Rapid Tests for Influenza

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A variety of antigen-capture assays are commercially available for the detection of influenza. In addition, real-time multiplex polymerase chain reaction (PCR) has been used to detect influenza A and B in clinical specimens. The commercial assays can be completed in less than 30 minutes and have a sensitivity of at least 70% and a specificity of 90%, compared with viral isolation. They are useful not only in the diagnosis and treatment of individual patients with influenza-like illness but also in surveillance for influenza, decreasing the time of nosocomial outbreaks, decreasing the use of laboratory tests, and decreasing antibiotic use in patients with influenza. Some of the rapid antigen assays, and PCR, can detect the H5N1 and H9N1 viruses. Real-time multiplex PCR also detects a variety of respiratory viruses within 6 hours, with only 1 hour of hands-on technician time. The widespread use of the rapid tests for influenza is changing the practice pattern of physicians who care for patients with influenza.

Introduction

In the United States, influenza A and B viruses cause yearly epidemic illness that results in 20,000 to 30,000 excess deaths per year, 100,000 hospitalizations, and an economic burden of 3 billion to 5 billion dollars [1–3]. Much of the morbidity and mortality from influenza infections occurs in persons aged older than 65 years and in persons with underlying chronic health problems. Hospitalization rates during influenza epidemics may be two- to fivefold higher than during nonepidemic periods [3]. In addition, healthy children aged younger than 1 year also bear excessive morbidity from influenza epidemics, with hospitalization rates attributable to influenza similar to those for high-risk adults [4].

Annually, global influenza surveillance monitors morbidity and mortality. Specimens are collected from patients with influenza-like illness so that the circulating influenza virus can be isolated, identified, and evaluated for antigenic changes. Novel strains of influenza are identified and saved for production of the subsequent year's vaccine. Influenza surveillance has been largely powered by the need to determine rates of infection, to establish public health policy for prevention strategies, and to assess morbidity and mortality from influenza; impact on individual practitioner's clinical decisions has been limited. The epidemic nature of influenza may be exploited to help diagnose influenza by linking circulation of the virus in the community with a clinical case definition. The presence of fever and cough as a clinical case definition has been shown to accurately identify influenza 77% to 77.6% of the time, with a negative predictive value of approximately 39.3% to 40% [5,6,]. However, availability of surveillance data lags 2 to 3 weeks behind the actual occurrence of the illness in the community; thus, surveillance information cannot provide timely diagnostic information (Unpublished observation). A clinical case definition may be inaccurate because other respiratory viruses circulate at the same time as influenza and produce similar symptoms.

Annually, influenza infections present a large-scale diagnostic and therapeutic challenge. Although influenza vaccination is the primary modality to prevent the infection, vaccine is not used optimally in the high-risk population, and insufficient supply of vaccine prohibits immunization of a larger population. Viral isolation, the traditional test to confirm the diagnosis of influenza, may take 9 days longer than rapid diagnostic testing and thus may not be helpful in assisting clinical decisions [7]. Commercially available rapid antigen testing for influenza can be used to establish a specific diagnosis for individual patients, and to identify clusters of cases, which facilitates timely intervention for treatment and prevention. This paper discusses the strengths and limitations of tests, and the development of real-time polymerase chain reaction (PCR). Their impact on individual treatment decisions, surveillance for influenza, and the cost of care is reviewed.

Discussion

Historically, rapid tests for influenza became more widely used and more readily available when high-quality monoclonal antibody was produced for the strains of circulating influenza viruses [8]. Indirect immunofluorescence, first described by Liu [9] in 1956, was a simple and quick technique to diagnose influenza. Epithelial cells from nasal swab or throat swab specimens were fixed on a microscope slide, and antibody to the virus (which was fluoresceinlableled) detected influenza in clinical specimens. Availability of the monoclonal antibody resulted in more widespread

use of antigen-capture enzyme immunoassays and production of commercial assay kits. Most of the tests are variations on dot blot immunoassays, with different colorometric endpoints. One of these uses a chromogenic substrate that is recognized by influenza neuraminidase. All detect influenza viruses within 30 minutes, are self contained, and have an easily recognizable endpoint. Some are point-of-care kits that may be performed outside of a certified laboratory. The tests differ in the types of influenza viruses that they can detect and their ability to distinguish between influenza types A and B (Table 1). The assays have different sensitivity to different influenza virus subtypes, but most have an overall sensitivity of at least 70% and a specificity of 90%, compared with viral isolation. There are few comparative data between the assays. In general, the predictive value of the test depends on the amount of influenza circulating in the community, the susceptibility of the patient, adequacy of the test used (sensitivity and specificity), and the adequacy of the specimen collection [10]. Other factors that affect performance of the assay are the patient population evaluated, pediatric versus adult patient specimens, and time after onset of symptoms when the specimen is collected [11]. The following text includes a discussion of applicability and limitations of the assays that have been reported in recent peer-reviewed journals.

Directigen Flu A (Becton-Dickinson, Sparks, MD) is an enzyme immunomembrane filter assay. Influenza A antigen is bound to the surface of a membrane. The antigen is detected by an enzyme-linked immunosorbent assay procedure using enzyme-conjugated monoclonal antibodies that are specific for a conserved epitope of the influenza nucleoprotein. Under some conditions, the sensitivity and specificity of the Directigen Flu A was 100% and 91.6%, respectively, compared with viral isolation and direct immunofluorescence; positive predictive value was 62.6%, and the negative predictive value was 100% [12]. The test may produce false-positive results when the reaction is weak, but it is reported that influenza A is not present if the test result is negative [12]. This is contrary to experience at the Edward Hines VA Hospital, where this test had been extensively used for surveillance and diagnosis of influenza in ambulatory and hospitalized patients. When Directigen Flu A was used on clinical specimens, its usefulness was limited by the sensitivity of the assay. Early in a community outbreak the test result was negative; viral isolation, which provided confirmation of the clinical diagnosis of influenza A, took up to 14 days (Unpublished observation).

Sensitivity of Directigen Flu A depends on the population tested, and type and timing of specimen collection. The sensitivity of Directigen Flu A was 86.8% when it was used to detect influenza during a nursing home outbreak [13]. Using nasopharyngeal swabs collected on day 2 of experimental influenza A virus infection in healthy volunteers, the sensitivity of the assay ranged from 64% to 78%. Testing throat gargle specimens from this same cohort decreased sensitivity to 24%, compared with viral isolation [14]. Viral titers in the specimens were highest on day 2 of illness and decreased thereafter until day 7 [14]. This illustrates an important problem that is encountered with use of all of the assays. Patients shed higher viral titers early in the illness and less as the illness progresses, and thus the sensitivity of the test depends on the duration of symptoms before seeking care and collection of a specimen for testing [14]. In addition, children shed higher titers of virus; thus, rapid testing in children may be more sensitive [11].

Directigen Flu A + B (Becton-Dickinson) is a rapid-membrane enzyme immunoassay that extracts viral antigen from patient specimens and binds it to a membrane surface. Antigens are detected by enzyme-conjugated monoclonal antibodies specific for influenza A or B virus nucleoprotein. This test differentiates between influenza A and B; thus, amantadine or rimantadine may be chosen for treatment of influenza A rather than oseltamivir or zanamivir. The test's sensitivity was 43.83% and specificity was 99.74% [15•]. The values make this a very good confirmatory test when clinical suspicion of influenza A infection is high but a less accurate test when used to screen large populations [15•].

QuickVue (Quidel Corporation, San Diego, CA) is a lateral-flow immunoassay that uses specific monoclonal antibodies in a strip for qualitative detection of influenza A or B antigens. Virus particles are disrupted so that the internal viral nucleoprotein is exposed. The nucleoproteins react with specific monoclonal antibodies and indicate the presence of influenza virus with a pink to red test line. If the color reaction is weak, the test is inconclusive, which may lead to false-positive results [16]. In a study in a pediatric population, 41 of 85 positive results were difficult to interpret when reading this test because of the presence of a very faint pink line [17]. In this study, using nasopharyngeal swabs, QuickVue had a sensitivity of 79.2%, specificity of 82.6%, positive predictive value of 49.4%, and a negative predictive value of 94.9% [17]. Sensitivity of the test was highest on day 1 of illness and decreased to 50% on day 2. Specificity decreased to 63.6% on day 2 when QuickVue was used on frozen nasopharyngeal swabs obtained from patients who had started antiviral therapy. False-positive results were reported, which decreased the specificity of the assay. Because antiviral therapy decreases viable virus in specimens, it is possible that the false-positive results derived from the detection of viral antigen [16]. Comparison of the assay with PCR could have confirmed this hypothesis. An endpoint that is difficult to read may be a problem for interpretation of results of this assay.

ZstatFlu-II (ZymeTx Inc., Oklahoma City, OK) is a chemiluminescent rapid test for influenza. The test is not able to distinguish between influenza A and B because it detects influenza viral neuraminidase. Viral neuraminidase acts on the substrate molecule and releases the chemiluminescent reporter groups. Sodium hydroxide is added to terminate the reaction and produce the chemiluminescence. Light from the reaction is captured on Polaroid high-speed detector instant film so that a positive reaction yields a white image against a

Table 1. Commercially available rapid tests for influenza

Test	Method	Types detected (discriminates between A and B)	Specimen	Waived by CLIA	Sensitivity	Specificity
Directigen Flu A & B (Becton-Dickinson, Sparks, MD)	Immunoassay membrane test	A, B (yes)	Nasopharyngeal swab, throat swab, nasal wash, nasal aspirate	No	Flu A 86.2%; flu B 80.8%	Flu A 90.7%; flu B 99.5%
FLU OIA A/B (Thermo Electron, Waltham, MA)	Optical immunoassay	A, B (no)	Nasopharyngeal swab, throat swab, nasal aspirate, sputum	No	Flu A nasal aspirates 88.4%*	Flu B nasal aspirates 69.4%*
Xpect Flu A/B (Remel Inc., Lenexa, KS)	Immunochromatographic test	A, B (yes)	Nasal wash, nasopharyngeal swab, throat swab	No	Flu A 92.2%; flu B 97.8%	Flu A and B 100%
QuickVue Influenza A + B (Quidel Corporation, San Diego, CA)	Lateral-flow immunoassay	A, B (yes)	Nasal wash, nasal aspirate, nasal swab	Yes	Nasal wash or nasal aspirates for A 77%, B 82%	Nasal wash or nasal aspirates for A 99%, B 99%
BinaxNOW Influenza A & B (Binax Inc., Portland, ME)	Immunochromatographic test	A, B (yes)	Nasopharyngeal swab, nasal wash, nasal aspirate	No	Flu A 75%, flu B 50%	Flu A 100%, flu B 100%
ZstatFlu (ZymeTx Inc., Oklahoma City, OK)	Neuraminidase assay	A, B (no)	Throat swab	Yes	Flu A 50.4% to 78.3%, flu B 39.2% to 74.5%	Flu A 94.9% to 100%, flu B 95.6% to 100%

*Same as Flu OIA [10,34–41].
CLIA—Clinical Laboratory Improvement Amendment.

black background and provides a permanent record of the reaction. The chemiluminescent substrate molecule is specific for the influenza viruses and has no crossreactivity with the neuraminidase in parainfluenza viruses. ZstatFlu-II tested on nasal aspirate specimens obtained from pediatric patients had a sensitivity of 88%, specificity of 92%, positive predictive value of 75%, and negative predictive value of 96%, compared with viral isolation. The assay was more sensitive than Directigen Flu A + B [18].

Lateral-flow chromatographic membrane immunoassay, Xpect Flu A/B (Remel Inc., Lenexa, KS), is a new twist on the immunoassay that uses separate membrane strips to detect viral antigen from influenza A or B [19]. The test had a sensitivity of 94.4%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 97.5% when used to test specimens from adults and children. Concurrent testing at three hospitals revealed no specific differences in performance statistics between the different sites [19].

A comparative review of commercially available rapid antigen testing is included in Table 1. Testing may or may not differentiate between an influenza A and B infection. The ability for the testing to detect novel influenza viruses is important, but only Directigen Flu A + B has been shown to detect H5N1 viruses. Assays that have been waived by the Clinical Laboratory Improvement Amendment may be performed at point of care, outside of a certified laboratory.

Reverse transcriptase PCR (RT-PCR) has been used as a rapid diagnostic method to detect influenza A and B. RNA is isolated in the specimens, and cDNA is synthesized with a reverse transcriptase and amplified with a segment of the conserved segment of the influenza virus gene. An end-labeled gene probe detects the PCR products that are visualized on agarose gel with ethidium bromide and/or radiograph film. Use of radiography increases the sensitivity of the test from detecting 5000 copies per mL to 50 copies per mL of RNA but requires overnight incubation; thus, the time for the test completion increases from 6 to 24 hours [20]. RT-PCR can distinguish the subtypes of influenza A and identify influenza B [20]. The gold standard for diagnosis of influenza has been viral isolation. However, RT-PCR may be more sensitive than tissue culture. There are approximately 100 to 1000 tissue culture infectious dose ($TCID_{50}$) per mL of influenza virus in respiratory secretions. It is estimated that 1 $TCID_{50}$ per mL of influenza virus contains approximately 100 copies of viral RNA, and thus respiratory secretions contain approximately 10^4 to 10^5 copies of viral RNA [21••]. PCR using the LightCycler detected 0.01 $TCID_{50}$ of influenza A virus and as low as 1.6×10^{-7} and 1.2×10^{-7} μg of influenza A and B viruses, respectfully [22]. $TCID_{50}$ as determined by viral isolation measures viable virus, but viral infection produces specific genomic nucleic acids from viable and nonviable virus. PCR will detect these nucleic acids because it measures a conserved segment of the matrix gene.

Polymerase chain reaction is expensive and requires specific reagents, specialized equipment, an experienced technician, and standardization at the laboratory that is performing the test. Because minute amounts of nucleic acids are detected with PCR, contamination from one specimen to the next may occur, from the environment or from the hands of the technician, which would result in false-positive results. Strict adherence to isolation methods that prevent cross-contamination of specimens during preparation and processing is essential in maintaining quality control. The validation of the PCR assay comes in part from assay of dilutions of the targeted virus, which defines the quantitative limits of the assay. In addition to a negative control containing no virus, every sample has a set of internal controls that are run concurrently. Every sample is spiked with eukaryotic RNA. Primers are added that will specifically amplify the eukaryotic RNA, and detection of this product serves as an internal control to ensure that conditions are correct for the synthesis of viral RNA. Specimens with an identical mixture to the experimental samples may be run without reverse transcriptase. The samples without reverse transcriptase should not detect amplified product; this is a marker for contamination or carry-over of RNA from another sample. PCR products are detected using gel electrophoresis, blotting, or hybridization. These methods are very labor intensive and lengthen the time of the assay.

Automated nucleic acid extraction and real-time PCR, which processes specimens in 6 hours with 1 hour of hands-on time, generate diagnostic results within 1 working day [21••,23]. PCR products are detected using fluorophore-labeled hybridization probes. Fluorescence values from amplification products formed during the PCR are measured in the LightCycler. A melting curve may also be produced to differentiate H1N1 and H3N2 subtypes [21••]. This method is able to detect 120 copies of RNA per specimen of H1N1 virus and 350 copies of RNA of the H3N2 virus [21••]. When testing this methodology with clinical specimens, freshly obtained specimens had higher sensitivity than did frozen specimens, probably because of degradation of viral RNA in stored specimens [21••].

Real-time PCR may be modified to perform multiplex amplification and detection where multiple pairs of primers are used in the same reaction so that multiple products can be identified after one reaction [23]. Multiplex PCR reduces the post-PCR processing, which increases the speed of the technique and reduces cross-contamination. Available technology can now detect four fluorophores in a single well so that four viruses can be detected. In an evaluation of 358 clinical specimens, seven viruses (influenza A, influenza B, respiratory syncytial virus, parainfluenza virus 1, 2, 3, 4) were assayed using two multiplex PCR assays [23]. This technique was more sensitive than tissue culture and detected virus within 6 hours of receipt of the specimen [23].

Use of the rapid diagnostic tests has positively impacted the care of patients with influenza. Rapid antigen testing of specimens from pediatric patients with influenza-like illness decreased the number of laboratory tests, decreased antibiotic use, decreased length of stay in the emergency room, and increased use of appropriate antivi-

ral therapy [24,25•]. In nursing home patients, use of rapid testing for flu decreased the overall duration of an outbreak of influenza A and resulted in a trend toward lower laboratory costs but an increase in hospitalizations [26]. In mathematical models of influenza infections in adults, rapid testing is beneficial when influenza B is prevalent and the probability of diagnosing influenza is low [27,28].

Public health officials have been concerned that use of the rapid detection methods would decrease the number of viral isolates obtained from surveillance. In Hawaii, when surveillance incorporated rapid testing, the number of specimens submitted and the number of influenza isolates obtained increased. This increase was independent of the activity of influenza infections in the community [29]. Increasing surveillance enhances the capacity to recover novel influenza isolates. Rapid testing has also made results of testing available much earlier than cell culture results, which is particularly important in controlling outbreaks in hospitals, nursing homes, and cruise ships [5]. Because a sophisticated virology laboratory is not needed, rapid testing could increase the availability of surveillance information. Surveillance done with rapid antigen testing provides clinicians with current information about outbreaks [5]. The impact of rapid testing on the availability and timeliness of epidemiologic data for number of cases of influenza, hospitalizations from influenza and pneumonia, and deaths from influenza needs to be assessed. At Edward Hines VA Hospital, use of rapid testing has made a great impact on diagnosing influenza in the ambulatory and inpatient population, facilitating the discovery of clusters of nosocomial influenza in the nursing home, and facilitating the initiation of infection control measures and the rational use of antiviral medication for treatment and prophylaxis (Unpublished observation).

Surveillance for influenza, traditionally done with viral isolation, provides a way to detect new strains of influenza that may cause pandemic illness. In 1997, an outbreak of highly pathogenic H5N1 influenza virus occurred among poultry in Hong Kong. Limited spread of the virus to humans occurred with a very high mortality [30]. Subsequently, outbreaks of H7N7 and H9N2 avian viruses, which spread to humans, occurred [31,32]. In 2003 and 2004, H5N1 re-emerged, causing disease among poultry throughout Asia, with limited transmission to humans. H5N1 virus was detected using a PCR with primers specific for the H5N1 virus [33]. Other RT-PCR assays use a segment of the matrix gene that is highly conserved among influenza A H1N1, H3N2, H2N2, H5N1, and H9N2; pairwise distance analysis showed more than 80% homology between all strains. Although this assay was tested only for H3N2 and H1N1 strains, it is likely that H1N2, H2N2, H5N1, and H9N1 strains would also be detected [22]. Directigen Flu A + B is licensed to detect H5N1 virus. Some avian strains cannot be supported in tissue culture; therefore, use of rapid antigen detection is important for surveillance and diagnosis.

Conclusions

A variety of rapid tests for the detection of influenza infection are available, including commercially available kits that can be used at the bedside, and real-time multiplex PCR, which can detect a number of antigens at one time. The commercially available tests are helpful in the diagnosis of influenza infections and detection of clusters of cases of nosocomial influenza. They not only facilitate the care of patients with flu by decreasing the amount of antibiotics used and increasing the appropriate use of antivirals but also improve surveillance for influenza. Rapid tests also may help in the detection of pandemic flu. Widespread use of the rapid antigen tests is changing the assessment and treatment of influenza A and B infections.

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