As influenza viruses constantly change, it’s nearly impossible to predict what the next flu season will be like. The timing, severity and length of each flu season alters year after year and often affects populations (i.e., the elderly, the young, etc.) quite differently. So, in true “it’s better to be safe than sorry” mode, the best bet for the clinical laboratory is to be prepared for whatever may present.

**Education is Key**
The first step should be education. Influenza can be difficult to diagnose based on clinical symptoms alone.

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**Table 1: Influenza Virus Testing Methods**

<table>
<thead>
<tr>
<th>Method</th>
<th>Types Detected</th>
<th>Acceptable Specimens</th>
<th>Test Time</th>
<th>CLIA Waived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral cell culture (conventional)</td>
<td>A and B</td>
<td>NP swab, throat swab, NP or bronchial wash, nasal or endotracheal aspirate, sputum</td>
<td>3-10 days</td>
<td>No</td>
</tr>
<tr>
<td>Rapid cell culture (shell vials; cell mixtures)</td>
<td>A and B</td>
<td>As above</td>
<td>1-3 days</td>
<td>No</td>
</tr>
<tr>
<td>Immunofluorescence, Direct (DFA) or Indirect (IFA) Antibody Staining</td>
<td>A and B</td>
<td>NP swab or wash, bronchial wash, nasal or endotracheal aspirate</td>
<td>1-4 hours</td>
<td>No</td>
</tr>
<tr>
<td>RT-PCR (singleplex and multiplex; real-time and other RNA-based) and other molecular assays</td>
<td>A and B</td>
<td>NP swab, throat swab, NP or bronchial wash, nasal or endotracheal aspirate, sputum</td>
<td>Varied (Generally 1-6 hours)</td>
<td>No</td>
</tr>
<tr>
<td>Rapid Influenza Diagnostic Tests (antigen)</td>
<td>A and B</td>
<td>NP swab, (throat swab), nasal wash, nasal aspirate</td>
<td>&lt;30 min.</td>
<td>Yes/No</td>
</tr>
</tbody>
</table>

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1. Serologic (antibody detection) testing is not recommended for routine patient diagnosis.
4. NP = nasopharyngeal
5. Reverse transcriptase polymerase chain reaction, including FDA-approved test systems, reference laboratory testing using ASR or lab-developed reagents
6. Chromatographic- and/or fluorescence-based lateral flow and membrane-based immunoassays
because the initial symptoms of influenza can be similar to those caused by other infectious agents, such as *Mycoplasma pneumoniae*, adenovirus, respiratory syncytial virus, rhinovirus, parainfluenza viruses and Legionella spp. The CDC provides extensive information on signs, symptoms, treatments, frequently asked questions and more for the public and healthcare workers (Resource Box).

### Available Tests, Technologies

Next, get familiar with what tests are available and be well-versed in new tools and technologies. A number of tests and methods can help in the diagnosis of influenza — conventional viral cell culture, rapid cell culture, immunofluorescence, direct or indirect antibody staining, RT-PCR and rapid molecular assays, for example.

Sensitivity and specificity of any test for influenza might vary by the laboratory that performs the test, the type of test used and the type of specimen tested. Among respiratory specimens for viral isolation or rapid detection, nasopharyngeal specimens are typically more effective than throat swab specimens. Results should be evaluated in the context of other clinical and epidemiologic information available to healthcare providers.

Of note: tests do not need to be done on all patients, according to the CDC website. “Tests are most useful when they are likely to give a doctor results that will help with diagnosis and treatment decisions. During a respiratory illness outbreak in a closed setting (e.g., hospitals, nursing home, cruise ship, boarding school, summer camp), however, testing for influenza can be very helpful in determining if influenza is the cause of the outbreak,” the site stated.

For those getting testing, preferred
The following links are CDC resources for clinical laboratory managers on the lab’s role and procedures, rapid diagnostic testing, influenza virus test methods and more.

- http://www.cdc.gov/flu/professionals/diagnosis/clinician_guidance_ridt.htm#figure1
- http://www.cdc.gov/flu/professionals/diagnosis/rapidclin.htm#table

respiratory samples for influenza testing, according to the CDC website, include nasopharyngeal or nasal swab and nasal wash or aspirate, depending on which type of test is used. Samples should be collected within the first four days of the illness. Rapid influenza diagnostic tests provide results within 15 minutes or less; viral culture provides results in 3 to 10 days. Most of the rapid influenza diagnostic tests that can be done in a physician’s office are approximately 50 to 70 percent sensitive for detecting influenza and approximately greater than 90 percent specific. Therefore, false negative results are more common than false positive results, especially during peak influenza activity.

Rapid Assays
The availability and use of commercial influenza rapid diagnostic tests by laboratories and clinics have substantially increased in recent years. Rapid influenza diagnostic tests (RIDTs) are screening tests for influenza virus infection and can provide results within 15 minutes.

More than 10 RIDTs have been approved by the FDA (see the Resource Box for links to these RIDTs).

The CDC noted that RIDTs differ in some important respects. For example, some can identify influenza A and B viruses and distinguish between them; others can identify influenza A and B viruses, but cannot distinguish between them. As well, some tests are CLIA waived.

Most tests, however, can be used with a variety of specimen types, but, as noted above, the accuracy of the tests can vary based on the type of specimen.

FDA approval is based upon specific specimen types. RIDTs vary in terms of sensitivity and specificity when compared with viral culture or RT-PCR. Product insert information and research publications indicated that sensitivities are approximately 50 to 70 percent and specificities are approximately 90 to 95 percent. Specimens to be used with RIDTs generally should be collected as close as possible to the start of symptoms and usually no more than 4 to 5 days later in adults. In very young children, influenza viruses can be shed for longer periods; therefore, in some instances, testing for a few days after this period may still be useful.

In June 2013, the FDA proposed a reclassification of Rapid Influenza Diagnostic Tests (RIDTs) from Class I to Class II. This proposal was in part due to the low sensitivity of many RIDTs used in medical practice. The FDA’s stance is that the general controls for Class I devices are insufficient for RIDTs and reclassification of RIDTs to Class II is needed. The FDA has proposed four special controls that include performance requirements compared to the reference methods of PCR or culture, and annual reactivity testing of contemporary circulating influenza strains.

On May 22, 2014, the FDA placed notice of the proposed change in the Federal Register and received public commentary for a 90-day period. The final order has been drafted and is under internal review.

If the proposed FDA reclassification is finalized after review, all manufacturers of RIDTs will be responsible for compliance to the new performance requirements. Package Insert claims for these products must meet the new criteria; otherwise, the manufacturers must cease distributing the non-conforming RIDTs within one year of the date the final order is published in the Federal Register.

References
2. Executive Summary Proposed Reclassification of the Rapid Influenza Detection Tests CDRH Microbiology Devices Advisory Committee Meeting June 13, 2013 Gaithersburg, Maryland