ABSTRACT

Objectives: To describe the strengths and limitations of the available influenza diagnostics, with a focus on rapid antigen detection assays and nucleic acid detection assays.

Methods: A case-based presentation is used to illustrate the potential limitations of rapid antigen detection assays for influenza.

Results: Influenza is a seasonal illness; estimates attribute influenza to approximately 200,000 hospitalizations and 41,000 deaths in the United States annually. Antigen detection assays for influenza are rapid and convenient, and thus are widely used in a variety of health care settings, even though the sensitivity of these assays may be suboptimal. The United States Food and Drug Administration has recently created new guidelines intended to improve the oversight and performance characteristics of influenza antigen detection assays. Molecular assays, although more costly and complex, are more sensitive and may be designed to simultaneously detect multiple respiratory pathogens within a single assay.

Conclusions: Diagnostic assays for influenza can vary greatly with regards to analytical performance characteristics, complexity, turnaround time and cost. This can have important patient care and infection prevention implications.

Case Scenario

During the month of January, a woman more than 90 years of age presented to the emergency department from a skilled nursing facility with hypoxia and complaints of shortness of breath. Her oxygen saturation was 85% on room air. Two days prior to the current presentation, a rapid influenza antigen assay was performed on a nasopharyngeal swab from the patient; the result was negative. Out of concern for bacterial pneumonia, antimicrobial therapy with ciprofloxacin was initiated at that time. In the emergency department, her complete metabolic panel and complete blood count were within normal limits, and a chest X-ray was negative for pleural effusion, pneumothorax and consolidation. She was admitted to the hospital for observation. A repeat nasopharyngeal swab was collected for influenza testing using a nucleic acid amplification assay, which was positive for influenza A. The primary physician from the skilled nursing facility called the laboratory to inquire about a possible explanation for the discrepant results observed between the rapid influenza antigen test and the nucleic acid amplification assay.

Questions

Influenza is a seasonal illness that is estimated to be responsible for approximately 200,000 hospitalizations and 41,000 deaths in the United States annually. This consult will highlight the different test methodologies that are available for influenza diagnosis, with the aim of answering the following questions:

- What is the clinical utility of influenza testing?
- Is a negative influenza rapid antigen assay result a reliable way to rule out disease?
• What is the relative sensitivity of a rapid influenza antigen assay compared to a molecular assay for detection of influenza?

Background

The influenza viruses are enveloped members of the family Orthomyxoviridae. They have segmented, negative-sense RNA genomes and are distinguished from one another based on the expression of the surface glycoproteins neuraminidase and hemagglutinin. These glycoproteins allow for the attachment of the virus to host respiratory tissue and contribute both to the specificity of the host that can be infected with a specific strain, as well as disease severity. The differential expression of these surface glycoproteins are used to further classify influenza A into various subtypes (ie, H1N1 or H3N2).

There are three different genera of influenza: influenza A, influenza B and influenza C. Influenza C causes disease primarily in animals, whereas the vast majority of disease in humans is caused by influenza A and influenza B. Both viruses are responsible for overlapping seasonal epidemics, with most disease in North America occurring between December and March. During “influenza season,” the proportion of disease attributable to influenza A (typically H1N1 or H3N2) and influenza B varies from year to year. Although it is common for a single subtype to predominate in a given influenza season, successive or overlapping waves of infection due to different subtypes of influenza can occur.

Influenza A and influenza B have segmented genomes that are able to undergo genetic reassortment. This is a process by which two distinct subtypes of influenza simultaneously infect a single cell, creating progeny virions with segmented genomes derived from random combinations of the original two viruses. Influenza A has a broader host range, including birds, humans, and other mammals, and as a result has a greater potential for reassortment events between unrelated subtypes. Such events can lead to the creation of strains with greatly increased pathogenicity and the potential for pandemic spread. In spring of 2009, a new strain of influenza, pH1N1, emerged from a recombination event in swine. This strain spread rapidly, eventually leading to a worldwide pandemic. This subtype continues to persist among the circulating subtypes of seasonal influenza. Other virulent subtypes of influenza persist at low levels with the potential to be a threat to public health globally; for example, avian-derived H5N1 has caused several outbreaks with high rates of mortality, and H7N9, first reported in 2013, has also been responsible for several outbreaks with high mortality.

Diagnostic Utility

Patients with influenza often experience some degree of respiratory symptoms such as cough, rhinorrhea, or sore throat. Respiratory symptoms are often accompanied by more generalized systemic symptoms including fever, malaise, weakness and fatigue. Diagnosis can be difficult since many of these symptoms are nonspecific and overlap with those caused by other common respiratory viruses, including rhinovirus, coronavirus, respiratory syncytial virus (RSV), parainfluenza virus, or adenovirus, and thus a specific clinical diagnosis can be challenging. However, identification of influenza specifically as the etiologic agent of disease can be important for several reasons, including initiation of antiviral therapy and appropriate infection control measures. The antiviral therapy available for the treatment of influenza includes neuraminidase inhibitors, such as oseltamivir, and M2 ion channel inhibitors, such as amantadine. Influenza B is inherently resistant to amantadine, and oseltamivir resistance has been associated with some strains of influenza A. Therefore, knowledge of the influenza subtype causing infection can provide information regarding antiviral resistance.

There are other benefits to the rapid and accurate diagnosis of influenza that may be less obvious. For example, one prospective study demonstrated that in patients with a confirmed influenza diagnosis, physicians were less likely to order invasive tests, prescribed fewer antibiotics and were more likely to discharge their patients sooner. Early diagnosis also allows for the identification of infected individuals capable of spreading disease. In hospitals without a rapid diagnostic modality for the recognition of influenza, it has been estimated that approximately 10% to 35% of cases were preventable had there been earlier recognition of infectious contacts. These studies support the use of isolation practices to limit the spread of influenza during outbreaks, a practice reliant upon the prompt and accurate identification of infected individuals.

Diagnostic Methods

Influenza infects respiratory epithelium; thus procedures that adequately sample this site provide the best diagnostic yield, and as a result, nasopharyngeal (NP) swabs or nasal washes are more sensitive compared to less invasive throat swabs. Once an adequate specimen is collected, a variety of methods can be used to diagnose influenza. Laboratory methods for the diagnosis of influenza include direct (or indirect) immunofluorescence, viral culture, rapid antigen detection and molecular detection. Table 1 highlights some of the key differences between these methodologies. Molecular diagnostics are becoming increasingly common.
Based on participation data from College of American Pathology (CAP) surveys, the number of laboratories utilizing molecular methods increased from approximately 217 in 2013 to approximately 360 in 2014. However, this pales in comparison to the use of rapid antigen tests which, according to CAP survey participation data, were used in at least 2,205 laboratories in 2013 and increased to 2,900 in 2014.

**Fluorescent Antibody Analysis and Viral Culture**

The most straightforward method for influenza diagnosis is the direct microscopic observation of infected epithelial cells. Several direct and indirect fluorescent antibody kits are available for the detection and differentiation of influenza A and B viruses. Some of these assays may simultaneously detect other respiratory viruses, such as adenovirus, parainfluenza, and RSV. Microscopic examination provides the opportunity to evaluate the quality of a specimen, which is approximated from the quantity of respiratory epithelial cells present per microscopic field. Significant disadvantages of these methods include the requirement for a fluorescent microscope, technologists skilled in interpretation and suboptimal sensitivity.

Viral culture has historically been used as a sensitive method of detection and still remains the gold standard for influenza diagnosis. Viral culture typically involves the inoculation of the patient specimen into a cell culture monolayer (such as rhesus monkey kidney, human fibroblasts, and A549 human lung carcinoma cells). Some culture systems combine multiple cell lines into a single monolayer (ie, R-Mix Freshcells, Athens, OH), allowing for increased sensitivity and breadth of respiratory virus detection. Following inoculation, cultures are typically incubated at 37°C and examined microscopically for evidence of viral cytopathic effect and/or stained with fluorescent antibodies to detect viral particles in the cells. Since viral culture requires extensive technical expertise, can have a prolonged turnaround time (up to a week) and is a potential biosafety concern as a result of viral propagation, many laboratories have abandoned it in favor of performing antigen testing and/or molecular methods directly on patient samples.

**Rapid Antigen Assays**

The initial introduction of antigen testing was an important breakthrough in the management of influenza, allowing physicians to obtain results in a time frame relevant to patient care. Early antigen detection assays were enzyme immunoassay-based utilizing membrane-bound capture antibodies and several incubation/washing steps. Compared to viral culture, early studies suggested sensitivities of the antigen-based methods of between 64% and 78%. Today, antigen-based testing has evolved into rapid, low-complexity, self-contained devices utilizing predominantly immunochromatographic methodologies. Advantages of these rapid influenza detection tests (RIDTs) include quick turnaround time (often less than 30 minutes) and ease of use. These features allow many of these assays to be offered as point of care testing devices. At the time of preparation of this manuscript, more than 15 RIDTs have been approved by the US Food and Drug Administration (FDA).

Although these assays are convenient, a significant drawback of RIDTs is a lack of sensitivity. Various studies have demonstrated that the sensitivity of rapid antigen detection assays ranges from 50% to 80%. There are several reasons for this suboptimal analytical performance characteristic. One factor is that the sensitivity of rapid antigen detection is inherently dependent on the amount of viral antigen present in clinical samples, often requiring $10^4$ to $10^5$ infectious particles. Adults shed less virus compared to infected children, and thus it is not surprising that the sensitivity of rapid antigen detection tests for respiratory viruses in general are greatly reduced in adults compared to children. Another factor contributing to the variable analytical performance characteristics of rapid antigen tests is the variation in the influenza strains themselves, which was best illustrated with the 2009 pH1N1 influenza A virus. A Centers of Disease Control study evaluating three different antigen assays demonstrated a 40% to 69% sensitivity for the 2009 pH1N1 influenza A virus compared to...

### Table 1
**Comparison of Methods for Influenza Detection**

| Method                | Sensitivity | Specificity | Potential to Detect Other Respiratory Viruses | Turnaround Time | Cost | Hands On/Expertise |
|-----------------------|-------------|-------------|----------------------------------------------|-----------------|------|-------------------|
| Culture               | ++++        | ++++++      | ++++                                         | +               | ++   | +                 |
| DFA                   | ++          | +++++       | +                                            | +++            | +++  | ++                |
| Antigen               | +           | +++++       | ++                                           | ++             | +++  | +++               |
| Nucleic acid detection| +++         | +++++       | Variable                                     | +++            | +++  | Variable          |

*aDirect fluorescent antibody; rating scale + to ++++, with ++++ indicating that the method is very favorable for a particular attribute.*
sensitivities ranging from 60% to 83% for strains circulating previously. These findings were supported by other studies, including a New York study citing an astonishing sensitivity of only 9.6% for the detection of 2009 pH1N1 influenza A virus by a commonly used rapid antigen detection test.25

While a lack of sensitivity can lead to difficulty in interpreting negative results, problems with specificity make positive results equally challenging to interpret. A small 2010 study of 45 specimens positive by RIDT demonstrated that 17 (37.8%) were actually false positives.26 A second study during the same year compared 336 RIDT results to polymerase chain reaction (PCR) results obtained from specimens tested at several different facilities. This study demonstrated an alarming RIDT specificity of 48.1% when compared to PCR.27

Despite these limitations, the appeal of a rapid, point-of-care diagnostic leads to the continued use of these assays in many settings. Following the 2009 H1N1 influenza A virus pandemic, a survey of hospital laboratories revealed that 84% continued to use RIDTs.28 Novel RIDTs are continuously in development, and efforts have been made to address concerns regarding sensitivity. One such recently developed assay, the BD Veritor System Flu A + B (BD Diagnostics, Sparks, MD), is a digital immunoassay that is purported to have improved sensitivity compared to other RIDTs. Comparative studies have demonstrated superior performance of this assay compared to other commonly used RIDTs using reverse transcription (RT)-PCR as a gold standard. However, the BD Veritor’s estimated sensitivity of 89.6% is still below that of RT-PCR.29

FDA Oversight of Rapid Antigen Tests

The inadequate sensitivity of commonly used RIDTs is a public health concern. The responsibility of test manufacturers to mitigate the risks of inaccurate influenza testing has been recognized by both professional and government agencies. As a result, the FDA has proposed a reclassification of these devices from Class I in vitro devices (IVDs) to Class II IVDs with special controls to create specific performance and evaluation criteria that must be adhered to by each RIDT manufacturer.32 The first standard established was that each RIDT, when compared to viral culture, must demonstrate 90% sensitivity for influenza A (lower bound of 95% confidence interval of at least 80%) and 80% sensitivity for influenza B (lower bound of 95% confidence interval of at least 70%). Specificity for RIDTs when compared to culture should be at least 95% (lower bound of 95% confidence interval of at least 90%) for both influenza A and influenza B. Specific minimal criteria for comparison to molecular methods were also given, including a sensitivity of 80% for both influenza A and influenza B (lower bound of 95% confidence interval of at least 70%). In addition, it is no longer acceptable to solely compare a RIDT to a previously existing RIDT to determine analytical performance characteristics for the purpose of FDA approval. As an additional quality measure, verification of assay performance must be conducted annually with contemporary circulating strain(s). Finally, in the event of an emergency, all manufacturers must be able to test emergent influenza strains within 30 days. It is hoped that the introduction of these new standards allows for ongoing quality assurance and improved patient safety.

Molecular Diagnostics

Nucleic acid amplification tests (NAATs) for the diagnosis of influenza have the advantage of superior sensitivity relative to RIDTs in addition to reduced turnaround time compared to viral culture. At the time of writing, 26 FDA approved molecular tests for the diagnosis of influenza are commercially available.33 In addition to increased sensitivity, NAATs can also be multiplexed to detect multiple targets, which can allow for simultaneous testing of both influenza A and B (and/or detection of additional respiratory viruses), and provide influenza A subtype information. Subtyping may be important for directing antiviral therapy, as well as for epidemiologic monitoring of currently circulating subtypes, and characterization of subtypes that may be associated with more severe disease and unusual disease presentation. Additionally, some NAATs have a high throughput, which can be of great importance during peak testing months, and these tests can be rapidly adapted for the detection of novel targets.34

PCR-Based Methods

There are a number of different chemistries used in commercially available influenza NAATs. RT-PCR is traditionally the most frequently used molecular method for the detection of RNA viruses. For influenza, the most commonly used gene targets for amplification are the matrix gene for influenza A and the hemagglutinin gene for influenza B. These genes are highly conserved within the different influenza types. The hemagglutinin gene is also important for the subtyping of influenza A strain. Although the hemagglutinin gene can undergo significant antigenic variation, this gene contains several conserved regions that can be targeted with PCR for identification of subtypes.35,36

Building upon the technology of RT-PCR, real-time PCR combines reverse transcription, DNA amplification and detection into a one-step assay. This decreases assay run time, allows for the visualization of results in real time and reduces contamination potential since the reaction and detection are carried simultaneously within a closed system. Most of the FDA-approved molecular diagnostic tests for

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influenza utilize multiplex PCR technology to identify both influenza A and B with some also having the ability to identify different influenza A subtypes. These tests vary with regards to complexity, hands-on time, and turnaround time.

“Syndromic” PCR Panels

Newly emerging multiplex PCR panels have been designed as “syndromic panels,” attributed to the fact that they simultaneously assay for a dozen or more pathogens associated with specific syndromes, such as respiratory or gastrointestinal illness. There are currently five FDA-approved syndromic panels for respiratory infections: FilmArray Respiratory Panel (BioFire Diagnostics, LLC, Salt Lake City, UT), eSensor Respiratory Viral Panel (GenMark Diagnostics, Carlsbad, CA), the Luminex x-TAG Respiratory Viral Panel and the Luminex x-TAG RVP FAST assay (Luminex Molecular Diagnostics, Austin, TX) and the Verigene Respiratory Pathogens Flex Test (Nanosphere, Northbrook, IL) Table 2. The Luminex xTAG Respiratory Viral Panel requires a nucleic acid extraction step, followed by multiplex RT-PCR amplification. Amplified targets with oligonucleotide tags are hybridized to beads, which are detected using Luminex 100/200 platform. This assay is capable of detecting 12 respiratory viruses, is usually performed in batch mode, and can be completed in approximately 8.5 hours for the Luminex xTAG RVP Assay, and can detect eight targets in approximately five hours for the Luminex xTAG RVP FAST Assay. The FilmArray Respiratory Panel, which detects 20 different respiratory pathogens in less than one hour, utilizes nested multiplex PCR, with the first stage PCR amplifying the specified targets and the second stage PCR amplifying targets using real-time PCR on an array. The software utilizes DNA melt-curve analysis to generate a result for each target. All steps, including sample lysis, nucleic acid purification, reverse-transcription, nested PCR and melt-curve analysis occur within the test pouch. The GenMark eSensor Respiratory Viral Panel can detect 14 respiratory viruses in approximately 6.5 hours with one hour of hands-on

| Table 2 |

Comparison of the FDA-Approved Multiplex “Syndromic” Respiratory Panels

| Assay                              | Influenza Type | Subtype          | Other Targets                                                                 | FDA-Approved Specimen Type | Assay Run Time/Complexity |
|------------------------------------|----------------|------------------|-------------------------------------------------------------------------------|---------------------------|--------------------------|
| eSensor Respiratory Viral Panel    | Influenza A and B | A/H1, A/H3, A/2009 H1 | RSV, parainfluenza 1, 2 and 3, human metapneumovirus, adenovirus, rhinovirus | NP swab                   | ~8 hr/high               |
| FilmArray Respiratory Panel        | Influenza A and B | A/H1, A/H3, A/2009 H1 | RSV, parainfluenza 1, 2 and 3, human metapneumovirus, rhinovirus, enterovirus, adenovirus, cornavirus, HKU1, NL53 Bordetella pertussis, Mycoplasma pneumoniae, Chlamydia pneumoniae | NP swab                   | 1.0 hr/moderate          |
| Verigene Respiratory Pathogens Flex Test | Influenza A and B | A/H1, A/H3 | Adenovirus, human metapneumovirus, parainfluenza 1, 2, 3 and 4, rhinovirus, RSV A and RSV B, B pertussis, Bordetella parapertussis/Bordetella bronchiseptica, Bordetella holmsieii | NP swab                   | <2 hours/moderate        |
| x-TAG Respiratory Viral Panel (RVP) | Influenza A and B | A/H1, A/H3 | RSV, parainfluenza 1, 2 and 3, human metapneumovirus, rhinovirus, adenovirus | NP swab                   | ~8 hr/high               |
| x-TAG Respiratory Viral Panel Fast (RVP FAST) | Influenza A and B | A/H1, A/H3, A/2009 H1 | RSV, human metapneumovirus, rhinovirus, adenovirus | NP swab                   | ~6 hr/high               |

NP, nasopharyngeal.

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time. Nucleic acids are extracted, followed by amplification on a thermocycler. Amplicons are then loaded into the eSensor cartridge and detected using electric current detection of the amplicons bound to gold-plated electrodes. Both the Luminex xTAG assays and the GenMark assays require separate steps for extraction, amplification, and detection. The direct manipulation of amplicons increases the risk for contamination in the laboratory and may require separate areas for pre- and post-amplification. The most recently FDA-approved panel, the Verigene Respiratory Pathogens Flex Test, has minimal hands-on requirements (less than 5 minutes), with amplification and detection occurring within a single cartridge (less than 2 hours run time). This test detects 16 different viral and bacterial targets. Unlike the other syndromic panels, the Verigene test offers a flexible option in which the user can order (and pay for) only a subset of the targets. If additional targets are needed later, these can be unlocked and do not require an additional sample or reagents.

In addition to the other viral and bacterial targets, all syndromic panels are capable of identifying both influenza A and B and influenza A subtypes (H1 and H3). The eSensor Respiratory Virus Panel and FilmArray Respiratory Panels are also capable of identifying the pH1N1 subtype.

**Other Methods**

In addition to PCR-based methods, other techniques have been developed to amplify and detect viral nucleic acids. Loop-mediated isothermal amplification (LAMP) utilizes reverse transcriptase and DNA polymerase in a single isothermal reaction. Specificity of this method is high due to the recognition of six different regions on the target DNA with four to six primers. This method can be used for the typing of influenza viruses and subtyping influenza A using melting temperature analyses. The Alere i Influenza A & B test (Alere Scarborough, Scarborough, ME) was developed using LAMP and can be completed in less than 15 minutes. The Alere instrument has a small footprint designed for the bench top and has recently (January 2015) been granted a Clinical Laboratory Improvement Amendments waiver by the FDA, allowing the test to be utilized outside of a diagnostic laboratory as a point-of-care device. The ability to use NAATs as point-of-care devices for influenza testing can potentially replace the use of RIDTs and lead to improved healthcare outcomes for smaller clinical areas and lower resource areas.

Another isothermal nucleic acid amplification method is nucleic acid sequencing-based amplification (NASBA). With this method, RNA is reverse transcribed using reverse transcriptase and RNase H. One of the primers in the reaction contains the promoter for T7 polymerase, which allows the RNA amplicons to undergo reverse-transcription again. In addition to influenza virus, this method has been used for the detection of other viruses including HIV, enterovirus, RSV, human metapneumovirus, and SARS-CoV.

**Molecular Diagnostic Testing for Influenza—Challenges and Limitations**

Molecular diagnostic testing for influenza has many advantages over other diagnostic methods, including improvements in sensitivity, specificity and turnaround time. There are also several limitations to these assays. First, the assays can only detect the specific viruses and subtypes that are targeted by the primers and/or probes. RNA viruses, such as influenza, are prone to high rates of mutations. Additionally, influenza viruses are known for genetic shift and drift, which can lead to the creation of “new” viral subtypes; these variants and subtypes may not be detected by current assays. This was especially apparent during the 2009 H1N1 influenza pandemic. At the time of the pandemic, there were three FDA-approved influenza molecular assays (Prodesse ProFlu+ assay, CDC real-time RT-PCR assay and the xTAG RVP assay), and these assays, in addition to many laboratory-developed tests in use at the time, were unable to detect the 2009 pH1N1 strain. As a result, the FDA cleared 16 tests for 2009 pH1N1 under emergency use authorization, four of which would ultimately gain FDA clearance for influenza testing.

Multiplexed assays for respiratory pathogens, including influenza, can allow for the detection of multiple pathogens from a single specimen, and while this can be advantageous, at times the results of this testing can be challenging to interpret. The detection of more than one pathogen within a single specimen has been reported in 10% to 30% of patients tested with multiplexed NAATs. The association of these mixed infections and disease is uncertain, with many studies showing conflicting results. Additionally, it is not fully understood what the detection of a viral pathogen means in an asymptomatic individual. Rhinovirus/enterovirus has been detected in healthy adults and children, in addition to immunocompromised individuals. Other respiratory viruses, including parainfluenza viruses, adenoviruses, coronaviruses and human metapneumoviruses are less commonly detected in asymptomatic individuals, and influenza and RSV are rarely detected. Coinfections and viral detection in asymptomatic individuals leads to questions and concerns regarding the management and treatment of these patients.

Finally, an important consideration in the widespread adoption of NAATs for influenza is cost. Many of the assay platforms require significant capital investment, and each assay may have a high cost. However, reduced hands-on time in the laboratory, as well as expedited and accurate diagnosis may achieve overall cost-effectiveness at the institutional level. To date, only a few articles have attempted to examine the cost-effectiveness of multiplex syndromic panels as
compared to other testing methods. Many of these studies are focused on specific tests and/or specific patient populations. Additional, larger studies comparing different panels and clinical outcomes are needed to fully understand the true cost-effectiveness of these syndromic panels. Also, awareness of future changes in reimbursement by the Centers for Medicare and Medicaid Services could greatly have an impact on the cost-effectiveness of this testing.

Case Summary

In light of the detection of influenza A in our patient, and a corresponding chest X-ray that was not suggestive of pneumonia, a five-day course of oseltamivir was prescribed, and ciprofloxacin was discontinued. The patient’s symptoms improved, and she was discharged from the hospital the next day. This case is an illustrative example of the suboptimal performance of RIDTs relative to NAAT for influenza testing, and the potential contribution to an inaccurate or delayed diagnosis, especially in adult patients.

Summary/Conclusion

A laboratory diagnostic assay should be both sensitive and specific in order to allow for an accurate diagnosis of a specific condition or disease. For influenza, this diagnosis is important for both appropriate treatment and infection control. The development of rapid antigen tests has allowed for a rapid diagnosis of influenza that can be performed in a point-of-care manner. Previous laboratory methods were complex, and typically required a high level of training and the laborious maintenance of cell lines. Unfortunately, RIDTs have decreased sensitivity, and due to this inadequate sensitivity, the FDA has reclassified these tests and set specific criteria for both sensitivity and specificity that must be recalculated each year based on the currently circulating subtypes. In the future, this should have a great impact on the performance of these diagnostic tests.

Many clinical laboratories have chosen to replace influenza antigen detection assays with molecular diagnostics. The molecular assays are sensitive and specific, but, with a few exceptions, are more complex and have longer turn-around times as compared to the RIDTs. Many of the molecular tests utilize multiplex PCR technology, which allows for the identification of multiple pathogens within one assay. This is used to provide subtype information for influenza A and is also used in the design of “syndromic” PCR panels, which can test for over a dozen different respiratory pathogens in one assay. While these panels have provided new challenges, they can provide rapid, accurate diagnostic information on a variety of different pathogens.

The case and review presented here illustrates the necessity of understanding the advantages and limitations of the diagnostic methods being utilized to diagnose influenza.

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