EDUCATIONAL COMMENTARY – RAPID TESTING FOR INFECTIOUS DISEASE: ANTIGEN VS. MOLECULAR

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Learning Outcomes

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

- identify the etiologic agents of strep throat and influenza;
- discuss the principles of antigen and molecular rapid tests used to detect the organisms that cause strep throat and influenza;
- explain the difference between antigen and molecular rapid testing;
- discuss pros and cons of rapid testing to detect group A strep and influenza.

Introduction

Strep throat and influenza are common infections worldwide; both are highly contagious and the illnesses caused by each can range from mild to severe, including death. Strep throat is caused by the bacterium *Streptococcus pyogenes* (group A strep) and is reported to be the cause of up to 30% of pharyngitis in children, and up to 20% in adults.1,2 Influenza is most commonly caused by influenza A and B, members of the virus family Orthomyxoviridae.3 The viruses constantly circulate and manifest in different strains from year to year owing to their ability to mutate.4 Annually, 5% to 20% of the U.S. population becomes ill as a result of influenza.4

Seasonal factors as well as increased interaction between people play a role in elevated incidences of group A strep and influenza infections. Individuals infected with one or, in the unlucky case, both pathogens will exhibit varied symptoms based on the severity and duration of their illness. Symptoms may include one or all of the following: fever, chills, muscle aches, cough, congestion, sore throat, runny nose, headache, and fatigue.4,5 Correct diagnosis of these two infections is important for prescribing the correct treatment, especially because one is caused by a bacterium and the other a virus. Illnesses caused by group A strep and influenza generally prompt individuals to seek medical attention,1 requiring health care providers to rely on diagnostic tests to help guide treatment decisions.5,6 To properly care for patients suffering with group A strep and influenza, accurate and efficient diagnostic tests are required.7
Rapid Antigen Tests for Group A Strep and Influenza

Most rapid antigen tests for group A strep and influenza use membrane-bound enzyme immunoassay principles. Membrane-bound enzyme immunoassay tests simplify the traditional enzyme immunoassay process by using a single-use, disposable cassette. An absorbent material on the underside of the membrane draws the liquid sample through the membrane. If antigen is present in the patient specimen, it will bind to the membrane-bound enzyme-labeled antibody as the sample passes through the membrane, activating the bound enzyme. The added substrate is broken down by the activated enzyme, which then produces a chromogenic reaction on the surface of the membrane, and indicates a positive result visually in the form of a line or dot.

Rapid Molecular Tests for Group A Strep and Influenza

Rapid molecular tests for group A strep and influenza use probe hybridization or amplification. Probe hybridization tests are developed by looking at a probe, a single nucleic acid strand derived from a known microorganism. The probe is used to seek a complementary nucleic acid strand, called the target, with which to combine. The target is the unidentified microorganism suspected in the patient’s specimen. A reporter molecule is attached to the probe to determine whether the hybridization reaction has taken place. The amount of the reporter molecule detected is directly proportional to the amount of hybridization that occurred and can be measured to determine a positive or negative result. Amplification tests use polymerase chain reaction (PCR) and have become popular for rapid molecular test development. Polymerase chain reaction can detect multiple microorganisms in a single test. In PCR, the patient’s specimen is heated to separate any nucleic acid material into single strands. For each target microorganism, the organism’s nucleic acid probe is added along with primer to the heated mixture to initiate the hybridization step. If the probe matches the single-stranded nucleic acid present in the specimen, they will combine. Next, nucleotides and Taq polymerase enzyme are added to the mixture to complete the formation of the double-stranded nucleic acid structure, known as an amplicon. This amplification process will be repeated for multiple cycles, depending on the test protocol, in a closed, automatically controlled system. During this process, the amplicon will be amplified exponentially. The probe is labeled with a reporter molecule, which can be measured to determine a positive or negative result. Further development of amplification has provided different approaches to this process; however, all of these use the principles of PCR to achieve rapid molecular detection of microorganisms.
Antigen vs. Molecular Testing

Rapid antigen and rapid molecular tests for group A strep and influenza vary with each assay, specimen type, methodology, and manufacturer. Both rapid antigen and molecular tests require that a predefined element be present in order to detect the microorganism of interest. Both rapid antigen and molecular tests require internal and external quality control elements to ensure adequate assay performance. Rapid antigen testing utilizes antigen-antibody interactions to detect the microorganism, whereas rapid molecular testing uses nucleic acid. The accuracy of rapid antigen testing depends on the quality of specimen collection and handling, whereas molecular testing is less dependent on specimen collection and handling requirements. In general, rapid antigen testing requires 5 to 15 minutes to perform, whereas rapid molecular testing requires 15 to 90 minutes.

Pros and Cons of Rapid Testing to Detect Group A Strep and Influenza

Pros and cons of rapid antigen and rapid molecular tests for group A strep and influenza can be subjective. Each clinical laboratory must select the testing method that is appropriate for the needs and circumstances of its own health care setting. Refer to the Table for the pros and cons associated with rapid group A strep and influenza testing.

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<th>Rapid Testing</th>
<th>Pros</th>
<th>Cons</th>
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| Antigen for group A strep and influenza | - Rapid turnaround time\(^6\)  
- Little to no instrumentation required  
- Lower cost per test  
- Decreased concern for cross-contamination | - Sensitivity varies depending on disease prevalence\(^6,9\)  
- May require culture confirmation  
- Can be difficult for testing personnel to interpret\(^3\)  
- Manual process requires hands-on time  
- Depends on specimen collection and quality\(^6,9\) |
| Molecular for group A strep and influenza | - High sensitivity and specificity regardless of disease prevalence\(^6\)  
- No need for culture confirmation  
- Can detect small amounts of genetic material\(^3\)  
- Decreased hands-on time  
- Automatic results interpretation | - Opportunity for cross-contamination\(^8\)  
- Inability to determine viable vs. nonviable organism\(^6\)  
- Longer turnaround times\(^6\)  
- Higher cost per test  
- Low- to high-complexity instrumentation |

Pros for both rapid tests for group A strep and influenza are the ability, when used properly, to reliably diagnose each illness, improve patient outcomes, and contribute to the appropriate use of antibiotics and
antiviral medications. A con for both rapid antigen and rapid molecular tests for group A strep and influenza is the inability to isolate and grow the microorganisms for surveillance purposes.3

Conclusion

Manufacturers of rapid diagnostic laboratory tests are required to perform extensive clinical testing before submitting their test data to the U.S. Food and Drug Administration to be cleared for clinical diagnostic use.6,7 Although rapid antigen tests are useful screening tools for the detection of group A strep and influenza, molecular confirmation may be required.2,6 Manufacturer recommendations and package inserts for individual assays will help determine the appropriate uses for each assay. The availability and use of rapid tests for group A strep and influenza will continue to evolve to meet patient needs. Rapid molecular tests for group A strep and influenza are relatively new concepts; however, each year more rapid molecular assays are added to the menu of available tests. Over the past decade, molecular tests have changed from highly complex, labor-intensive tests to simple, closed-system tests.3 The ability to rapidly detect and accurately identify group A strep and influenza is important for patient care and for the overall health of our communities. Laboratories and health care providers will need to investigate which rapid testing method is most appropriate for their practices and patient populations.

References


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