent, then filtered into a vial containing dried substrate. The vial is then capped with a rubber stopper, mixed, and incubated in a heat block at 41°C for 30 minutes (not to exceed 35 minutes). In the final step, stop reagent is added to the vial, and the contents are poured into the collection device for visualization of any blue color. The total time required to perform a single test is approximately 45 minutes. Positive and negative control reagents are included with each kit, but the only internal control is a pH indicator on which a drop of the final solution is added and shows whether the reagents were added in the correct proportions. The specimen recommended for this assay is a special foam swab that is included in each kit. In addition, kits come in 2 formats: POL and diagnostic laboratory formats. The difference between the 2 is that the diagnostic laboratory format kits include a special tube into which the swab is placed for transport to the laboratory. There are no published

studies examining the performance characteristics of this assay, but according to the manufacturer, the sensitivity is 65% for influenza A and 57% for influenza B, with 95% to 100% specificity.[36] Although this assay detects both influenza A and B, it is does not differentiate between them.

An additional influenza antigen detection kit utilizes a unique mechanism for visualization of antigen-antibody complexes: FLU OIA® is an enhanced optical immunoassay in which influenza A or B nucleoproteins that are present in the specimen are extracted and placed on a silicon wafer in the test device. This wafer is an optical surface on which anti-nucleoprotein antibody is immobilized. Antigen in the specimen binds to this antibody, the surface is then washed, and a substrate is added that binds to the complex. This increases the thickness of the molecular complex—a process referred to as mass enhancement—and alters the path of light reflected by the optical surface. The alteration in light is visualized as a change in color that is purple against a gold background. The assay procedure is quite simple and takes approximately 20 minutes for a single specimen. The sample is extracted, added to the silicon wafer, and incubated, washed, and blotted dry; the substrate is then added and incubated, washed, and blotted dry. All steps are performed at room temperature. A positive result is a purple circle that reflects off the silicon wafer. An internal positive control appears as a dot in the center of the larger circle. External positive and negative control reagents are included with each kit. Specimens recommended by the manufacturer for use in this assay include throat swabs, nasopharyngeal swabs, nasal aspirates, and sputum. However, it is not recommended that a swab or specimen be placed in the viral transport medium, because the dilution affects assay performance. An alteration in specimen collection procedures would therefore be required for cases in which a culture of the specimen is desired. Whereas this assay detects both influenza A and B in clinical specimens, like ZstatFlu® it does not differentiate between these viruses. The performance characteristics of this assay, as described in the package insert, have recently been published.[37] This study showed that in comparison with 14-day cultures, the sensitivity of the assay varied with the specimen type: throat swabs were the least sensitive specimens (62%), nasal aspirates the most (88%), with an overall sensitivity of 80% for this assay. Specificity ranged from 52% (sputum) to 80% (throat swab), with an overall value of 73%. Although no data were presented regarding the assay's relative sensitivity in detecting each type of virus, 2 recent studies in which influenza B was the predominant isolate demonstrated performance characteristics similar to those presented by Covalcuic and associates.[37] Yamazaki and colleagues[38] showed that compared with culture and polymerase chain reaction (PCR), the sensitivity of FLU OIA® was 89% and 82%, and the specificity was 65% and 72%, respectively; all isolates in this study were influenza B. Similarly, in a pediatric study in which 88% of the isolates were influenza B, FLU OIA®, when compared with culture, exhibited an 80% sensitivity and a 69% specificity for nasopharyngeal aspirates and a 37% sensitivity and an 84% specificity for throat swabs.[39]

Two new antigen detection tests have recently become available that may have the operational characteristics needed for use in offices or laboratories staffed by individuals with a wide range of experience and technical skill. The QuickVue® Influenza A/B Test (Quidel Corporation, San Diego, California) and the Influenza A/B Rapid Test (Roche Diagnostics Corporation, Indianapolis, Indiana) use similar test principles and procedures for the

detection of type-specific influenza antigens in clinical material. These tests are both capable of detecting but not differentiating between influenza A and B. In the Quidel test, nasal swab, nasal wash, and/or nasal aspirate specimens are added to a tube containing an extraction reagent that disrupts the viral particles and releases internal viral antigens. A test strip is added to the extraction tube through which the viral antigens can migrate. Antigen is captured by type-specific antibody impregnated in the strip and is visualized by the development of a pink-red line. A blue control line will also be visualized if the test was performed properly. The entire test can be performed in approximately 10 minutes. The manufacturer reports better performance when using nasal washes or aspirates (81% sensitive and 99% specific) than when using nasal swabs (73% sensitive and 96% specific). The overall performance of this test compared with culture had a sensitivity of 75% (101/135) and a specificity of 97% (227/235).[40] In the Roche test, the only recommended specimens are throat swabs, which are added to lysis/elution solution that releases the viral nucleoprotein in the specimen. Type-specific, conjugated anti-nucleoprotein antibodies in the solution bind to the viral antigen, forming a complex that migrates through a test strip which is added to the reaction cup and is captured to form a line across the strip to indicate a positive result. A second, positive control line forms if the sample migrates properly. The test can be performed in approximately 12 minutes and is reported to be 68% sensitive and 81% specific, according to the manufacturer.[41]

Molecular Methods

Further attempts to shorten the turnaround time for laboratory diagnosis have utilized molecular methods aimed at detecting viral nucleic acids in patient specimens. Reverse transcriptase-polymerase chain reaction (RT-PCR) is a widely used molecular tool that has been applied to both the detection of influenza virus and the subtype characterization of virus isolates. Viral nucleic acids (NA) are extracted from specimens using commercially available kits or in-house prepared guanidinium isothiocyanate. Complementary DNA (cDNA) is synthesized from the viral RNA by an in vitro reverse transcriptase reaction using either virus-specific oligonucleotides or random hexamers as primers for the reaction. The cDNA template generated in the RT reaction is then amplified in PCR by a heat-stable DNA polymerse using influenza-specific primers. The amplified product can then be visualized directly in an agarose gel or indirectly using one of a variety of available detection systems. A number of assays developed in house have been shown to be comparable to culture for the detection of influenza virus in clinical specimens.

Atmar and associates retrospectively analyzed combined nasal wash/throat swab specimens obtained from an outpatient pediatric population by RT-PCR and found that this method was 95% sensitive and 98% specific when compared with culture.[24] In addition, Claas and colleagues prospectively analyzed nasopharyngeal aspirates and bronchoalveolar lavages from pediatric patients over a winter season and found that their RT-PCR method was 92% sensitive relative to culture.[42] This tool has been developed further to screen for multiple viral respiratory pathogens in the same specimen. Hexaplex® (Prodesse, Milwaukee, Wisconsin) is a multiplex, quantitative RT-PCR enzyme hybridization assay that has been shown to detect and quantitate NA of respiratory syncytial viruses A and B, influenza viruses A and B, and human parainfluenza virus types 1, 2, and 3.[43] The major advantage to these molecular assays is the potential to obtain a result on the same day with-

out compromising sensitivity. The methodologies, equipment, and level of training required to perform PCR assays is not yet widespread; therefore, molecular assays are not part of today's diagnostic armamentarium, but they will undoubtedly play a role in the routine diagnosis of viral infections in the future.

Practical Guidelines

Recent enthusiasm for using antiviral drugs to treat influenza has increased interest in rapid diagnostic assays. It is clear from the data discussed above that the specificity of the antigen tests is high enough to regard positive results as "true positives." Therefore, it is appropriate to initiate antiviral therapy for antigen-positive patients. However, some of the currently available tests do not differentiate between influenza A and B, so some positive antigen tests are less useful than others for selecting among the available therapies. The choice of drug to use should be based on what is known about the types of virus currently circulating in the community. Furthermore, the Food and Drug Administration (FDA) has recently issued a public health advisory reminding clinicians of factors to consider before prescribing antiviral drugs for the treatment of influenza. This notice stressed the importance of considering concomitant or secondary bacterial infection, especially in patients with chronic medical conditions. Appropriate antibacterial therapy should therefore be initiated whenever bacterial infection is suspected. Finally, it was noted that antiviral drugs have not been shown to prevent or effectively treat viral complications of influenza such as viral pneumonia.

The use of influenza tests to differentiate a nonspecific febrile illness from influenza in a patient with an influenza antigen-negative test result is more difficult. The currently available data has shown that these tests are between 60% and 80% sensitive, so 20% to 40% of patients truly infected with influenza virus will be missed by those who rely on the results of the antigen test alone. Although some currently available antigen detection tests are of sufficient complexity to make their use impractical for most POLs, the newer tests are more user friendly and are approved by the FDA for use in POL settings. However, it is important to remember that test performance in clinical practice often does not match claims in manufacturers' package inserts. Furthermore, during influenza season, a clinical diagnosis has been shown to be as effective as laboratory testing in diagnosing influenza infection. Therefore, a negative antigen test result should be interpreted with caution, and taken in the context of the patient's clinical symptoms and local epidemiology. It should be emphasized that tests vary in performance and complexity and that several of the more recently introduced tests have not been independently evaluated. Clearly, rapid tests are useful for defining and managing outbreaks of influenza in closed populations. However, the rational use of rapid tests in other clinical settings has not been evaluated.

Conclusions

Making an accurate and rapid diagnosis of influenza is often a combination of the index of clinical suspicion, epidemiological information, and the availability of appropriate laboratory tools. The choice of tools to use will depend on the population of patients being seen and the skill level of the laboratory staff, as well as the level of sensitivity needed and the speed required to obtain a result. In the current assortment of diagnostic tests, many are suitable for use in the hospital laboratory as well as the POL setting. Practices with ready access to a laboratory may elect to perform a rapid test and base decisions on these results, delaying therapy until the results return from the laboratory (if this could occur within 4 to 6 hours).

Rapid diagnostic tests may have more current applicability in the hospital or institutional setting where testing may facilitate early recognition of influenza in hospitalized patients, by differentiating this disease from other causes of fever in patients with a complex medical history (eg, compromised immunity). This would allow 1) the avoidance of antibacterial drugs, 2) informed discussions regarding isolation of infected patients, and 3) earlier discharge. In addition, rapid tests could allow early recognition of outbreaks in institutions and more effective utilization of prevention strategies. We must continue to explore strategies for the prevention and control of infection, as well as pursue the development of improved diagnostic tools, because most experts agree that the clock is ticking for the arrival of the next pandemic.

··· REFERENCES ···

1. Glezen WP, Couch RB. Interpandemic influenza in the Houston area, 1974-1976. *N Engl J Med* 1978;298:587-592.
2. Glezen WP, Payne AA, Snyder DN, Downs TD. Mortality and influenza. *J Infect Dis* 1982;146:313-321.
3. Marine WM, McGowan JE, Thomas JE. Influenza detection: A prospective comparison of surveillance methods and analysis of isolates. *Am J Epidemiol* 1976;104:248-255.
4. Keech M, Scott AJ, Ryan PJ. The impact of influenza and influenza-like illness on productivity and healthcare resource utilization in a working population. *Occup Med* 1998; 49:85-90.
5. Betts RF. Influenza virus. In: Mandell GL, Bennet JE, Dolin P, eds. *Principles and Practice of Infectious Diseases*. New York, NY: Churchill Livingstone;1995:1546-1567.
6. Centers for Disease Control and Prevention. Prevention and control of influenza: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 1998;47(RR-6):1-28.
7. Treanor JJ, Hall CB. Influenza and infections of the trachea, bronchi, and bronchioles. In: Reese RE, Betts RF, eds. A Practical Approach to Infectious Diseases. Boston, MA: Little, Brown;1996:240-257.
8. Hurwitz ES, Barrett MJ, Bregman D, et al. Public Health Service study of Reye's syndrome and medications. Report of the main study. *JAMA* 1987;257:1905-1911.
9. Couch RB. A new antiviral agent for influenza—Is there a clinical niche [editorial]? *N Engl J Med* 1997;337:927-928.
10. Hayden FG, Treanor JJ, Fritz RS, et al. Use of the oral neuraminidase inhibitior oseltamivir in experimental human influenza: Randomized controlled trials for prevention and treatment. *JAMA* 1999;282:1240-1246.
11. Centers for Disease Control and Prevention. Neuraminidase inhibitors for treatment of influenza A and B infections. *MMWR Morb Mortal Wkly Rep* 1999;48(RR-14):1-10.
12. Wingfield WL, Pollack D, Grunert RR. Therapeutic efficacy of amantadine HCl and rimantadine HCl in naturally occurring influenza A2 respiratory illness in man. *N Engl J Med* 1969;281:579-584.
13. Knight V, Fedson D, Baldini J, et al. Amantadine therapy of epidemic influenza A2-Hong Kong. *Antimicrob Agents Chemother* 1969;9:370-371.
14. Galbraith AW, Oxford JS, Schild GC, et al. Therapeutic effect of l-adamantamine hydrochloride in natrually occurring influenza A2/ Hong Kong infection. *Lancet* 1971;2:113-115.
15. Rabinovich S, Baldini JT, Bannister R. Treatment of influenza. The therapeutic efficacy of rimantadine HCl in naturally occurring influenza A2 outbreak. *Am J Med Sci* 1969;257:328-335.
16. Van Voris LP, Betts RF, Hayden FG, et al. Successful treatment of naturally occurring influenza A/USSR/77 H1N1. *JAMA* 1981; 245:1128-1131.
17. Younkin SW, Betts RF, Roth FK, et al. Reduction in fever and symptoms in young adults with aspirin or amantadine. *Antimicrob Agents Chemother* 1983;23:577-582.
18. Atkinson WL, Arden NH, Patriarca PA, et al. Amantadine prophylaxis during an institutional outbreak of type A (H1N1) influenza. *Arch Intern Med* 1986;146:1751-1756.
19. Zeigler T, Cox NJ. Influenza viruses. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. *Manual of Clinical Microbiology*. Washington, DC: American Society for Microbiology Press;1999:928-935.
20. Espy MJ, Smith TF, Harmon MW, Kendal AP. Rapid detection of influenza virus by shell vial assay with monoclonal anitbodies. *J Clin Microbiol* 1986;24:677-679.
21. Mills RD, Cain KJ, Woods GL. Detection of influenza virus by centrifugal inoculation

of MDCK cells and staining with monoclonal antibodies. *J Clin Microbiol* 1989;27: 2505-2508.
22. Stokes CE, Bernstein JJ, Kyger SA, Hayden FG. Rapid diagnosis of influenza A and B by 24-h fluorescent focus assays. *J Clin Microbiol* 1988;26:1263-1266.
23. Dominguez EA, Taber LH, Couch RB. Comparison of rapid diagnostic techniques for respiratory syncytial and influenza A virus respiratory infections in young children. *J Clin Microbiol* 1993;31:2286-2290.
24. Atmar RL, Baxter BD, Dominguez EA, Taber LH. Comparison of reverse transcription-PCR with tissue culture and other rapid diagnostic assays for detection of type A influenza virus. *J Clin Microbiol* 1996; 34:2604-2606.
25. Reina J, Munar M, Blanco I. Evaluation of a direct immunofluorescence assay, dot-blot enzyme immunoassay, and shell vial culture in the diagnosis of lower respiratory tract infections caused by influenza A virus. *Diagn Microbiol Infect Dis* 1996;25:143-145.
26. Ellis JS, Fleming DM, Zambon MC. Multiplex reverse transcription-PCR for surveillance of influenza A and B viruses in England and Wales in 1995 and 1996. *J Clin Microbiol* 1997;35:2076-2082.
27. Stockton J, Ellis JS, Saville M, Clewley JP, Zambon MC. Multiplex PCR for typing and subtyping influenza and respiratory syncytial viruses. *J Clin Microbiol* 1998;36:2990-2995.
28. Directigen® Flu A package insert. Becton Dickinson and Co; Cockeysville, MD;1996.
29. Waner JL, Todd SJ, Shalaby H, Murphy P, Wall LV. Comparison of Directigen FLU-A with viral isolation and direct immunofluorescence for the rapid detection and identification of influenza A virus. *J Clin Microbiol* 1991;29:479-482.
30. Chomel JJ, Remilleux MF, Marchand P, Aymard M. Rapid diagnosis of influenza A. Comparison with ELISA immunocapture and culture. *J Virol Methods* 1992;37:337-343.
31. Ryan-Poirier KA, Katz JM, Webster RG, Kawaoka Y. Application of Directigen® FLU-A for the detection of influenza A virus in human and nonhuman specimens. *J Clin Microbiol* 1992;30:1072-1075.
32. Johnston SL, Bloy H. Evaluation of a rapid enzyme immunoassay for detection of influenza A virus. *J Clin Microbiol* 1993;31:142-143.
33. Leonardi GP, Leib H, Birkhead GS, Smith C, Costello P, Conron W. Comparison of rapid detection methods for influenza A virus and their value in health-care management of institutionalized geriatric patients. *J Clin Microbiol* 1994;32:70-74.
34. Marcante R, Chiumento F, Palu G, Cavedon G. Rapid diagnosis of influenza type A infection: Comparison of shell-vial culture, Directigen Flu-A and enzyme-linked immunosorbent assay. *New Microbiol* 1996;19:141-147.
35. Newton DW, Mellen CF, Menegus MA. A practical and sensitive screening strategy for influenza virus [abstract]. Presented at: 99th Annual Meeting of the American Society for Microbiology, Chicago, IL, 1999. Abstract C-319.
36. ZstatFlu package insert. ZymeTx, Oklahoma City, OK;1998.
37. Colvalcuic KA, Webb KH, Carlson CA. Comparison of four clinical specimen types for detection of influenza A and B viruses by optical immunoassay (FLU OIA test) and cell culture methods. *J Clin Microbiol* 1999; 37:3971-3974.
38. Yamazaki M, Kimura K, Watanabe S, et al. Use of rapid detection assay for influenza virus on nasal aspirate specimens. *Kansenshogaku Zasshi* 1999;73:1064-1068.
39. Mitamura K, Sugaya N, Shimizu H, et al. Optical immunoassay test for rapid detection of influenza A and B viruses: An evaluation. *Kansenshogaku Zasshi* 1999;73:1069-1073.
40. QuickVue® Influenza A/B Test package insert. Quidel Corporation, San Diego, CA;1999.
41. Influenza A/B Rapid Test 510(k) summary for the US Food and Drug Administration. Roche Diagnostics Corporation, Indianapolis, IN;1999.
42. Claas EC, van Milaan AJ, Sprenger MJ, et al. Prospective application of reverse transcriptase polymerase chain reaction for diagnosing influenza infections in respiratory samples from a children's hospital. *J Clin Microbiol* 1993;31:2218-2221.
43. Fan J, Henrickson KJ, Savatski LL. Rapid simultaneous diagnosis of infections with respiratory syncytial viruses A and B, influenza viruses A and B, and human parainfluenza virus types 1, 2, and 3 by multiplex quantitative reverse transcription-polymerase chain reaction-enzyme hybridization assay Hexaplex®). *Clin Infect Dis* 1998;26: 1397-1402.