Influenza Testing in the Diagnostic Laboratory

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DOI: 10.1309/TFED6CLTYW4A6V06

Laboratory professionals need to understand the prevalence of influenza in the community, clinical manifestations, and test characteristics for the proper use of rapid diagnostic tests.

Rapid diagnosis of influenza is essential for reducing ancillary tests, decreasing inappropriate antibiotic use, and guiding appropriate antiviral therapy. Differences in rapid influenza tests stem mainly from which type of virus is identified and whether it can differentiate between influenza A and B viral antigens.

After reading this article, the reader should be able to understand the significance of viral influenza from an epidemiologic perspective, appreciate when tests to determine a microbiological diagnosis of influenza are indicated, and select optimum methods for laboratory detection for use in physician offices and hospitals. Microbiology exam 70601 questions and corresponding answer form are located after the CE Update section on p. 375.

Influenza is caused by single-stranded, enveloped, RNA viruses with helical nucleocapsids in the family Orthomyxoviridae. In the center of the viruses are segments of negative-stranded RNA, each with a closely associated nucleoprotein (NP). The viruses for which humans are the primary host that cause widespread disease include influenza A and influenza B, separated based on differences in NP and matrix (M) proteins. Influenza C viruses sometimes cause mild upper respiratory infections, but disease is limited primarily to local outbreaks. Influenza A viruses are subtyped and named on the basis of their surface glycoprotein hemagglutinin (HA) and neuraminidase (NA) antigens that are involved in viral attachment and release from host cells, respectively.1 Common strains of influenza A circulating in humans include H1N1, H1N2, and H3N2. The strain of avian influenza of current interest, which is spreading readily among birds and rarely from birds to humans, is designated H5N1. Antibodies developed against HA and NA antigens through natural infection or as a result of immunization are protective against disease. However, influenza viruses change over time as a result of point mutations or exchange of genetic material from another strain of influenza resulting in antigenic drift. A major genetic change in influenza A, referred to as antigenic shift, will result in expression of different HA and NA antigens which are immunologically distinct. Such changes enable pandemic influenza to occur due to lack of protective antibody in susceptible populations. Influenza B is less antigenically variable than influenza A, and it is not known to infect animals or birds.

Epidemics of influenza are responsible for a significant amount of morbidity and mortality in the United States with approximately 133,900 hospitalizations and 36,000 deaths attributable to influenza or influenza-related complications annually.2 During winter months, the peak of influenza activity in temperate climates, clinicians are challenged to distinguish influenza from other viral and bacterial respiratory illnesses, as nonspecific signs and symptoms may mask the underlying pathogen.3,4

Major epidemics of influenza A occur every 2 to 3 years, due mostly to antigenic drift variants, while influenza B epidemics occur every 3 to 4 years.5 In 2004-2005, the World Health Organization (WHO) and National Respiratory and Enteric Virus Surveillance System (NREVSS) Collaborating Laboratories reported 23,549 positive specimens for influenza out of a total 157,759 collected. Of the positive specimens, 75.4% were influenza A and 24.6% were influenza B.6 These data differed sharply from the preceding year in which 99.0% were influenza A viruses.7 There are also data showing that the distribution of circulating viruses varies widely based on geographic location.8

Influenza vaccines are the mainstay of disease prevention. The parental formulation contains inactivated strains of influenza A and influenza B, whilst the nasal spray formulation contains live attenuated viruses. During 2003 and 2004, there were shortages of the inactivated vaccine in the United States, as well as concerns of diminished protection due to lack of inclusion of current circulating strains. Recent news reports concerning a potential avian influenza A pandemic have further intensified interest and concern by the public, as well as health care providers in accurate diagnosis of influenza. Thus, understanding current indications and alternatives for laboratory diagnosis of influenza is essential.

Clinical Manifestations and Rationale for Laboratory Diagnosis of Influenza

Influenza is transmitted from person to person primarily by airborne droplets generated by coughing, sneezing, and even talking. The signs and symptoms of influenza are nonspecific, appearing after an incubation period of 1 to 4 days. When influenza is circulating in the community, the differential diagnosis
must include other common bacterial and viral respiratory pathogens, which may produce overlapping clinical manifestations. However, several collective characteristics identified in the history and physical examination should be utilized to include or exclude the possibility of infection with influenza. Criteria that assist in the diagnosis of influenza include fever, rigors, and sweating, as well as the onset of symptoms within 3 days of the physician's visit. Pertinent negative symptoms suggestive of another diagnosis include no systemic symptoms, not coughing, and being able to perform daily activities. A major complication of influenza that is responsible for much of the mortality associated with the illness is development of secondary bacterial pneumonia in the respiratory tract damaged by the influenza viruses. The benefits of making a microbiological diagnosis of influenza early in the course of illness include: (1) selection of the most appropriate antiviral agent to lessen the duration and severity of illness; (2) avoiding the use of unnecessary antibiotics unless secondary bacterial infection is suspected; (3) reducing the likelihood of spreading infection to others; (4) guidance for administration of antiviral prophylaxis; and (5) epidemiological surveillance. A negative test is also important during outbreaks of influenza-like illnesses since it guides physicians to search for other infectious agents. However, the possibility of coinfection or secondary infection with other pathogens should always be considered in persons suspected or proven to have influenza.

Current Alternatives for Laboratory Diagnosis of Influenza

Viral propagation in cell culture and measurement of acute and convalescent antibody titers are accurate and reliable means for detecting influenza. Their drawbacks include the length of time (days to weeks) necessary for their completion and the complexity of technology that limits their performance to specialized laboratories. Detection of viral antigens in exfoliated respiratory epithelial cells by immunofluorescent antibody (IFA) staining can theoretically provide same day results, assuming a fluorescent microscope and an experienced microscopist are present. Immunoassays for viral antigen detection may also provide results within 2 hours, but some of them can be technically demanding and unsuitable for performance in physician office laboratories. Reverse transcriptase PCR-based methods (RT-PCR) are technically the most sensitive types of assays for detection and genetic characterization of influenza viruses. PCR assays can be performed in 1 day, but performance requires laboratory facilities with molecular biology capabilities. None of the above methods is suitable for point-of-care testing to aid physicians in immediate diagnosis and management of persons suspected of having influenza.

Rapid Influenza Testing

Several rapid diagnostic tests for detection of influenza A and B are now commercially available for use in clinical laboratory and physician office laboratory settings. The tests detect influenza viruses within 10 to 30 minutes, making them suitable for use as point-of-care tests (Table 1). Rapid influenza tests based on detection of immunological determinants such as viral NP can detect both viable and nonviable virus in clinical specimens. Some rapid tests detect the presence of influenza A only, others identify both influenza A and B but do not differentiate between them, and some detect both influenza A and B as well as differentiate between them. Several commercially sold products in the United States are considered waived tests, with the remainder falling under the moderate complexity category according to the Clinical Laboratory Improvement Act (CLIA). Considering the fact that 60% of the more than 154,000 diagnostic laboratories registered through CLIA possess a Certificate of Waiver, those tests which can be performed under this designation will be the ones most widely used. Rapid tests also differ in methodology and the types of specimens (throat swabs, nasal swabs, nasopharyngeal secretions, or nasal washes) appropriate for collection. It is important to emphasize that the amount of virus found in different samples varies which can affect analytical sensitivity. Nasal samples are generally preferred over throat swabs because of higher quantities of detectable virus. Specimens should be collected as soon as possible after the onset of symptoms because virus shedding is at its peak during the first few days of illness, and antiviral drugs work best if initiated within 48 hours of onset of illness. During peak influenza season, busy clinics may find the most rapid tests that can be performed in 10 minutes to be preferable to those that require longer times of up to 30 minutes since they allow patients to be processed more quickly.

When compared with viral isolation, rapid diagnostic tests as a group have had sensitivities of approximately 70% and specificities of approximately 90%. Their variable performance has been attributed to the timing, type, and quality of specimens collected, the population sampled, and the level of experience of the test performer. For example, studies performed in children may yield higher sensitivities than adults since children tend to harbor larger quantities of virus in their respiratory tracts making them more easily detectable. Purchase prices for rapid influenza tests are generally between $10 to $30 per test, supplied in kits containing reagents to perform 10 to 25 tests, depending on the manufacturer. The rapid tests cannot provide information on viral subtypes, but this is not generally important for individual patient care unless avian influenza is suspected.

Use of a test that does not include both influenza A and B is not recommended since the types of virus circulating changes over time, and it is impossible to predict which one may be predominant at any given time. A previous argument for the use of rapid tests that differentiate between influenza A and B was that this information could justify treatment with more expensive neuraminidase inhibitors if type B was present. However, in light of current treatment recommendations by the CDC, the distinction of influenza A from influenza B in the laboratory may not be as important as in the past. All of these factors, in addition to shelf life, ease of use, sensitivity, and specificity are important to consider when choosing a rapid influenza test.

Persons who may benefit the most from rapid influenza testing include children and adults with lower respiratory tract illness who have underlying medical conditions placing them at risk for secondary complications of influenza. The value of rapid influenza tests on patients with flu-like illnesses, who present outside an influenza outbreak period and are considered to be at low risk for influenza-related complications is diminished by their low positive predictive value in this setting. If a positive result is achieved, confirmation by IFA tests, viral culture, or RT-PCR is suggested before initiating antiviral therapy. In the beginning of influenza season, rapid testing is of clinical value for detection and guiding management of persons with typical manifestations. However, once influenza has been well documented in the community, and if epidemiological information is available about the types of viruses in circulation, clinical diagnosis may be sufficient. Still,
rapid tests may be helpful in making decisions concerning use of antiviral agents. When influenza activity in the community is unknown, rapid testing will have an unknown predictive value, so confirmatory testing is recommended. Thus, it is prudent for clinicians to monitor influenza activity through local health departments and weekly surveillance reports issued by the Centers for Disease Control and Prevention (CDC) available on their Internet Web site (http://www.cdc.gov/ncidod/diseases/flu/weekly.htm). WHO recommendations for optimum use of rapid influenza tests during periods when influenza activity is known or unknown are summarized in Figures 1A and 1B.

### Impact of Laboratory Diagnosis on Patient Management and Choice of Antiviral Drugs

Rapid diagnostic testing for influenza has increasingly demonstrated an impact on patient management by reducing ancillary tests performed, decreasing inappropriate antibiotic use, decreasing length of stay in emergency departments, and increasing utilization of antiviral therapy.\(^3\)\(^4\)\(^1\) Four antiviral drugs are currently approved for influenza treatment–amantadine, rimantadine, zanamivir, and oseltamivir. Amantadine and rimantadine inhibit the M2 viral protein, which is involved with hydrogen ion transport. These drugs are approved only for treatment and prophylaxis for influenza A since the M2 protein is not present in influenza B.\(^4\)\(^1\) Zanamivir and oseltamivir, inhibitors of viral neuraminidase, are effective against both influenza A and B, preventing viral release from infected host cells. Oseltamivir, administered in capsule form or as a liquid suspension is available for treatment of influenza in persons who are 1 year of age and older, and for prophylaxis of influenza in exposed persons who are 13 years of age and older. Zanamivir, administered as an inhalant, is approved only for treatment and is limited to persons who are 7 years of age and older. Both classes of drugs have been shown to produce beneficial effects by reducing the duration of influenza symptoms by 1 to 2 days. However, to achieve maximum efficacy, they must be started within 48 hours of the onset of illness.\(^3\)\(^4\)\(^1\)\(^5\)
The primary advantage of amantadine is its relatively low cost. However, development of viral resistance during administration is very common, and untoward side effects such as nervousness, confusion, and nausea may occur. Rimantadine has a better side effect profile, but it also carries a greater cost. Due to a significant increase in amantadine resistance in influenza A in the United States from 1.9% in 2004 to 14.5% during the first 6 months of the 2004-2005 influenza season, the CDC has provided an interim recommendation for 2005-2006 that neither amantadine nor rimantadine be used for treatment or prophylaxis of influenza A. Ninety-one percent of H3N2 influenza A viruses tested at the CDC in early 2006 were resistant to amantadine and rimantadine. The neuraminidase inhibitors have fewer adverse effects; they are the only option for the treatment of influenza B; and they are less likely to induce resistant strains. However, they are substantially more expensive. The cost of oseltamivir may be as much as 20 times that of amantadine. Resistance to the neuraminidase inhibitors has been observed, but thus far this does not occur as often as with amantadine.

**Concern for Avian Influenza**

The viruses implicated in the great influenza pandemics of the 20th century originated directly from avian strains through genetic reassortment between human and avian strains or possibly through adaptation of purely avian strains to infect and replicate in humans. The ongoing outbreak of avian influenza in poultry, aquatic birds, and even pigs in several Asian countries with numerous occurrences of direct bird-to-human transmission in association with mortality exceeding 50% has heightened awareness of this potential global threat. Moreover, a limited number of possible human-to-human transmissions of avian influenza have also been reported. Epidemiologic exposures that may place a person at risk for infection with avian influenza H5N1, even if he/she has not traveled to countries where these viruses have been identified, include close contact of a symptomatic individual with an ill traveler from one of the affected regions or residence in an area where there are rumors of the death of domestic fowl and one or more of the following: (1) contact with live or dead domestic fowl or wild birds; (2) exposure to settings in which domestic fowl have been confined within the previous 6 weeks; (3) exposure within 6 weeks of a person confirmed to have avian influenza; (4) contact with persons with unexplained acute respiratory illness that resulted in severe pneumonia or death; and (5) occupational exposure in poultry processing plants, live animal markets, restaurants dealing with recently killed domestic fowl, pet shops dealing with birds, health care settings, and laboratories where samples possibly containing avian influenza were handled.

Avian influenza viral subtypes belong to the influenza A group, and the strain now circulating in Asia has the H5N1 designation. Although some differences in clinical manifestations between avian influenza and human influenza A have been suggested, differentiation should be based on epidemiologic history and genetic verification. Rapid tests which detect the presence of influenza A should be positive if avian influenza is present, but none can subtype the virus to confirm it is indeed H5N1. Persons suspected of having avian influenza should be tested by methods that can determine the subtype of the virus such as PCR. Moreover, some manufacturers of rapid influenza tests have not conclusively determined the accuracy of their tests in the diagnosis of H5N1 avian influenza. The sensitivity and specificity for rapid tests in the diagnosis of H5N1 avian influenza is unknown.
at this time and their use in patients with suspicion of avian influenza is not recommended unless the facility where it is performed has the capability to perform confirmation RT-PCR or culture for the H5N1 subtype so that human influenza A can be satisfactorily excluded. If PCR facilities are not available, samples should be sent to a reference laboratory immediately. Development of test kits aimed at detection of H5N1 is currently underway. Limited data suggest that oseltamivir is effective against avian influenza. However, there is evidence that in some cases its administration does not completely suppress viral replication, allowing resistance to develop during the course of therapy. These recent observations suggest that higher dosages, longer duration of treatment, and/or combination treatments should be considered. The concern for development of resistance to the current neuraminidase inhibitor agents in avian influenza underscores the need for new antiviral agents that may act at different targets within the virus. Currently circulating strains of H5N1 are resistant to M2 inhibitors, so these drugs have no role in treatment.

Summary

Influenza is the only infectious disease that causes pandemics at frequent intervals, inflicting considerable morbidity and mortality. Concern for accurate and timely detection of influenza is greater now than in previous years, largely because of the concern for spread of avian influenza to humans. A variety of relatively simple and moderately priced rapid assays are now available to enable physicians to identify patients with influenza A and B in an ambulatory care setting, to provide guidance for administration of specific antiviral agents, and to reduce the unnecessary administration of antibiotics. The value of rapid testing is influenced by the underlying prevalence of influenza in the community at any given time. All physician office laboratories and hospitals should be familiar with current recommendations by the WHO and CDC regarding laboratory diagnosis of human influenza and avian influenza. Hospitals should have a comprehensive written plan in place developed in consultation with their local and state health departments to deal with detection, management, and limiting spread of infection in the event avian influenza strikes in the United States.

Some Internet Links to Additional Sources of Influenza Information:

CDC Influenza Homepage: at http://www.cdc.gov/flu/

