Rapid Diagnosis of Viral Infections

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After reading this article, the reader should be able to identify the common rapid tests used to diagnose viral infections, and describe how these tests vary with regard to sensitivity, specificity, ease of use, and time to results.

Molecular diagnostic exam 0201 questions and the corresponding answer form are located after the "Your Lab Focus" section, p. 307.

- Viruses that are commonly detected using rapid test methods.
- Rapid tests used to diagnose viral infections.
- Factors to consider when comparing rapid tests.

The laboratory diagnosis of viral infections traditionally has been considered a lengthy process, providing results that may have minimal clinical impact. Specimens inoculated into cell culture tubes typically require incubation for several days prior to development of cytopathic effect or detection by methods such as hemadsorption. Culture tubes are often observed for 2 weeks or longer before reporting negative results. However, significant advances during the past 10 to 15 years in the development of viral diagnostic tests provide rapid and more useful results. Cell culture incubation times have been shortened significantly with the use of centrifugation enhanced shell vials. Methodologies that detect viral antigens or nucleic acids directly in patient specimens can be completed in minutes or hours. Decreased sensitivity and specificity may accompany improved time to detection. The costs required to perform newer, rapid tests versus traditional culture methods are additional considerations. Rapid tests may have unique specimen requirements. All of these issues must be weighed carefully when considering which tests to offer.

Respiratory Viruses

Rapid tests have had more impact on the detection of respiratory viruses than any other group of viruses. Indeed, studies have demonstrated a number of positive outcomes of rapid detection of respiratory viruses in hospitalized patients, including decreased length of hospitalization, patient isolation, costs, and better use of antibiotics. Respiratory viruses that are frequently identified by both traditional and rapid methods include influenza virus, respiratory syncytial virus (RSV), adenovirus, and parainfluenza virus. All of these viruses will grow in commonly available cell lines in tube cultures and are usually detectable within 2 weeks or longer after several days of incubation. However, influenza and parainfluenza viruses may not produce discernible cytopathic effect (CPE), requiring blind hemadsorption or immunofluorescence staining of cells. Respiratory syncytial virus is more labile than other respiratory viruses. Titer decreases rapidly during specimen transport, even at 4°C. Culture is therefore a relatively insensitive test for RSV. Non-culture methods perform well in comparison.

Rapid non-culture tests used routinely to detect respiratory viruses include direct immunofluorescence assay (DFA) and antigen/enzyme assays employing solid membrane surfaces. Centrifugation-enhanced shell vials provide rapid culture detection of respiratory viruses. The DFA procedure utilizes fluorochrome (usually fluorescein isothiocyanate)-labeled monoclonal antibodies for the staining of viral antigens within infected cells. Following excitation, fluorochromes emit light at specific wavelengths. Fluorescein isothiocyanate appears apple green when excited by ultraviolet light. The DFA procedure can be performed on a variety of respiratory specimens including swabs in viral transport medium, nasal washes, and nasal aspirates. Washes and aspirates are more optimal than swabs because they usually contain a greater number of epithelial cells from the posterior nasopharynx. However, well-collected swabs of the posterior nasopharynx will usually yield an acceptable number of epithelial cells, and swabs are often preferred by older patients and those who collect specimens. Specimens should be centrifuged and washed with phosphate-buffered saline to remove material that may affect fluorescence or interfere with adherence of cells to the slide.

Smears are prepared on slides by placing a drop of washed, concentrated specimen directly on the slide, or by cytocentrifuging the specimen onto the slide. Cytocentrifugation produces a spot that contains more cells, and is more easily read than smears prepared by placing a drop on the slide. Smears are allowed to air dry completely, fixed in cold acetone, then are stained with fluorochrome-labeled monoclonal antibodies according to the manufacturers’ instructions. Antibody reagents are either direct (primary antibody is also labeled with the fluorochrome) or indirect (includes an unlabeled primary antibody and a fluorochrome-labeled secondary antibody). Since each antibody incubation step is typically 30 minutes, direct reagents shorten the assay time substantially. After applying mounting fluid and a cover slip, slides are examined using a fluorescent microscope with the appropriate filter matched to the fluorochrome.

The DFA procedure can be streamlined by the use of a single screening reagent containing monoclonal antibodies specific for several viruses. Specimens exhibiting fluorescence are then stained with individual antibodies to determine the specific agent. The respiratory virus screening approach has been further simplified by the use of dual fluorochromes in a single reagent (Simulfluor, Chemicon...
M, Temecula, CA). This allows not only detection of multiple viruses within a single smear, but also identification, depending on which antibodies are labeled.

Drawbacks of DFA include the requirement for a fluorescent microscope, the subjective interpretation of fluorescence, and the requirement for skilled technologists. Staining patterns of viral infected cells must be differentiated from non-specific staining. Fluorescence may be nuclear, cytoplasmic, or both, and may appear more or more uniform. Control slides containing viral-infected cells must be stained and read alongside patient specimens.

An advantage of DFA is the short assay time. The time required to wash a specimen, apply it to a slide by cytocentrifugation, and to fix in acetone is under 1 hour. As stated earlier, staining time is 30 or 60 minutes, depending on whether direct or indirect antibodies are used. Shortening the staining time may result in unsatisfactory staining. A skilled technologist can read a slide in several minutes or less, for a total time to results of less than 2 hours. Another advantage of DFA is the ability to assess specimen quality. Stained smears containing fewer than 20 total epithelial cells are generally considered to be inadequate. Negative DFA results from these specimens should be reported only with a statement that an insufficient number of cells might contribute to a false negative result.

While DFA is a relatively inexpensive procedure, prices of DFA reagents may vary considerably among vendors. Reagents and other consumables are few with minimal volumes. Four major vendors of DFA reagents are Chemicon, Bartels/Trinity Biotech M (Carpinteria, CA), and Diagnostic Products Corporation M (Los Angeles, CA). Fluorescence intensity and staining characteristics will vary not only among reagents from different vendors, but between direct and indirect formats, and between reagents containing a single antibody versus screening reagents containing several antibodies.

Medical technologists may develop a comfort level with a particular reagent and its staining characteristics, and therefore have difficulty in interpreting staining patterns of reagents from other vendors or formats. Not all commercially available, fluorochrome-labeled monoclonal antibodies are FDA-cleared for direct specimen detection; some are cleared only for culture confirmation.

The specificity of DFA is consistently high. Sensitivity varies depending, in part, on the virus detected. Sensitivity is highest for RSV reagents (equivalent to, or greater than that of culture) due to the lability of the organism during specimen transport, and resultant low sensitivity of culture, against which DFA is compared. Sensitivity of DFA reagents for other respiratory viruses is generally less than that of culture. For this reason, negative DFA results should be confirmed with culture.

Rapid antigen/enzyme membrane tests are commonly used for the detection of influenza virus and RSV. Five influenza A and B membrane tests are currently commercially available in the United States. These include the Directigen Flu A and Flu A+B tests (Becton Dickinson M, Sparks, MD), ZstatFlu (ZymeTx M, Oklahoma City, OK), QuickVue (Quidel M, San Diego, CA), and Flu OIA (Thermo Biostar M, Boulder, CO). The Directigen tests are true EIAs, using enzyme conjugated monoclonal antibodies and substrate to generate a color in the presence of influenza virus antigen. The ZstatFlu test is an endogenous viral-encoded enzyme assay that detects influenza virus neuraminidase activity. The QuickVue test is a lateral flow immunoassay. The Flu OIA test is an optical immunoassay. Assay times are all under 30 minutes. The Directigen, Flu OIA, and QuickVue tests can be performed on multiple specimen types. The ZstatFlu test can only be performed on throat swabs using the swab provided in the kit, which prevents reflexive testing or adding other viral tests from a single specimen. There is currently 1 commercially available rapid EIA test for RSV (Directigen, Becton Dickinson). The format of this test is virtually identical to that of the Directigen influenza virus tests. In 4 published studies, the sensitivity and specificity of the RSV membrane test ranged from 71% to 86% and 76% to 91%, respectively.

Advantages of all of these tests include simplicity [the ZstatFlu and QuickVue influenza tests are classified as waived tests by the FDA under the Clinical Laboratory Improvement Amendments (CLIA) of 1988], speed, and convenience for point-of-care testing. However, speed and simplicity come at a price which is increased performance. All of the antigen/enzyme membrane assays are less specific than culture or DFA, resulting in decreased positive predictive value outside of the influenza or RSV “seasons” when disease prevalence is low. Out-of-season positive test results should be interpreted with caution and confirmed by culture. During the height of influenza or RSV season or when disease prevalence is high, the predictive value of a positive result is correspondingly higher. The sensitivity of rapid membrane assays is variable, depending in large part on the specimen source (as with DFA, washes and aspirates are generally better than swabs), 16-18 Most studies have shown that membrane tests are less sensitive than culture. In spite of their relative inaccuracy, rapid membrane tests are performed by many laboratories because their rapid time to results offers greater potential impact on patient management.

The centrifugation-enhanced shell vial assay is a rapid culture method for the detection of respiratory and other viruses. In this modification of tube cultures, the cell monolayer is grown on a coverslip in a flat-bottom vial. Vials are centrifuged after adding specimen to enhance viral infection of the cells. Vials are incubated for 24 to 48 hours after which the coverslips are stained with fluorochrome-labeled monoclonal antibodies that are specific for early viral antigens. Alternatively, a cell suspension can be prepared from the coverslip and dropped on a slide well. This substantially reduces the amount of monoclonal antibody required but may potentially decrease sensitivity since less of the cell monolayer is stained and examined. To increase the detection speed (but at greater cost), multiple vials are inoculated. A vial is stained after 24 hours incubation, and the result is reported if it is positive. If the 24-hour vial is negative, other vial(s) are incubated an additional 24 to 48 hours, and stained. Cell lines used in shell vial assay for respiratory viruses include primary monkey kidney, Madin-Darby canine kidney, and others. In a recent modification, a single coverslip
containing a mixed monolayer of 2 cell lines (mink lung and A549 human lung carcinoma) supported the growth of all significant respiratory viruses (R-Mix, Diagnostic Hybrids M, Athens, OH).22

Advantages of the shell vial assay include rapid time to results (though not as rapid as non-culture methods), sensitivity, and specificity. Evaluations of the shell vial assay have demonstrated variable sensitivity compared to conventional culture. Factors affecting shell vial assay sensitivity include the cell line used, monolayer age, incubation time, immunofluorescence staining reagents, and others. Disadvantages of the shell vial assay include the labor required to inoculate vials, and (as with conventional tube culture) a lack of standardization.

Due in part to the variety of rapid methods available for detection of respiratory viruses, there has been less reliance on nucleic acid amplification tests (NAATs) such as polymerase chain reaction (PCR). This is in spite of the greater sensitivity of NAATs, which makes the point that sensitivity is not the only factor to be considered when implementing a diagnostic test. Nucleic acid amplification tests have several disadvantages relative to other rapid non-culture tests: they are labor intensive; time to results is several hours; there is limited availability of commercial or standardized tests; and none are FDA approved. Real-time PCR using a rapid hot air thermal cycler and simultaneous detection of fluorescent PCR product reduces assay time to less than 2 hours (including sample preparation time), but is not available in a standardized format. A commercially available NAAT is the Hexaplex test (Prodesse M, Waukesha, WI). This multiplex PCR assay contains 7 different primer sets specific for influenza virus types A and B; parainfluenza virus types 1, 2, and 3; and RSV subgroups A and B in a single PCR reaction tube. Following amplification, PCR product is hybridized to probes in different microtiter plate wells and detected colorimetrically. The Hexaplex test has been shown to be both specific and highly sensitive.24

Herpesviruses

Among the herpesviruses, herpes simplex virus (HSV), varicella-zoster virus (VZV), and cytomegalovirus (CMV) are commonly detected using rapid methods. Herpes simplex virus grows rapidly in cell culture. Specimens with high viral titters (eg, swabs of genital lesions) show characteristic CPE often after only 24 to 48 hours of incubation. However, non-genital and low titer genital specimens may require incubation for several days prior to development of CPE. For these specimens, rapid HSV detection methods offer a distinct advantage. Varicella-zoster virus grows more slowly than HSV, and CMV grows slower still. Tube cultures for CVm detection are usually observed for 4 weeks. Additionally, tube cultures are generally an insensitive method for detection of CMV, with the exception of urine specimens from neonates. Rapid methods frequently used to detect HSV and VZV include shell vial culture, DFA, and NAATs. Rapid detection methods used to detect CMV include shell vial culture and NAATs.

The DFA procedure is commonly used to detect HSV and VZV, particularly in dermal lesion specimens. Dual fluorochrome reagents that distinguish HSV and VZV in a single smear are available (Simulfluor, Chemicon).25 Alternatively, 2 smears can be prepared and stained separately with HSV- and VZV-specific monoclonal antibodies. These reagents can be obtained from the same vendors that provide DFA reagents for detection of respiratory viruses. Reliable detection of HSV and VZV in dermal lesions by DFA requires the presence of cells, which are obtained by vigorous swabbing of the base of the lesion. The DFA procedure is more sensitive than culture for the detection of VZV,26 but less sensitive for detection of HSV.

The shell vial assay is used routinely for the detection of HSV, VZV, and CMV. The procedure is similar to that used for detection of respiratory viruses, except that cell lines susceptible to infection by herpesviruses are used (eg, human diploid lung fibroblasts). Shell vials are substantially more sensitive than tube cultures for detection of CMV, and time-to-results is reduced from weeks to 1 to 3 days. A modification of the shell vial assay utilizes a monolayer of genetically engineered baby hamster kidney cells containing an HSV-specific promoter and the E. coli lacZ gene [enzyme linked virus inducible system (ELVIS), Diagnostic Hybrids M, Athens, Ohio]. Cells infected with HSV type 1 or 2 produce galactosidase, which turns a substrate (X-gal) blue. This system is specific for HSV. It is rapid (16 to 24 hour incubation prior to staining), results are easier to interpret than fluorescence, and sensitivity is equivalent to conventional tube culture.27

Nucleic acid amplification tests are used more routinely to detect herpesviruses than respiratory viruses. Nucleic acid amplification tests are the most reliable method for detection of herpesviruses in cerebrospinal fluid (CSF) of persons with central nervous system infections. As previously discussed, NAATs are labor intensive and technically demanding relative to other rapid non-culture methods. There is currently 1 FDA-approved test for qualitative detection of a herpesvirus (CMV). This is the Hybrid Capture test (Digene M, Gaithersburg, MD) which is based on signal amplification to achieve adequate sensitivity.28 Specimens (peripheral white blood cells) are lysed to release DNA, and combined with a CMV specific RNA probe. The DNA:RNA hybrids are captured in microtiter plate wells and detected with multiple anti-RNA:DNA antibodies conjugated to alkaline phosphatase. Most NAATs for herpesviruses are developed and validated in-house, resulting in little standardization among methods. A rapid PCR assay (approximately 2 hours versus 4 to 6 hours for conventional PCR) using rapid temperature cycling and simultaneous detection of fluorescent PCR product has been used to detect HSV and VZV in a variety of specimens.2930 Nucleic acid amplification tests are highly sensitive, and can also be very specific when proper measures are taken to avoid carryover contamination. A drawback of qualitative NAATs for detection of CMV is the questionable correlation of a positive result with active disease. For this reason, quantitative tests may have more utility.
Enteroviruses

Enteroviruses are pathogens that cause a myriad of clinical diseases. Most enteroviruses can be detected in conventional cell culture, using susceptible cell lines. The hardiness of these viruses makes culture a reasonably sensitive method. Unlike HSV, enteroviruses can be reliably detected in CSF specimens using culture. Because of typical slow development of CPE in cell lines, NAATs have been used for more rapid detection.34 There are no commercially available, FDA-approved NAATs for enteroviruses.

Another rapid method used for detection of enteroviruses is the shell vial assay. Monolayers containing single cell lines and mixed cell lines (E-Mix, Diagnostic Hybrids) have been described.32

Rotavirus

Rotavirus is an important cause of acute gastroenteritis, primarily in young children. Rotavirus cannot be grown in cell lines commonly used in virology laboratories, leaving antigen-detection EIA (microtiter plate and membrane formats) as the predominant method. Because of high virus load in stool, EIAs have reasonable sensitivity.33 Membrane EIAs are commercially available (Meridian Diagnostics M, Cincinnati, OH). These tests are rapid and simple to perform. Latex agglutination is another rapid, commercially available method for detection of rotavirus (Wampole Laboratories M, Cranbury, NJ). Latex agglutination tests are generally less sensitive than EIA.34

The PCR technique has been described for the sensitive detection of rotaviruses, but due to the availability of easier, faster, commercial antigen detection tests there is little need for NAATs for routine diagnosis.

Conclusions

Rapid methods are a significant part of the diagnostic virology test menu, greatly enhancing the impact of diagnostic virology on patient management. The sensitivity and specificity of rapid (faster than traditional cell culture) methods vary greatly depending on the methodology. It is incumbent upon the laboratory professional to be aware of this variability and to communicate test limitations to clients.