

AN UPDATE OF RAPID DIAGNOSIS OF INFECTIOUS DISEASES II - VIRUS

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ABSTRACT

Diagnosis of viral infections has, until recently, been considered to be retrospective and epidemiological in nature, of little practical significance to patient management and a futile exercise as far as the clinician is concerned. Recent advances have enabled the development of new techniques and more sensitive and specific reagents and assays. These allow viral diagnosis to be made within one to two days of receipt of a patient's specimen, a time frame which permits meaningful management to be instituted since effective antiviral agents are now available and the epidemiology of many viral illnesses known.

Keywords: rapid diagnosis, infectious diseases, viral infections

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INTRODUCTION

Traditionally, diagnostic virological methods have been too expensive and laborious and too slow to be of much benefit to direct patient management. The self-limiting nature of most viral illnesses and the limited therapeutic options have not encouraged requests for a specific viral diagnosis either. However, the availability of effective antiviral agents in recent years as well as the increase in the number of immunocompromised patients resulting from transplant programmes and diseases such as AIDS, in whom severe viral infections are frequent complications, have resulted in an increased demand for rapid viral diagnosis.

This is a review of the laboratory tests now available for the diagnosis of viral infections within a day or two of receipt of a specimen. A similar review covering bacterial, fungal and parasitic infections is presented in Part I of this paper (published in the June 1994 issue).

DIAGNOSIS OF VIRAL INFECTIONS

Rapid methods depend upon the visualisation of virus particles, the detection of viral antigen and nucleic acid directly in the specimen, the detection of virus-specific antibodies and amplification of virus culture. Such methods include immunofluorescence (IF), immunoperoxidase (IP) and enzyme immunoassays (EIA), radio-immunoassay (RIA), particle and whole blood agglutination, shell vial culture, electron microscopy (EM) and molecular methods such as the polymerase chain reaction (PCR).

Respiratory viruses

Ribavirin is an antiviral agent which is of significant clinical benefit for infections caused by respiratory syncytial virus (RSV)⁽¹⁾, a major respiratory pathogen of infants causing both bronchiolitis and pneumonia. Another antiviral agent, amantadine, is effective against influenza A, the cause of multiple epidemics worldwide. In hospitals, nosocomial infections by RSV and parainfluenza viruses are common in

children. However, it is often difficult to distinguish clinically, illnesses caused by respiratory viruses from those caused by bacteria, for instance, *Bordetella pertussis* and *Mycoplasma pneumoniae*. For these reasons, rapid identification of these viruses is of practical use.

The two most frequently used rapid tests for RSV are IF and EIA. RSV is the respiratory virus for which direct IF has been most successfully applied, the sensitivity being equal to or greater than that of cell culture⁽²⁾. Results are available in less than 2 hours. In this method, the processed respiratory specimen is spotted onto slides and after drying and fixing, the smears are reacted with fluorescein-conjugated virus-specific antibodies and examined under the fluorescence microscope for fluorescence in respiratory epithelial cells. Both monoclonal and polyclonal antibodies are available for RSV detection but monoclonal antibodies, being directed against precisely defined epitopes on viruses, increase the specificity of identification.

EIA can detect antigen or antibody depending on whether antigen or antibody is the capture agent on the solid phase. In EIAs for the detection of solubilised RSV antigen, RSV specific antibodies adsorbed onto a solid phase will bind RSV antigen if this is present in the specimen. RSV specific antibodies conjugated with an enzyme are then reacted with the bound antigen. On addition of substrate, a positive colour change in the reaction well indicates a positive result. When compared with virus isolation, RSV antigen EIAs can approach sensitivities of at least 95% and specificities of about 98%⁽³⁾. Although some of these assays take 3 to 4 hours to perform, there are membrane EIA systems for RSV which work on the same principle as the standard EIA except that the antigen is adsorbed onto a membrane held in an assay cartridge and these require only 15 to 20 minutes of assay time. The sensitivity ranges from 62% to 94% and specificity from 69% to 100% with virus culture as the reference⁽⁴⁾.

IF is preferred to EIA by some because the former allows for testing, on the same specimen, for other respiratory viruses for which reagents are available. These include parainfluenza virus types 1, 2 and 3, adenovirus, influenza virus A and influenza virus B. Monoclonal antibodies are usually used. IF for parainfluenza virus detection has been found to be superior to isolation while for adenovirus, the reported sensitivity of detection by IF relative to virus isolation is between 28% to 60%⁽⁵⁾.

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A membrane EIA kit for influenza A virus (Directigen FLU-A, Becton Dickinson Microbiology Systems) ranges in sensitivity from 65% to 100% and in specificity from 91.6% to 96% in comparison with cell culture^(6,7). Such a kit allows specimens to be tested singly without the need for batching and results are obtained in under 15 minutes. However, it is expensive and IF is still preferred.

The disadvantage of antigen detection systems for influenza viruses is that strain characterisation of the circulating virus is not possible. Virus isolation is required for this. The shell vial culture amplification is a technique in which culture cells, instead of being grown in culture tubes, are grown in a monolayer on cover slips within small plastic bottles. After inoculation with the specimen, the bottles are centrifuged at low speed. This process of centrifugation is thought to enhance the uptake of viruses by the cells and for reasons yet unknown, it accelerates viral replication and the appearance of cytopathic effects. After overnight incubation, the cell cultures are stained with immunofluorescent monoclonal antibodies against the early and immediate early antigens of the virus. This culture method permits amplification of small numbers of virus that may be present. One study using antibodies for detecting influenza viruses A and B reported a sensitivity of 84% and specificity of 100% compared with standard culture⁽⁸⁾.

Herpesviruses

The availability of acyclovir, and more recently, ganciclovir, for treatment of infections by herpes simplex virus (HSV), varicella-zoster virus (VZV) and cytomegalovirus (CMV) has added impetus to the development of rapid methods for their diagnoses.

Rapid identification of HSV is required in critical situations such as infections in the newborn, pregnant women with genital infections at term, patients with encephalitis and immunocompromised patients who are at risk of severe HSV infections. Antigen from infected cells obtained from the base of vesicular lesions or from biopsy of the affected organ can be detected by IF or IP. The IP method is similar to the IF but instead of fluorescein isothiocyanate, horseradish peroxidase is conjugated to the detecting antibodies. Upon the addition of substrate, a reddish brown deposit is formed in the cells where the antigen has reacted with the antibodies. This coloured product is visualised with a light microscope, dispensing with the need for the more expensive fluorescence microscope. The sensitivity of IF compared with tissue culture isolation may be as high as 88%⁽⁹⁾ and a specificity of 85% has been reported⁽¹⁰⁾. Since both false positive and false negative results can occur with IF as well as IP, a sample should also be obtained for viral culture.

EIA detects both extracellular and intracellular antigens, and hence may be more sensitive than IF. Using EIAs for the detection of HSV antigen, sensitivity of 95% and specificity of 100% have been reported for the diagnosis of genital herpes⁽¹¹⁾. The test can be completed in under 4 hours but because with EIAs, specimens are usually batched, their turnaround time may be longer.

DNA hybridisation is a technique whereby viral nucleic acid is heat denatured to become single stranded, then detected by its binding to a complementary nucleotide sequence (the probe). Probes may be labelled with a radio-isotope and the viral nucleic acid-probe complex then detected by autoradiography, or with a non-isotope such as biotin and the assay for the complex formatted like an EIA. DNA hybridisation is sensitive for demonstrating infected cells in

tissue sections but is not widely used in clinical laboratories partly because of the cost and expertise involved.

Immunocompromised as well as adult patients with VZV infections would benefit from acyclovir and varicella-zoster immune globulin if these are given early in the course of the illness. As the virus is extremely labile, virus isolation is often difficult. Detection of the antigen from vesicular contents is the most sensitive method. With direct IF using monoclonal antibodies, a sensitivity of 92% has been reported which is greater than those obtained with shell vial culture and standard cell culture⁽¹²⁾. Although electron microscopy demonstrates VZV in vesicular fluid, it does not distinguish among the herpesviruses because of their similar morphology.

Rapid diagnosis of acute or past VZV infection is effected by detecting IgM or IgG antibody to the virus. Fluorescent antibody to membrane antigen is the gold standard for the demonstration of VZV IgG but is too cumbersome for routine use. Instead, the immune status of immunocompromised patients exposed to chickenpox can be ascertained in 10 minutes by a latex agglutination test where specific viral antigens are bound to polystyrene beads and after mixing with sera, examined for agglutination. A recent study comparing one such test with an EIA for VZV⁽¹³⁾ found it to produce both false negative and false positive results. The EIA is reasonably sensitive and specific⁽¹³⁾ and takes 2 to 3 hours to perform.

Rapid detection of CMV infection is essential when a decision to initiate ganciclovir therapy has to be made and to determine if donor organs are suitable for use. With the standard method of virus isolation, cultures may not be positive until after 4 weeks. The shell vial amplification assay detects viral antigens rapidly within 24 hours. It is as specific and may be more sensitive than the conventional cell culture⁽⁴⁾.

Direct CMV antigen detection in clinical specimens such as blood and bronchoalveolar specimens can be accomplished in about 3 hours by IF. The technique, which involves separation of the white blood cells (buffy coat) or bronchoalveolar cells, and preparation of these cells on slides using a cytocentrifuge prior to IF staining, has a sensitivity ranging from 59% to 93% depending on the type of specimen and disease⁽¹⁰⁾.

Donor organ screening for CMV antibodies can be carried out by latex agglutination such as the CMV Scan™, which has a sensitivity of 98% and specificity of 97%⁽⁴⁾. In the microparticle EIA, CMV antigen is adsorbed onto microparticles as the solid phase. This has a turnaround time of about an hour and sensitivity and specificity are reported to be greater than 98% and 99% respectively.

Rubella, Measles, Mumps

The significance of rubella lies in the risk of foetal damage when pregnant women are infected especially in the first trimester. Early diagnosis is required in cases where termination of pregnancy is being considered. EIA for rubella-specific IgM can be completed in about 3 hours. However, a negative result may not exclude a diagnosis of the infection if specimens are obtained very early before the appearance of specific IgM. Conversely, a false positive result will arise if rheumatoid factor is present. Therefore, in all cases, the result must be confirmed with a second specimen taken 7 to 10 days later for demonstration of a four-fold or greater rise in titre by tests such as the haemagglutination-inhibition assay.

In suspected cases of measles, the viral antigen can be directly demonstrated in infected cells in nasopharyngeal secretions or skin biopsies by IF. Results can be ready in 2

hours. Mumps and measles IgM can be detected in a few hours by IF or EIA.

Hepatitis

Hepatitis A to E are routinely diagnosed serologically using assays which require from one to 18 hours for completion. Most laboratories diagnose hepatitis A by detecting for anti-hepatitis A virus IgM using EIA which is almost 100% sensitive⁽¹⁴⁾. Diagnosis of hepatitis B infection relies on serological markers of the virus which can be detected by EIA, microparticle EIA or RIA. Current assays are able to detect as little as 100 pg/ml of hepatitis B surface antigen (HBsAg)⁽¹⁵⁾. At the present moment, immunoassays for hepatitis C virus (HCV) and hepatitis E virus (HEV) IgG only are commercially available but an EIA for HCV IgM is now being evaluated^(16, 18).

Dengue

Dengue infection is most commonly diagnosed by serology. Rapid methods include the IgM EIA, which is an overnight test, and the immunoblot assay. Dengue IgM is detectable from the fifth day after the onset of fever. Although uncommon, false positive results can occur in the EIA as a result of cross reactions with other flaviviruses. The immunoblot assay⁽¹⁹⁾ is essentially an EIA in which dengue antigens are bound onto a nitrocellulose membrane and reacted with virus-specific antibodies in the patient sample. A positive reaction is indicated by a purple dot of a colour intensity equal to or greater than that given by a positive control serum on the membrane. The assay is designed to yield a positive result when the dengue antibody titre by the haemagglutination-inhibition test is equal to or exceeds 1,280. Since such titres in the acute phase are found only in secondary infections, this test is more useful for the rapid diagnosis of secondary rather than primary dengue infections.

Enteric Viruses

The most feasible way of detecting viruses that cause gastroenteritis is by electron microscopy because they are either difficult or impossible to propagate in routine cell cultures. Indeed, electron microscopy is the only means of identifying some of these pathogens such as the calicivirus⁽²⁰⁾. The viruses in faecal specimens are identified by their morphology under the electron microscope in 20 minutes to 2 hours⁽²¹⁾. Immunoelectron microscopy is a modification of the method where immune serum is used to coat or aggregate virus particles to concentrate small numbers of viruses⁽²⁰⁾.

Commercially available standard EIAs for the detection of rotaviruses have total incubation times that range from 75 minutes to several hours. The kits use monoclonal or polyclonal antibodies but the former appears to be more sensitive⁽²²⁾. Some false positive results have been obtained with these kits⁽²²⁾.

Other rotavirus assays provide results in 15 minutes or less and these include latex agglutination and membrane EIAs. Results are read visually as clumped particles or by a colour reaction. EIAs are usually more sensitive than latex agglutination tests⁽²²⁾. With fresh stool specimens, sensitivity of 95% and specificity of 90% have been obtained for a membrane EIA⁽²³⁾.

Human immunodeficiency virus

Rapid diagnosis of human immunodeficiency virus (HIV) infection is required in donor organ screening. Recently, a number of instrument-free assays allowing rapid and accurate

detection of antibody of HIV have been made available. As with EIAs for screening for anti-HIV antibody, specimens which test positive on such rapid techniques must be confirmed by supplementary assays.

Rapid tests include membrane EIAs such as HIVCHEK in which the recombinant HIV-1 envelope protein is used as the antigen on the membrane. After reaction with the patient's anti-HIV antibodies and substrate, a dark red dot appears on the membrane. The whole procedure takes 10 minutes or less. Spielberg et al⁽²⁴⁾ reported a sensitivity of 97.2% compared with Western Blot and a specificity of 96.6%.

Another rapid assay is the gelatin particle agglutination test which uses a lysate of HIV-1 infected cells as antigen coated on gelatin particles to agglutinate specific antibodies in the specimen. The result is determined by the settling pattern of the particles after two hours of incubation. The sensitivity and specificity are 97.2% and 98.2% respectively compared with Western Blot⁽²⁴⁾ and 100% and 99% respectively compared with IP⁽²⁵⁾. The test detects total (that is, both IgM and IgG) antibodies.

In the latex agglutination assay for anti-HIV antibody detection, recombinant proteins from a highly conserved region of the HIV-1 genome are used as antigens on the polystyrene beads. One group of workers has reported the sensitivity to be as high as 99.3% and specificity, 100% compared with Western Blot⁽²⁶⁾. However, these estimates were obtained from populations where HIV-1 infection is endemic and the usefulness of this test in screening low risk populations is uncertain.

The autologous red cell agglutination assay which takes only two minutes to perform is equally rapid⁽²⁷⁾. This uses a non-agglutinating monoclonal antibody to human red blood cells which is conjugated to a synthetic peptide antigen derived from an HIV-1 envelope protein. Addition of this complex to whole blood from a patient will cause the patient's red cells to become coated with the complex. If HIV-1 antibodies are present in the patient's sample, they will bind to the cell-bound antigen and cause agglutination of the red cells. Although the original workers reported a false positivity rate of 0.1% in HIV-1 seronegative blood donors and a false negativity rate of about 1%⁽²⁷⁾, subsequent experience has shown its sensitivity to be less because the assay uses only one antigen, gp 41, and individuals who are infected with HIV but do not have detectable anti-gp41 antibody will be missed.

Polymerase chain reaction

The polymerase chain reaction (PCR) amplifies target nucleic acid sequences by repeated cycles of DNA denaturation by heat, followed by annealing of oligonucleotide primers to the respective recognition sequences flanking the target nucleic acid sequences on the separated DNA strand and extension of the primer by DNA polymerase and oligonucleotide bases. The target sequences which may be present in minuscule quantities are amplified to a level that is readily detectable by means such as hybridisation with oligonucleotide probes or gel electrophoresis followed by ethidium bromide staining⁽²⁸⁾. PCR is exquisitely sensitive and results can be obtained within 5 to 7 hours. The limitations of this method are its susceptibility to contamination by nucleic acid resulting in false positive results, the need for an expensive thermal cycler and the requirement for trained personnel to perform the test.

A particularly useful application of the PCR technique is in the diagnosis of HIV-1 infection in infants in whom the presence of maternally transferred HIV-1 antibodies, blunted

endogenous synthesis of HIV-1 IgM and a viral load below the level of detection by culture are some reasons for the difficulties encountered in diagnosis below 18 months of age²⁹. Although data about the usefulness of PCR in infants under 3 months of age are scarce, its sensitivity probably approaches 100% with infants older than 6 months and data have also shown that specificity exceeds sensitivity with infants of any age²⁹.

PCR and other nucleic acid amplification techniques for the detection of HCV, HEV and other viruses which cannot be cultured or are difficult to culture are available in research laboratories.

CONCLUSION

Virological diagnosis has travelled far to become a practical means of providing information to aid in patient management. For herpesviruses for which effective treatment is available, rapid diagnosis leads to improved outcome in patients. Rapid identification of RSV and rotavirus, which can cause nosocomial infections and epidemics, is necessary for infection control. Rapid viral diagnosis has also contributed to the rational use of antibiotics and the judicious application of invasive diagnostic investigations.

Although nucleic acid probes are available commercially for in-situ detection of viruses, these are no faster than assays that use monoclonal antibodies. Of greater promise is the PCR which, although presently confined to reference and research laboratories, will probably come into routine use in the clinical laboratory in the near future.

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