Practical considerations in respiratory virus testing

By Margaret Ford, MT(ASCP) — Legacy Virology, Technical Specialist
Claudia Atherton, MT(ASCP), SM(AAM) — Laboratory Manager, Microbiology

Years ago, the long delay involved in tissue culturing for respiratory viral pathogens precluded using the laboratory as part of the real-time diagnosis of viral disease. Clinicians relied on being familiar with the likely organism present in the population depending on what time of year it was and the clinical symptoms. With the advent fairly recently of “rapid turnaround” respiratory virus tests, this scenario has changed.

Respiratory viruses are mostly seasonal in their presentation, especially in a temperate climate like the Pacific Northwest. In late fall and winter, influenza types A and B appear in the population. The year 2009 saw a new strain of influenza A, and the normal seasonality was upset while the strain was introduced into the general population. That “pandemic” presentation is over; for the 2010 to 11 respiratory virus year we expect “normal” influenza seasonality. The determination of the presence of influenza virus specifically can be important, since there are neuraminidase antivirals used for treatment in severe cases. Also, the epidemiological importance of tracking hemagglutinin types of influenza A in the human population (H1, H2, H3, H5) is a consideration.

Another organism in a definite seasonal pattern is Respiratory Syncytial Virus (RSV). The RSV season begins in early winter and continues through late spring. Typically no RSV is detected in the Legacy Laboratory patient population outside of this season. Again, there is a specific monoclonal antiviral to use as a prophylaxis against RSV called Synagis (palivizumab). Thus, the determination of RSV specifically is important during the season the virus is present, especially due to the possible severity of infection in the pediatric population.

The advent of PCR (polymerase chain reaction) nucleic acid detection methods has widened the scope of viruses possible to identify in a respiratory specimen to include human Metapneumovirus, human Bocavirus, coronaviruses NL63, OC43, 229E and HKU1, as well as the possibility of differentiating RSV A from RSV B, and detecting the very rare parainfluenza type 4. The incidence of detect-
ing two or more viruses in the same sample has also become much more common using the PCR method. This increase in possible organisms to identify is possible because of the greater sensitivity of this test method, and the greater increase in R&D to make the test primers and probes available for a greater variety of organisms. However, even though it is possible to detect these virus types using the PCR method, the clinical significance of detecting any one of these is not clear. Legacy Laboratory Services is studying whether this testing aids clinicians in care management.

During our last “normal” respiratory virus year, 2008 to 2009 (before the introduction of the novel influenza A H1N1 — swine flu), influenza season ran from January to March. This is a pattern seen in most years. While influenza and RSV typically are the most commonly found respiratory viruses during the “season” by all the methods, from November 2008 to March 2009 Legacy saw the following isolates detected in respiratory samples ordered for Culture Virus: adenovirus (17 percent), influenza A (4 percent), influenza B (17 percent), parainfluenza type 2 (11 percent), RSV (25 percent), rhinovirus (26 percent).

### Respiratory viruses and associated common diseases

<table>
<thead>
<tr>
<th>Family</th>
<th>Virus</th>
<th>Common diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoviridae</td>
<td>Human adenovirus</td>
<td>Pharyngitis, pneumonia, gastroenteritis, conjunctivitis</td>
</tr>
<tr>
<td>Coronaviridae</td>
<td>Human coronavirus, SARS coronavirus</td>
<td>Common cold, severe acute respiratory syndrome (SARS)</td>
</tr>
<tr>
<td>Orthomyxovirida</td>
<td>Influenza A</td>
<td>Influenza, bronchiolitis, pneumonia, severe lower respiratory tract infection</td>
</tr>
<tr>
<td></td>
<td>Influenza B</td>
<td>Influenza</td>
</tr>
<tr>
<td>Paramyxovirida</td>
<td>Human metapneumovirus (hMPV)</td>
<td>Pharyngitis, bronchiolitis, pneumonia</td>
</tr>
<tr>
<td></td>
<td>Human respiratory syncytial virus (RSV)</td>
<td>Croup, bronchiolitis, pneumonia (in infants and young children)</td>
</tr>
<tr>
<td></td>
<td>Human parainfluenza virus, types 1 and 3</td>
<td>Croup, non-specific upper respiratory tract infections, bronchopneumonia (primarily in children)</td>
</tr>
<tr>
<td></td>
<td>Human parainfluenza virus, types 2 and 4</td>
<td>Croup, pharyngitis, and colds (mainly in children)</td>
</tr>
<tr>
<td>Parvoviridae</td>
<td>Human bocavirus</td>
<td>Thought to be associated with a variety of respiratory infections, including bronchiolitis and pneumonia</td>
</tr>
<tr>
<td>Picornavirida</td>
<td>Human rhinovirus A and B</td>
<td>Common cold</td>
</tr>
</tbody>
</table>

Most respiratory viruses, except the adenoviruses and paroviruses, are RNA viruses. The principle molecular method for detecting RNA viruses is reverse-transcriptase polymerase-chain reaction (RT-PCR). Commercial applications of RT-PCR are available to detect respiratory viruses, particularly influenza A & B and RSV. Multiplex PCR methods detect more than one nucleic acid sequence, and therefore more than one virus type, in the same assay.
The advantages of PCR testing are time, sensitivity and specificity. A result can be turned out in 24 hours rather than days for cell culture. The complex PCR technology is costlier for the patient; we advise physicians to use PCR-molecular methodology selectively.

New testing developments at Legacy

PCR testing available for influenza and RSV
Legacy provides PCR testing for influenza and RSV on nasal swabs, aspirates and washes. This test is run once a day, Monday through Saturday during the influenza season, with an expected turnaround time of 24 hours.

Anti-CCP added to in-house testing menu
Anti-CCP aids in rheumatoid arthritis (RA) diagnosis. Until recently, the early diagnosis of RA relied on clinical manifestations and the serological marker, rheumatoid factor. Rheumatoid factor is rather sensitive for RA (50-90 percent), but has limited specificity (70-90 percent). In comparison, anti-CCP has a diagnostic sensitivity for RA of 68 percent and a diagnostic specificity of 96 percent.

Faster turnaround times for cancer antigen CA 19-9
Cancer antigen 19-9 (CA 19-9) has been added to our in-house testing menu with improved turnaround times of 24 to 48 hours. CA 19-9 is useful in monitoring pancreatic, hepatobiliary, gastric, hepatocellular and colorectal cancer. Due to the change in methodology, patient results may vary. Re-baseline testing is strongly recommended. Results obtained with different assay methods or kits cannot be used interchangeably. Re-baseline testing will be offered at no additional cost until March 1, 2011. For further information, refer to the Laboratory Update mailed in November or call client services at 503-413-1234.

Questions and Answers

Don Toussaint, vice president of Legacy Laboratory Services and Juan Millan, M.D., clinical vice president of Legacy Diagnostics

Send questions to legacylaboratoryservices@lhs.org. One of our team will respond.

Q: What are the goals for Legacy LabAdvisor?
A: We want to give our providers the perspective of our laboratory’s technical and medical staff on laboratory technologies and how they impact a physician’s ability to manage their patients.

Q. How will this publication benefit physicians?
A: We believe we can help physicians understand how to best use our services and how our technology choices add value to the information we provide. We have seen significant advances in clinical and anatomic pathology, including the use of molecular diagnostics to personalize treatments and in the use of medical informatics. Legacy LabAdvisor presents the current technologies, how they are employed at Legacy Laboratory Services and explains how they are best used in diagnosis and treatment.

Q: What role can LabAdvisor play in communicating the impact of the significant advancements in laboratory testing to physicians and patients?
A: In recent years we have seen significant advances in technology used in clinical and anatomic pathology. The most pronounced areas of development include the use of molecular diagnostics to personalize treatments and in the use of medical informatics. Legacy LabAdvisor aims to present the current technologies available, how they are employed at Legacy Laboratory Services and explains how they are best used in diagnosis and treatment decisions.
Go paperless

Are you receiving *Legacy LabAdvisor* on paper, but would like to go paperless?

Or would you like to start an electronic subscription? Simply fill out the short form at www.legacyhealth.org/labadvisor to receive the next issue of *LabAdvisor* in your inbox.