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Opportunities for Rapid Viral Diagnosis

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Rapid viral diagnostic test procedures vary in processing time from several minutes to hours depending on the method used to detect an unknown virus. Several rapid viral diagnostic assays are reviewed in this paper. The assays are all available commercially or can be performed using readily available supplies. The assay methods include enzyme immunoassay (EIA), direct immunofluorescence (DFA), and latex agglutination. In most of the assays reviewed, the mechanism of identification involves detection of viral antigen in clinical specimens. Except for DFA, the assays require minimal training or specialized equipment. Many of the procedures can be performed in laboratories during off hours and without specialized facilities. The viruses represent those that are clinically important and have rapid diagnostic tests available.

Respiratory Syncytial Virus

Rapid identification of respiratory syncytial virus (RSV) is important for two reasons: (i) timely cohorting of patients can prevent nosocomial spread of disease and (ii) appropriate antiviral therapy can be initiated early. In winter months when the incidence of RSV infection is high and hospital admissions of patients increase, viral culture techniques do not allow for expedient cohorting of patients because 5 to 7 d are required before test results become available.

Rapid identification techniques include DFA and several EIA systems. Methods that will be discussed include DFA, Directigen RSV (Becton Dickinson Microbiology Systems), and TestPack RSV (Abbott Laboratories). DFA, Directigen, and TestPack can all be processed in under 1 h. Ortho RSV, Kallestad Laboratories EIA, and Abbott Laboratories RSV EIA will not be reviewed because these "rapid" tests require several hours to process. EIA systems are more suitable during the RSV "off season," when tests can be batched, or in high-volume laboratories.

DFA is considered by some to be the most sensitive technique for the identification of RSV from clinical specimens (1–3). The method requires approximately 30 min for incubation and additional time to examine the slide (4). Although DFA is rapid and sensitive, the technique requires a fluorescence microscope, high quality specific reagents, and trained, experienced personnel to prepare, examine, and interpret the stained specimen.

There are two EIA test kits that provide rapid diagnosis, are self-contained, and do not require specialized training or equipment. The Directigen and TestPack kits are membrane enzyme immunoassay systems with assay times of 15 and 20 min, respectively. Both test kits contain internal controls. Directigen RSV shows a lower sensitivity and specificity than TestPack when fresh specimens are used (2) (Table 1). Wrenn et al. report that TestPack is more sensitive than viral culture (5). Directigen specimen samples, after a period of freezing and thawing, show increased sensitivity and specificity when compared to culture (6). The increase in sensitivity and specificity is thought to result from increased disruption of cellular material with the result that more antigenic material is available for detection (3). Current Directigen assay instructions include modifications that suggest that increased cellular disruption may be required for optimal test results (6). The Directigen assay, in one study, shows uninterpretable patterns in

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TABLE 1. Comparison of EIA test kits to cell culture for the detection of RSV

Test kit	Mean % sensitivity (range)	Mean % specificity (range)	Mean % PPV (range)	Mean % NPV (range)	References
Directigen	76 (62–91)	81 (69–90)	63 (38–81)	89 (75–97)	2–4, 6
TestPack	87 (73–94)	93 (84–100)	89 (81–100)	92 (85–95)	1, 2, 4, 5

8.5% of specimens tested. The inability to interpret the test arises from difficulties with filtration of the specimen into the device. The filtration problem can be overcome by diluting the specimen (3). Additional processing of the specimen potentially increases the test turnaround time and may reduce the sensitivity of the test by diluting the specimen. The Directigen test kit has an improved filtration system that should help resolve the filtration problems reported previously.

Influenza A

Influenza A virus is a pathogen associated with respiratory tract infection. The disease spectrum ranges from mild to severe and may cause fatal pneumonia. Influenza A is associated with epidemics and can be lethal in the elderly and immunocompromised individuals. Rapid detection of the influenza A virus is important because effective antiviral agents, amantadine and rimantidine, are available (7, 8).

Commercially available identification techniques include membrane EIA and DFA. A membrane EIA kit is Directigen FLU-A (Becton Dickinson Microbiology Systems). The test can be completed in under 15 min and does not require a fluorescence microscope or specially trained personnel to be interpreted. The sensitivity and specificity of Directigen FLU-A, compared to cell culture, range from 65 to 100% and 91.6 to 96%, respectively. Positive (PPV) and negative predictive values (NPV) range from 62.6 to 85% and 89 to 100%, respectively (7, 9). The sensitivity, specificity, PPV, and NPV of Directigen FLU-A compared to DFA are 100, 91.6, 75, and 100%, respectively (7). In contrast to the Directigen RSV membrane EIA, there is evidence that freeze-thawing may compromise the ability of the Directigen FLU-A test to identify positive specimens. Specimens should not be frozen prior to testing with the Directigen test until the freeze-thaw issue has been resolved (7).

Although the Directigen test is a valuable tool, it must be used appropriately in laboratory practice. Directigen FLU-A is more expensive per test than cell culture and should be used when rapid results are likely to affect patient management.

Rotavirus

In the cooler months of the year in the United States, rotavirus infections account for approximately 50% of pediatric hospital admissions due to diarrhea and dehydration (10). Each year rotavirus epidemics follow a regional sequence from East to West. Outbreaks occur in November and December in the Southwestern states and spread to the New England states in April and May (11). The rapid detection of rotavirus aids in the diagnosis of gastroenteritis and in the identification of infected individuals who are sources of outbreaks in settings such as day-care centers and hospital wards. Healthcare workers must be aware that other viruses, including the fastidious adenoviruses, Norwalk and Norwalk-like viruses, caliciviruses, astroviruses, and possibly coronaviruses, also may be etiologic agents of gastroenteritis (10).

Commercial kits for the rapid detection of rotavirus antigen in stool specimens have been available for many years. These include those with enzyme-linked immunosorbent assay (ELISA), membrane EIA, and latex agglutination methodologies. Comparisons of results from evaluations of commercial rotavirus assays are quite difficult because of the variety of gold-standard reference tests used by investigators and the variability of results (12). Several monoclonal and polyclonal antibody–based ELISA kits are available for use in laboratories that receive many requests for rotavirus testing. Monoclonal antibody–based ELISA kits appear to be superior to polyclonal antibody–based kits (13, 14).

Rapid rotavirus assays that have a 15 min or less performance time include latex agglutination and a membrane EIA from Abbott Laboratories (TestPack Rotavirus). Latex agglutination tests from Wampole Laboratories (Virogen Rotatest), Meridian Diagnostics, Inc. (Meritec Rotavirus), Murex Diagnostics, Inc. (formerly Wellcome Diagnostics, Wellcome Rotavirus), BioMerieux (Slidex Rota-kit), and Medical Technology Corporation (Rotalex) have lower sensitivity than an ELISA; however, they are good tests for laboratories that receive few requests for rotavirus testing or for off-season testing (Table 2).

TestPack Rotavirus membrane EIA is also useful for small volume and off-season testing. Brooks et al. demonstrated that the TestPack performed better with fresh stool specimens (sensitivity 95%, specificity 90%, PPV 86%, NPV 97%) than with frozen specimens (16). When receiving evaluations of TestPack, readers should note whether fresh or frozen specimens were used.

Herpes Simplex

The rapid detection of herpes simplex virus (HSV) antigen is useful in cases of pregnant women with genital

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TABLE 2. Comparison of five latex tests for detection of rotavirus in stool specimens

Test kit	Mean % sensitivity (range)	Mean % specificity (range)	Mean % PPV (range)	Mean % NPV (range)	References
Virogen	89 (85–93)	90 (80–100)	88 (76–100)	90 (85–94)	13, 14
Meritec	81 (75–89)	95 (92–100)	91 (85–100)	88 (87–89)	12–14
Wellcome	83 (70–95)	100 (100)	100 (100)	90 (85–95)	13, 14
Slidex	82 (73–91)	97 (95–99)	96 (95–97)	90 (88–91)	12, 13
Rotalex	62	95	84	84	12

infections and individuals who are particularly susceptible to severe infections. Several enzyme immunoassay kits are available that provide more rapid test results than the "gold standard" technique, cell culture, for detecting HSV antigen (Table 3). Cell culture techniques may require up to 14 d for a definitively negative result (16).

HERPCHEK (DuPont) is a microdilution plate-based enzyme immunoassay that requires approximately 50 min of hands-on time by the technologist and 4 h to complete. The assay does not require specialized laboratory equipment or highly trained personnel. Specimens must be collected in the proprietary transport media, HERPTRAN. There is no requirement for rapid transport of the specimen to the laboratory (16, 17). Verano et al. suggest that HERPCHEK is a sensitive ELISA for symptomatic herpes lesions even if the specimen is transported in viral transport medium instead of HERPTRAN (18). The combined labor and reagent costs for HERPCHEK are comparable to the total cost of HSV antigen detection using shell vial culture techniques. The HERPCHEK test does not differentiate between HSV types 1 and 2 (19).

The Vitek ImmunoDiagnostic Assay System (VIDAS, Vitek Systems Inc.) is a qualitative enzyme-linked fluorescent immunoassay system. The VIDAS system is fully automated and can carry out internal washes and incubation steps. Final result calculations are also performed by the system. The test results can be available in less than 3 h. The VIDAS system is limited to 13 samples per run, whereas the HERPCHEK assay can perform up to 94 samples per run. The assay does not provide virus typing. Cell culture backup is recommended for negative HSV results obtained by VIDAS (19).

The IDEIA (NovoNordisk Ltd.) kit is an EIA that detects HSV antigen. The assay can be completed in less than 4 h. Sillis suggests that results obtained using brain tissue should be interpreted cautiously in the absence of other supporting evidence of infection (20).

SureCell (Kodak), a filtration EIA system, is performed directly with the clinical specimen. Results of the test are available within 15 min. No instrumentation or special transport media, other than viral transport media, are required. Dorian and others suggest that all negative test results should be followed up by inoculation of cell culture (21, 22).

Cytomegalovirus

Rapid detection of cytomegalovirus (CMV) infection is useful in preventing overtreatment of patients who are receiving immunosuppressive therapy, to initiate antiviral therapy, and to determine if donor organs are acceptable for transplantation.

The current standard method for the detection of CMV infection is cell culture. The test is slow, requiring an average of 9 d (range: 7 to 21 d) to complete. The shell vial assay is more rapid than conventional tube cell cultures, requiring an average of 16 h to complete (23). Gleaves et al. suggest that the shell vial technique is as specific and more sensitive than the conventional tube cell culture (23). The shell vial assay uses a commercially available monoclonal antibody directed against CMV immediate early antigen (23).

An antigen detection system has been described that uses monoclonal antibodies directed against immediate early CMV antigen in cytocentrifuged blood leukocytes. The process requires 3 to 5 h to complete (24). The technique has an overall sensitivity of 93% and specificity of 92% compared to viral isolation, IgG and IgM ELISA, and clinical symptoms (25). van der Bij et al. suggest that the process is as specific as and more sensitive than current isolation techniques (24). Reagents and equipment necessary to perform the assay are readily available. The clinical virology laboratory should be able to perform the procedure without difficulty.

A commercially available kit (CMV-vue kit: INCSTAR Corp.) contains all of the reagents required to detect CMV antigenemia. The CMV-vue kit does not require the use of a cytocentrifuge and does not require a fluorescence microscope because CMV-infected polymorphonuclear blood leukocytes are stained with horseradish peroxidase. In a recent evaluation Erice et al. (26) reported sensitivities, specificities, PPVs, and NPVs of 87, 92, 65, and 98%, respectively, for CMV-vue and 69, 96, 73, and 93% for the shell vial assay compared to cell culture isolation. Erice et al. recommend CMV antigenemia detection as the method of choice

TABLE 3. Comparison of EIA test kits to cell culture for the detection of HSV

Test kit	Mean % sensitivity (range)	Mean % specificity (range)	Mean % PPV	Mean % NNP	References
HERPCHEK	94 (90–99)	96 (92–100)	86	96	16–19
VIDAS	92	89	83	95	19
IDEIA	94	97	84	99	20
SureCell	73 (64–81)	99 (99–100)	97	84	21, 22

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for rapid diagnosis of CMV viremia (26). Results of CMV antigenemia testing should be verified with cell culture.

The standard method used for donor organ screening consists of antibody detection. A commercially available antibody detection kit is CMV Scan. (Becton-Dickinson). CMV Scan is a latex agglutination test performed on donor sera and can be performed in under 20 min. In a prospective comparison to Abbott ELISA (CMV TOTAL AB ELISA), CMV Scan has a sensitivity of 98% and specificity of 97% (27). PPV and NPV are 98% and 97%, respectively (27). Zbinder et al. suggest that negative results should be retested against a "highly sensitive EIA" and that sensitivity can be improved by double-checked reading (27).

Concluding Remarks

Rapid nucleic acid technology is not yet available for the clinical virologist. Probes are commercially available for viral detection in situ and for staining shell vial coverslips, but these reagents are no more rapid than monoclonal antibody assays. The polymerase chain reaction (PCR) has been applied to the detection of several viruses in clinical specimens but PCR technology will be limited to reference laboratories until commercial kits are available.

Clinical viral testing has evolved from an epidemiologic exercise to a practical tool providing useful diagnostic information. The more rapidly available test results allow physicians to make better patient management decisions. Clinical microbiologists need to educate the health care community with regard to availability and appropriate use of viral testing.

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Editorial

Are Sputum Screens Still Relevant?

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Expectorated sputum is the specimen most commonly collected for microbiologic diagnosis of bacterial pneumonia because it is considered easy and safe to collect. Better quality specimens, such as transtracheal aspiration, transthoracic needle aspiration, and bronchoscopic aspirates are uncomfortable for the patient, uncommonly associated with significant risk, or are contraindicated in certain patients. Thus the indication for such specimens remains a clinical decision in each patient's case.

Analysis of the expectorated sputum, however, is fraught with controversy. Many authorities in the field became skeptical of expectorated sputum culture over 20 yr ago when Barrett-Conner (1) reported that the yield of *Streptococcus pneumoniae* in patients with pneumococcal pneumonia was only 50%, which prompted her to say, "The routine sputum culture for the diagnosis of acute bacterial pneumonia may be a sacred cow."

This accusation resulted in multiple attempts to improve the quality of the sputum culture by washing, quantitation, and microscopic screening. Washing the sputum with sterile saline to remove salivary contamination followed by quantitatively culturing the retained purulent portion (after straining through a tea strainer) seemed to work reasonably well (2). Even though the method is popular in Europe, it never caught on as a routine laboratory procedure in the United States. The method most often employed in the United States' clinical laboratories is the microscopic screening of Gram-stained sputum samples viewed under low-power (10× objective) to determine the extent of salivary contamination by determining the relative numbers of polymorphonuclear leukocytes (PMNs) and squamous epithelial cells (SEC) (3–7). Although some authorities advocate microscopic examination as a useful gauge of the specimen's quality and to decide whether to "reject" or culture specimens, there is no consensus regarding the specific criteria needed. In addition, experience between laboratories is sometimes very different.

The original report by Bartlett (7) scored sputum specimens on the basis of the gross appearance and the numbers of PMNs and SEC. Generally those specimens containing mucus, at least 10 PMNs, and <25 SEC per low-power field (LPF) were acceptable for culture, resulting in rejection of 21% of the specimens. In the next report from the Mayo Clinic, Murray and Washington (4) used the acceptability criteria of >25 PMNs and <10 SEC per LPF and rejected 75% of the specimens. In this report, the appearance of sputum was considered an "unreliable indicator of oropharyngeal contamination." Shortly after, in another report from the Mayo Clinic by Van Scoy (6), the criteria were modified to accept any sputum specimen with >25 PMNs per LPF so that only about 25% of the sputum specimens were discarded. The most recent report was from Duke University Medical Center (8) using the criteria of <10 SEC per LPF and the presence of organisms on the Gram-stain to accept endotracheal suctions for cultures. In this study the "rejection" rate was 55%. The authors noted that the quantity of PMNs did not predict the quality of the endotracheal suctions.

The utility of Gram stain for interpretation of expectorated sputum is also controversial. Gram stain is a subjective analysis, and there is considerable variation in expertise. In view of CLIA '88 and the lowered standards for technical personnel, it is technical expertise that must be emphasized. Critical therapeutic decisions based on these stains probably should not be entrusted to personnel who do not have special training. The main problem is the failure to recognize coccobacillary gram-negative bacilli resembling *Haemophilus influenzae* and *Pseudomonas cepacia* on Gram stains of sputum and endotracheal suctions.

It is crucial not to overlook the fact that pneumonia is primarily a clinical diagnosis. Clinicians have several sources of information for evaluating a patient's pulmonary problem, including history, physical examination, and nonmicrobiologic laboratory tests such as the chest radiograph and white blood cell count. The sputum culture is used to establish etiologic diagnosis rather than to serve as a test for confirming or disproving the diagnosis of pneumonia. The sputum culture is not pathognomonic of pneumonia or completely reliable in demonstrating the etiologic agent if pneumonia exists. This is best demonstrated in a study conducted at

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