EDUCATIONAL COMMENTARY - ADVANCES IN DIAGNOSING INFLUENZA BY MOLECULAR METHODS

LEARNING OUTCOMES

On completion of this exercise, the participant should be able to

- discuss the disadvantages of conventional methods for diagnosing influenza.
- name 3 molecular methods for diagnosing influenza.
- discuss the advantages and limitations of molecular methods for diagnosing influenza.

Educational Commentary

Conventional tests to diagnose influenza include tube viral culture, shell vial culture (SVC), hemagglutination inhibition, complement fixation, enzyme immunoassay, and direct fluorescent antibody (DFA) staining. Traditional tube culture was replaced in the early 1990s by SVC, and hemagglutination inhibition is no longer used in the clinical laboratory. DFA staining of cells obtained by nasopharyngeal swab or aspirate was introduced in the 1980s, and SVC combined with DFA has been considered the criterion standard in recent years.

All of the conventional methods have disadvantages that limit their usefulness in monitoring and controlling disease outbreaks and in making treatment decisions for patients. Traditional viral tube cultures require 4 to 14 days to isolate the virus, which is too late to begin therapy with antiviral drugs. The SVC method can yield results in 1 to 2 days, but some virus strains do not replicate well in the cells used in this method. Also, neither culture method can detect virus that has been inactivated by improper collection or handling. Enzyme immunoassay tests yield rapid results, but with reported sensitivities of 70% to 75%, they are substantially less sensitive than culture or DFA. DFA staining of cells obtained from nasopharyngeal swabs or aspirates yields rapid results, but the sensitivity of these tests varies from 70% to 100%. DFA combined with SVC has higher sensitivity than DFA alone, but neither this method nor DFA alone is well suited to detect mixed viral infections.

Molecular Methods

Since the early 1990s, 3 molecular methods have been developed to detect influenza virus. These methods, collectively termed nucleic acid amplification tests (NAATs), include reverse-transcription polymerase chain reaction (RT-PCR), nucleic acid sequence–based amplification (NASBA), and loop-mediated isothermal amplification (LAMP).
Of the 3 NAATs, RT-PCR assays are the most frequently used in the clinical laboratory. In these tests, the enzyme reverse transcriptase is used to synthesize DNA from the viral RNA template. Then, routine PCR techniques amplify (replicate many times) and detect the amplicon (nucleic acid target).

RT-PCR assays have 4 important advantages over conventional tests.

1. RT-PCR can provide results within one day, which can help clinicians decide whether to treat patients with antiviral drugs.

2. RT-PCR can detect multiple viruses. Most commercially available RT-PCR assays can detect seasonal influenza type A and type B viruses. In addition to the type A and type B viruses, some assays can also detect type C viruses or distinguish seasonal influenza A virus subtypes. RT-PCR assays have also been developed to detect the novel H1N1 virus that caused the pandemic in 2009 and the H5N1 (avian) influenza virus. Finally, multiplex RT-PCR assays have been developed that can detect not only influenza viruses but also other respiratory viruses, such as respiratory syncytial virus. The ability to screen for a number of respiratory viruses in a single test can shorten the time needed to identify the cause of illness and begin appropriate therapy.

3. RT-PCR is better able to detect mixed infections than conventional methods such as DFA.

4. RT-PCR is highly sensitive and specific. In a review of molecular methods used to detect respiratory viruses, Mahoney cited studies that found that RT-PCR tests have specificity and sensitivity equal to or greater than those of culture.

NASBA and LAMP assays are not as widely used as RT-PCR, and their performance has not been as widely evaluated. However, studies cited by Mahoney found that NASBA was more sensitive than culture or DFA, and LAMP and culture were equally sensitive. In general, NAATs are the most sensitive tests available to detect influenza, followed by SVC, DFA, tube culture, and enzyme immunoassay.

Laboratories that are contemplating adding NAATs to their test menu need to address 3 issues necessary for reliable results. First, staff must be trained in molecular biology techniques, regulatory requirements, and good laboratory practices for molecular methods. Second, the laboratory should have adequate space to allow separation of crucial steps in the testing process. Third, proper technique must be used to avoid contamination that could lead to false-positive results. Potential sources of contamination include

- mislabeled specimens;
- aerosols from processing, splashes, loop and pipette transfers, and safety cabinet airflows;
- contaminated solutions and equipment; and
- carryover, if specimens are processed, cultured, or examined in the area where molecular testing is performed.
EDUCATIONAL COMMENTARY - ADVANCES IN DIAGNOSING INFLUENZA BY MOLECULAR METHODS (cont.)

The Clinical Laboratory Standards Institute publishes approved guidelines for molecular methods, which can be ordered from their website at www.clsi.org.

Impact of Molecular Testing

The introduction of NAATs is transforming diagnostic testing for influenza and other respiratory viruses, and many experts believe that they will soon replace SVC combined with DFA as the criterion standard. The high sensitivity and specificity of NAATs surpass that of conventional tests, and the development of multiplex RT-PCR tests enables laboratories to test for multiple viruses with a single assay with a turnaround time as short as one day. Further, NAATs can detect dual or triple infections, which occur in 8% to 11% of cases of respiratory illness. These advantages over conventional methods mean that patients can now be diagnosed more quickly, more accurately, and more often. In fact, the use of NAATs has been shown to increase the diagnosis of respiratory viruses by 30% to 50% over conventional methods.

NAATs are important not only in the diagnosis of respiratory illness but also in the surveillance and monitoring of circulating viruses, because they quickly provide accurate information about which viruses are in the community. For diagnosis of influenza in a surveillance network, Pérez-Ruiz and colleagues recommend a combination of SVC and multiplex RT-PCR on the SVC supernatant. In this method, the SVC recovers the virus for further study, and the multiplex RT-PCR simultaneously detects, types, and subtypes the virus.

Conclusion

Even with the advantages of molecular methods, implementation of NAATs is not feasible for every laboratory. The cost of implementing and performing molecular testing is higher than the cost of conventional methods, and this must be weighed against the potential savings in technologist time and the advantage of performing fewer tests to arrive at a diagnosis. The expense may prohibit implementation of molecular testing even if properly trained staff and adequate space are available. However, despite the higher cost, many laboratories now find that the advantages of NAATs justify implementing molecular methods.
EDUCATIONAL COMMENTARY - ADVANCES IN DIAGNOSING INFLUENZA BY MOLECULAR METHODS (cont.)

Suggested Reading


© ASCP 2012