RAPID DIAGNOSIS OF VIRAL INFECTIONS

Thomas F. Smith

Section of Clinical Microbiology
Mayo Clinic and Mayo Foundation
Rochester, MN 55905

INTRODUCTION

Rapid tests for use in the virology laboratory have evolved because of the recognition of serious complications produced by viruses and the availability of antiviral therapy such as acyclovir and gancyclovir to influence the clinical course of the patient. Accordingly, many of these systemic viral infections occur as a result of impaired host immune defense mechanisms. Thus, the importance of the herpesviruses in particular, producing life-threatening disease in organ transplant recipients (kidney, bone marrow, liver, heart, lung, and pancreas) has been recognized for several years. The severe immunosuppression induced in patients infected with human immunodeficiency virus (HIV) has amplified the importance of underlying infection with herpesviruses, and hence laboratory diagnosis of these infections in this population.

Key technologic developments such as monoclonal antibodies and genetic probes, many of which are now commercially available, have broadened the scope of laboratories to detect medically-important viruses with greater sensitivity and specificity than in years past. Importantly, rapid tests are now in place for routine diagnosis that may produce results faster than available in other areas of clinical microbiology.

The extent, direction, and function of diagnostic virology depends on the type of medical practice that is supported by that laboratory. For example, rotavirus and many agents causing respiratory tract infections (respiratory syncytial virus (RSV), influenza virus, parainfluenza virus) are of much greater concern in primary care medical centers and children's hospitals than in tertiary care facilities in which the herpesviruses are likely to predominate. Similarly, practical considerations involving resources (physical space, laboratory personnel), demography, and numbers of patients will figure into the equation of the type and extent of laboratory tests performed for virology. Unfortunately, a single rapid technique is not universally appropriate for the rapid detection of every virus. This review emphasizes tests primarily used for the laboratory detection of the herpesviruses.

Laboratory Tests

Cell culture. Recovery of viruses in cells grown *in vitro* continues

Rapid Methods in Clinical Microbiology
Edited by B. Kleger *et al.*
Plenum Press, New York

to be the standard by which the performance of other tests are measured. Recognition of cytopathic effects (CPE) produced by agents such as herpes simplex virus (HSV), some enteroviruses, and influenza virus can yield diagnostic reports within a 1 to 3 d period. Importantly, these results are highly specific but frequently, in order to achieve the maximum sensitivity, cultures must be incubated and examined for several days.

In general, the disadvantages of cell culture technology include: (i) dependence on efficient transport of specimens to ensure active virus for replication, (ii) preparation of cultures is expensive and labor-intensive, (iii) recognizable CPE may require several days to develop, and (iv) limited susceptibility of host cells to viruses such as rotavirus, Norwalk-like agents, papillomavirus, parvovirus, many coxsackievirus type A strains, hepatitis viruses, and Epstein-Barr virus and many others. Because of these problems, laboratories have sought other more rapid methods that may be used preferably independently, but usually to supplement the conventional tube cell culture technique.

Cytology. For many years, the rapid diagnosis of cytomegalovirus (CMV) was achieved by the detection of characteristic inclusion-bearing cells in stained tissue sections. However, ultimate recovery of the virus in cell cultures, although much slower than the histopathologic technique, has been more sensitive. For example, CMV was recovered from 34 (6.8%) of 502 lung specimens from an unselected autopsy population (Macasaet et al., 1975). Histologic examination revealed the typical intranuclear CMV inclusion bodies in several organs from 12 cases (35%), but in lung tissue from only 6 (18%).

"Similarly, (Moseley et al., (1981)"enrolled 103 consecutive patients presenting to a sexual transmitted disease clinic for suspected genital HSV infection. Specimens from each patient were processed in the laboratory to compare viral isolation, indirect immunoperoxidase, and cytology. HSV was isolated from 81% of these patients and was detected by indirect immunoperoxidase in 57.3%. Papanicolaou smears from the genital lesions revealed giant cells or cells with intranuclear inclusions in 38% of specimens. The maximum sensitivity of detecting a viral infection by cytology depends on the collection of an adequate number of intact cells (25-50) for examination, quality of reagents, and the expertise required for accurate interpretation of results. Conversely, cell culture isolation of the virus is more sensitive, owing to the amplification of virus replication in the host cells over several days period of time.

Electron Microscopy (EM). The technique of negative staining of specimens preparatory to examination of the specimen by the electron microscope was first used to obtain fundamental information about virus morphology. More recently, the procedure has been applied for diagnostic purposes. A drop of a virus suspension is mixed with a 25% aqueous solution of glutaraldehyde on a piece of dental wax. A Formvar-coated, carbon-stabilized grid is floated on the drop for approximately 30 seconds. The grid is then placed on a drop of heavy metal solution, such as phosphotungstic acid. By this procedure, the virus particles are surrounded by heavy metal atoms which act as an electron stain. The electron beam passes through the low density of the virus but not through the metallic background, resulting in the negative stain (Almeida, 1980).

EM has probably been used most extensively for the examination of feces from patients with gastroenteritis. The technique allows recognition of several viruses that are not easily cultivated in cell cultures: Norwalk agents, rotaviruses, adenoviruses, coronaviruses, astroviruses, and caliciviruses (Chernesky et al., 1982). Although EM techniques have been used for the diagnosis of HSV and CMV, most laboratories recognize that the

procedure is too expensive, cumbersome, and insensitive for routine viral diagnosis and its utility is limited to special situations (Miller and Lang, 1982; Montplaiser et al., 1972; Richman et al., 1984).

Enzyme Immunoassay (EIA). Conjugation of horseradish peroxidase to immunoglobulin was reported over 20 years ago (Nakane and Pierce, 1966). Enzyme labels are less costly, simpler to use, safer, and have a long reagent shelf life relative to radioisotope tags. In addition, the amplification resulting from the enzyme-mediated conversion of many substrate molecules to a visible chromogenic product by a single enzyme molecule has produced a sensitivity in assay comparable to that of radioimmunoassays (Josephson, 1985). The principle of EIA tests is like that of fluorescent antibody methods, except that the end-point of the assay is a color change that can be objectively measured spectrophotometrically. Similarly to the fluorescent antibody system, both direct and indirect tests can be used to detect viral antigens. The EIA procedure is most often used for detection of viral antigens that are captured by homologous antibodies bound to a solid phase such as a microtiter plate or a bead. The captured antigen is then detected (in the direct test) with another viral-specific antibody conjugated to an enzyme.

EIA's for the direct detection of HSV from clinical specimens have been developed, but generally have lacked the high sensitivity that is needed for the diagnosis of this important virus. For example, an evaluation of the Ortho EIA test (Ortho, Diagnostic Systems, Inc., Raritan, NJ) in our laboratory found a sensitivity of 64% compared to recovery of the virus in conventional tube cell cultures (Morgan and Smith, 1984). Another evaluation of a non-marketed research prototype assay for detection of HSV yielded a sensitivity of 77% (van Ulsen et al., 1987). Amplification of HSV in specimens by replication in cell cultures, however, yielded a much more sensitive system (98%) (Michalski, 1986). Interestingly, another commercial system, "Herp Chek™, DuPont Co., Billerica, MA) was able" to achieve a high detection rate (sensitivity, 95%) of HSV, presumably because of the presence of a viral lysing agent incorporated into the transport medium (Baker et al, 1987).

Nerurkar et al. (1984) modified an EIA test for the direct detection of HSV in clinical specimens by using an enzyme labeled with streptavidin that combined with a biotin-linked anti-HSV antibody that was used as the second antibody (sensitivity, 96%). The biotin-streptavidin system has a very high binding constant (10^{13}) that apparently increases the sensitivity of the EIA. Nevertheless, use of the biotin-avidin EIA in other studies did not necessarily assure the laboratory of highly sensitive and specific results (Adler-Storthz et al., 1983). Variation in test performance may be due to the type of specimen (i.e. titer of virus present) and the relative susceptibility of the assay systems used to detect the virus. In this regard, it is probably much more difficult to develop a highly sensitive EIA test for direct detection of HSV since this virus is very easily recovered in cell cultures. On the other hand, the performance characteristics of similar tests for varicella-zoster virus (VZV) and RSV may be apparently better because the "gold-standard" of cell culture isolation may not be optimal (Smith, 1987).

Immunofluorescence. Cell scrapings from several sources (eye, skin, throat washings), impression smears, and tissue sections may be examined for the presence of viral antigens using fluorescence microscopy. The indirect test, theoretically, is considered to be more sensitive than the direct test since an antispecies antibody reacts with a viral-specific antibody in a sandwich technique. The merit of immunofluorescence tests for antigen detection is the speed of results. The success of the assay, however, depends on the control of many variables: (i) like cytology,

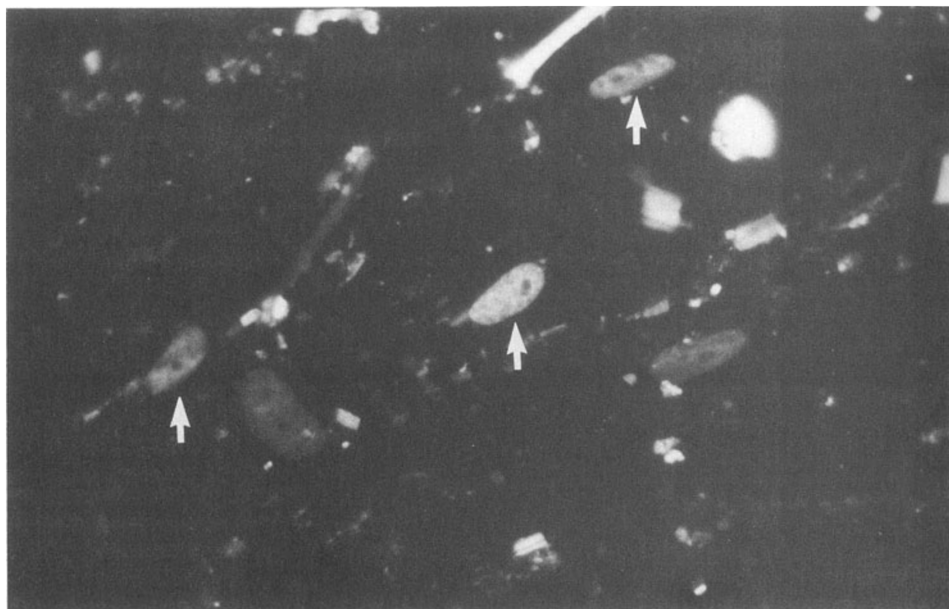

Fig. 1. Foci of CMV-infected MRC-5 cells 16 h postinoculation stained with monoclonal antibody in the indirect immunofluorescence test.

fluorescent antibody tests require an adequate number of intact cells, (ii) quality reagents, (iii) subjective interpretation based on expertise of technologist, and (iv) high-quality fluorescence equipment with optimal filter systems.

Typical experience with the fluorescent antibody test in a pediatric population found that the assay had a sensitivity of 86% for detecting measles, 77% for RSV, 68% for parainfluenza type 3, and 50% or less for adenovirus and influenza virus (Hallsworth and McDonald, 1985). Results were generally less productive when specimens from adults were obtained. Therefore, although the fluorescent antibody test performed on intact cells obtained directly from the patient can be rapid (although tests are performed in batch form in most laboratories), another assay must be performed as backup to provide maximum sensitivity.

Shell Vial Assay. This test is based on the amplification of virus in specimens by short-term infection of cell cultures and the immunologic detection of early antigens by highly specific monoclonal antibodies (Gleaves et al., 1984). The method takes advantage of the immunologic assays (enzyme and fluorescence) to detect viral antigens in their initial stages of production. Early antigens in infected cells are identified 16 h postinoculation by the presence of homogeneous fluorescence throughout the nuclei (Fig. 1). Both the smooth regularity of the nuclear membrane and the characteristic shape of the CMV-infected cell nuclei allow for the specific recognition and distinction of this viral infection from background nonspecific debris that may fluoresce. The availability of a monoclonal antibody with these characteristics for the rapid detection of CMV and the importance of the diagnostic results to clinicians involved in managing organ transplant patients provided impetus to develop a rapid laboratory test for this virus infection.

Many of the steps used in the procedure for isolation of *Chlamydia trachomatis* have been instructive for achieving maximum sensitivity in the

shell vial assay. Several technical aspects of the test must be followed to achieve the desired results and have been discussed in detail in other publications (Gleaves et al., 1984; Paya et al., 1987; Shuster et al., 1985; Smith, 1987). These variables include: age, cell density, and number of cell cultures (shell vials or microtiter plate wells) used per specimen), centrifugation, specificity of monoclonal antibodies for immediate-early or early antigens, type of specimen (urine, blood, bronchoalveolar lavage, tissue), quality of fluorescence equipment, and technical experience with the assay to subjectively evaluate specific results. While adherence to optimal conditions for all these variables is required for optimal performance results, centrifugation of the specimen onto the monolayer is probably the single most important step. Without this enhancement procedure, the sensitivity can be reduced up to 60% (Gleaves et al., 1984). One common problem that many laboratories have experienced during the course of establishing shell vial techniques is the toxicity produced by some specimens. Specimen toxicity of shell vial cell monolayers is likely due to the use of cultures from commercial sources that are already several days old by the time they arrive in the laboratory. For example, Thiele et al. (1987) found that the sensitivity of detection of CMV decreased precipitously with cell cultures that were prepared over 11 days prior to use.

Experience of almost 5 years in our laboratory has indicated that the shell vial assay is more rapid (16 h postinoculation), sensitive, and as specific than conventional tube cell cultures (mean of 9 d for detection) for detection of CMV. Similarly, the technique has been expanded for the diagnosis of HSV, VZV, adenovirus, and influenza virus. These tests are routine procedures in our laboratory in that urine (CMV) specimens and genital specimens (HSV types 1 and 2) are inoculated into 2 shell vials. Dermal specimens are inoculated into 4 shell vials: HSV types 1 and 2 and VZV (stained at 2 d and 5 d postinoculation). Specimens from the eye are inoculated into four shell vials; cells on two coverslips are stained for detection of HSV 1 and 2 the day following inoculation. The remaining two vials are processed for the detection of adenovirus 2 and 5 days postinoculation.

Nucleic Acid Hybridization. Techniques involving nucleic acid hybridization have been known for several years. For example, almost 8 years ago, an assay was described that could accurately differentiate HSV type 1 from type 2 (Brautigan et al., 1980). The principle of the hybridization assay is based on the detection of genetic sequences of viruses present in clinical material with homologous fragments of nucleic acid. These probes are particularly valuable for the detection of many viruses that are not routinely recovered in cell cultures. Their commercial availability will add a new dimension to the diagnostic tests now available in the clinical laboratory.

CONCLUSIONS

Diagnostic virology is in a dynamic state. The incidence of infections in immunocompromised hosts, the rapidly evolving immunologic and nucleic acid probe reagents for diagnosis, and the development of effective antiviral therapy offer limitless opportunity in this area of laboratory practice.

REFERENCES

Adler-Storthz, K., Kendall, C., Kennedy, R. C., Henkel, R. D., and Dreesman, G. R., 1983, Biotin-avidin-amplified enzyme immunoassay for detection of herpes simplex virus antigen in clinical specimens, J Clin Microbiol., 18:1329.

Almeida, J. D., 1980, Practical aspects of diagnostic electron microscopy, Yale J Biol Med., 53:5.

Baker, D. A., Pavan-Langston, D., Gonick, B., Milch, P. O., Dunkel, E. C., Berkowitz, A., Vander-Mallie, R., Woodin, M. B., Philip, A., Bobrow, M. N., and Cukor, G, 1987, Multicenter clinical evaluation of DuPont's Herpchek™, a new rapid diagnostic test for herpes simplex virus (HSV), Abstract, Eastern Pennsylvania Branch, ASM, Philadelphia, PA.

Brautigan, A. R., Richman D. D., and Oxman, M. N., 1980, Rapid typing of herpes simplex virus isolates by deoxyribonucleic acid: deoxyribonucleic acid by hybridization, J Clin Microbiol., 12:226.

Chernesky, M. A., Ray, C. G., Smith, T. F., and Drew, W. L., 1982, Laboratory diagnosis of viral infections, Cumitech, 15:1.

Gleaves, C. A., Smith, T. F., Shuster, E. A., and Pearson, G. R., 1984, Rapid detection of cytomegalovirus in MRC-5 cells inoculated with urine specimens by using low-speed centrifugation and monoclonal antibody to an early antigen, J Clin Microbiol., 19:917.

Hallsworth, P. G., and McDonald, P. J., 1985, Rapid diagnosis of viral infections with fluorescent antisera, Pathology, 17:629.

Josephson, S. L., 1985, Immunoenzyme techniques, Clin Microbiol Newsletter, 7:81.

Macasaet, F. F., Holley, K. E., Smith, T. F., and Keys, T. F., 1975, Cytomegalovirus studies of autopsy tissue. II. Incidence of inclusion bodies and related pathologic data, Am J Clin Pathol., 63:859.

Michalski, F. J., Shaikh, M., Sahraie, F., Desai, S., Verano, L., and Vallabhanine, J., 1986, Enzyme-linked immunosorbent assay spin amplification technique for herpes simplex virus antigen detection, J Clin Microbiol., 24:310.

Miller, S. E., and Lang, D. J., 1982, Rapid diagnosis of herpes simpex infection: amplification for electron-microscopy by short-term in vitro replication, J Infect., 4:37.

Montplaisir, S., Belloncik, S., Leduc, N. P., Angi, P. A., Martineau, B., and Kurstak, E., 1972, Electron microscopy in the rapid diagnosis of cytomegalovirus: ultrastructural observation and comparison of methods of diagnosis, J Infect Dis., 125:533.

Morgan, M. A., and Smith, T. F., 1894, Evaluation of an enzyme-linked immunosorbent assay for the detection of herpes simplex virus antigen, J Clin Microbiol., 19:730.

Moseley, R. C., Corey, L., Benjamin, D., Winter, C., and Remington, M. J., 1981, Comparison of viral isolation, direct immunofluorescence and indirect immunoperoxidase techniques for detection of genital herpes simplex virus infection, J Clin Microbiol., 13:913.

Nakane, P. K., and Pierce, G. B., 1966, Enzyme-linked antibodies: preparation and application for localization of antigens, J Histochem Cytochem., 14:929.

Nerurkar, L. S., Namba, M., Brashears, G., Jacob, A. J., Lee, Y. J., and Sever, J. L., 1984, Rapid detection of herpes simplex virus in clinical specimens by use of a capture biotin-streptavidin enzyme-linked immunosorbent assay, J Clin Microbiol., 28:109.

Paya, C. V., Wold, A. D., and Smith, T. F., 1987, Detection of cytomegalovirus infections in specimens other than urine by the shell vial assay and conventional tube cell cultures, J Clin Microbiol., 25:755.

Richman, D. D., Cleveland, P. H., Redfield, D. C., Oxman, M. N., and Wahl, G. M., 1984, Rapid viral diagnosis, J Infect Dis., 149:298.

Shuster, E. A., Beneke, J. S., Tegtmeier, G. E., Pearson, G. R., Gleaves, C. A., Wold, A. D., and Smith, T. F., 1985, Monoclonal antibody for rapid laboratory detection of cytomegalovirus infections: characterization and diagnostic application, Mayo Clin Proc., 60:577.

Smith, T. F., 1987, Rapid methods for the diagnosis of viral infections, Lab Med., 18:16.

Thiele, G. M., Bicak, M. S., Young , A., Kinsey, J., White, R. J., and Purtilo, D. T., 1987, Rapid detection of cytomegalovirus by tissue culture, centrifugation, and immunofluorescence with a monoclonal antibody to an early nuclear antigen, J Virol Meth., 16:327.

van Ulsen, J., Dumas, A. M., Vagenvoort, J. H. T., van Zuuren, A., van Joost, T., and Stolz, E., 1987, Evaluation of an enzyme immunoassay for detection of herpes simplex virus antigen in genital lesions, Eur J Clin Microbiol., 6:410.