Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Pharmac. Ther. Vol. 40, No. 2, pp. 287–328, 1989
Printed in Great Britain.

0163-7258/89 $0.00 + 0.50

Specialist Subject Editor: DAVID SHUGAR

RAPID AND ACCURATE VIRAL DIAGNOSIS

M. L. LANDRY,[1,2,3] D. R. MAYO[2,4] and G. D. HSIUNG[1,2]

[1] *Virology Reference Laboratory, Veterans Administration Medical Center, West Haven, CT 06516, U.S.A.; Departments of* [2] *Laboratory Medicine, and* [3] *Medicine, Yale University School of Medicine, New Haven, CT 06510, U.S.A.; and* [4] *Virology Laboratory, Connecticut State Department of Health Services, Hartford, CT 06144, U.S.A.*

Abstract—In recent years, there has been increased recognition of the importance of viral infections. In addition, new antiviral agents have become available. These factors have led to a marked increase in utilization of viral diagnositc services. In this review, both conventional and rapid methods for viral diagnosis are presented, with emphasis on recent advances. The antiviral agents currently available and the major drugs under investigation are also briefly discussed. It is hoped that this review will serve as a useful adjunct for the management of patients with virus infections.

CONTENTS

1. INTRODUCTION

Despite the prevalence of viral infections, viral diagnostic laboratories have traditionally existed only as part of either regional health departments or university research laboratories. Conventional viral diagnostic methods have been considered time-consuming, expensive and inaccessible to the practising physician (Herrmann, 1974; Herrmann and Herrmann, 1976; Hsiung, 1977). Thus an accurate viral diagnosis was infrequently attempted. However, in recent years the importance of viral infections has been increasingly recognized particularly as a cause of morbidity and mortality in the immunosuppressed patient (Muller *et al.*, 1972; Ho, 1977; Shields *et al.*, 1985), of both severe and subtle disease in the neonate (Stagno *et al.*, 1975; Whitley *et al.*, 1980b), as well as a cause of venereal disease (Ng *et al.*, 1970; Jordon *et al.*, 1973; Handsfield *et al.*, 1985). The epidemic of acquired immunodeficiency syndrome (AIDS) has focused the world's attention on viruses as potentially serious pathogens (Barre-Sinoussi *et al.*, 1983; Popovic *et al.*, 1984; Shaw *et al.*, 1984). In addition, viruses may be etiologically linked to cancer (Rawls *et al.*, 1969; Henle *et al.*, 1969; Hanto *et al.*, 1981; Andiman *et al.*, 1983; Durst *et al.*, 1983; Wong-Staal, 1983). Most importantly, promising new antiviral agents are becoming available. Therapy, if it is to be effective, must be instituted early in the course of the disease; thus, there has been increasing interest in viral diagnosis and particularly in the development of more rapid diagnostic procedures for viral infections (Gardner, 1977; Yolken, 1980; Richman *et al.*, 1984a,b).

The awareness on the part of the medical community and the public of the significance of herpes infections in particular has led to the establishment of viral diagnostic laboratories in an increasing number of community hospitals and a tremendous increase in utilization of viral diagnostic services previously available in regional laboratories or university hospitals. There has also been a burgeoning of commercial laboratories offering viral diagnostic tests to those hospitals or practitioners without such services readily available. The number of commercial companies and products available to aid in viral diagnosis has also greatly increased. With the advent of antiviral therapy, it is no longer acceptable for lack of accurate viral diagnosis to hinder or delay the treatment of patients. Thus, physicians are beginning to demand laboratory diagnosis of their patients' illnesses in order to have specific and proper treatment. To accomplish this, viral diagnostic facilities are becoming more accessible and, additionally, health practitioners must be more knowledgeable regarding procedures used for viral diagnosis. The purpose of this review, therefore, is to discuss both new and standard methods for virus recognition and identification with special reference to rapid diagnosis and the advances made in the last few years. The selection of antiviral agents currently used is also briefly discussed. It is hoped that this review will serve as a useful adjunct for the management of patients with virus infections.

2. NEW AND STANDARD METHODS FOR VIRUS RECOGNITION AND IDENTIFICATION

Conventional methods of viral diagnosis consist of virus isolation and serology; light and electron microscopy are performed in certain situations. Although there is tremendous interest in the development of rapid diagnostic techniques, conventional diagnostic

methods remain the most widely used and are essential in confirming the usefulness of newer techniques. However, it must be emphasized that only standard virus isolation and electron microscopy allow for success in recognition of unexpected or 'new' viruses.

2.1. Specimen Collection

The critical first step in making a successful viral diagnosis is obtaining the proper specimens. This includes the choice of specimens, and proper collection and transport. If these initial steps are not appropriately undertaken, the subsequent time and effort spent in attempting virus isolation will be wasted.

The choice of specimens depends upon the clinical syndrome and the viruses suspected. Since one syndrome can be associated with many viruses, a set of specimens is often recommended. In general, specimens for virus isolation should be collected early in illness as many viruses are excreted for only a few days. However, certain viruses, such as cytomegalovirus (CMV), enteroviruses and adenoviruses, can be excreted for prolonged periods.

Table 1 contains the commonly encountered clinical syndromes, the associated viruses and the appropriate specimens to be obtained. For throat swabs, a vigorous swab of the posterior pharynx and of any visible lesions should be obtained. Stool specimens are preferred over rectal swabs because the larger sample size results in greater yield of virus isolates. First-voided morning urines are best and two or three specimens are optimal for CMV isolation. Aspiration of nasopharyngeal mucus has been found to be superior to nasal swabs or nasal washes for isolation for respiratory syncytial virus (RSV) (Bromberg *et al.*, 1984) and bronchoalveolar lavage specimens have been found superior to bronchial washings for CMV (Stover *et al.*, 1984; Martin and Smith, 1986).

2.2. Specimen Transport

Since viruses are obligate intracellular organisms, they require living cells in which to replicate. As a result, a significant decrease in virus infectivity titer will occur if clinical specimens are allowed to stand for any period at room temperature. For best results, direct inoculation of cell cultures at the bedside should be done. Generally this is not feasible, therefore swabs and tissues should be placed in viral transport media containing a balanced salt solution and a protein stabilizer, gelatin or calf serum. A variety of collection and

Table 1. *Examples of Specimens to be Collected for Virus Studies*

Clinical syndrome	Viruses commonly associated	Specimens to be collected*
Respiratory syndromes	Influenza	Nasal washing or aspirate, throat swab
	Parainfluenza	
	Respiratory syncytial virus	
	Adenovirus	
	Rhinovirus	
Gastroenteritis	Rotavirus	Stool
	Norwalk agent	
	Adenovirus	Throat swab
	Enterovirus	Stool
Hepatitis	Hepatitis A	Serum
	Hepatitis B	Serum
	Cytomegalovirus	Throat swab, urine, blood
	Epstein–Barr virus	Serum
Cutaneous and mucous membrane lesions	Varicella zoster	Vesicle fluid or swab
	Herpes simplex virus	
	Enterovirus	Throat swab, stool
Encephalitis and Aseptic Meningitis	Herpes simplex virus	Brain tissue
	Togaviruses	Serum
	Enteroviruses	Throat swab, stool, CSF†

*Acute and convalescent sera to be collected in each case.
†Cerebrospinal fluid.

transport devices are now available commercially and have been the subject of several recent studies (Johnson *et al.*, 1984; Warford *et al*, 1984). Urine, stools, spinal fluids and other body fluids should be placed in sterile containers. Prompt transport to the laboratory is imperative. If a delay is necessary, specimens can be held at 4°C until inoculation into cell culture. If a long delay is necessary, specimens should be frozen at −70°C. For transport to a distant laboratory, specimens can be shipped by rapid delivery service on wet ice; frozen samples can be shipped on dry ice. If swabs dry out or specimens are left at room temperature for any period, virus infectivity will deteriorate markedly. Serum is usually obtained for serodiagnosis and is helpful if no virus is isolated or to confirm an unusual isolate. Whole blood or leukocytes can also be useful in virus isolation, e.g. for CMV or Epstein–Barr virus.

2.3. VIRUS ISOLATION

Once specimens arrive in the laboratory, they must be inoculated promptly into sensitive test systems. Since viruses require living cells in which to replicate, the inoculation of cell cultures or laboratory animals is necessary. Unfortunately, no single culture system will support the growth of all viruses. Thus a variety of cell cultures are routinely used in a diagnostic laboratory. In certain circumstances, embryonated eggs or small animals may be utilized. It is apparent that laboratory personnel must know the clinical syndrome and/or the viruses suspected in order to choose the appropriate system. If an insensitive system is utilized, it is unlikely that any virus will be isolated even though virus is present in the specimen.

2.3.1. *Cell Cultures*

It was the discovery that poliovirus could replicate in nonneural tissue culture (Enders *et al.*, 1949) that revolutionized diagnostic virology. Currently, cell cultures are the mainstay of most viral diagnostic laboratories and, for many labs, they are the only system employed. Embryonated eggs, small animals and serology are reserved for the larger reference laboratories. There has been a proliferation in the types of cell culture available. In general, three main types of cell culture are used: primary cell cultures, diploid cell strains and continuous cell lines. Primary cell cultures are made directly from animal or human tissues and can be subpassaged only a few times. Diploid cell strains are generally derived from human embryonic tissues, particularly embryonic lung, and can be subcultured for about 50 passages. Continuous cell lines are usually derived from human or animal tumors and can be propagated indefinitely. A wide variety of primary cell cultures (e.g. monkey kidney, rabbit kidney), human diploid fibroblast (HDF) cell strains (e.g. WI-38, MRC-5) and continuous cell lines (e.g. HeLa, HEp-2) are now available commercially. The choice of types of cell cultures employed in any laboratory is dependent upon the viruses sought, the patient population and the economic constraints. A fairly broad spectrum of viruses can be cultured if one set of the following cell cultures are used: primary monkey kidney (MK), HDF and HEp-2 cells. The use of several cell types facilitates the chances of recovering a variety of virus types from clinical specimens.

2.3.2. *Recognition of Virus-Induced Cellular Changes*

After inoculation into cell culture, the presence of a virus may be detected in several ways. Most commonly, virus-induced changes such as rounded refractile cells or grape-like clusters are noted (Fig. 1, C–H). These changes are called cytopathic effect (CPE) and vary depending on the causative virus. The formation of syncytia is characteristic of certain viruses, such as respiratory syncytial virus and measles, as well as parainfluenza types 2 and 3 when inoculated in continuous cell lines (Fig. 1, J). Some viruses produce no visible change, therefore indirect tests for their presence are necessary. For influenza and parainfluenza viruses, the hemadsorption test is utilized whereby a dilute solution of

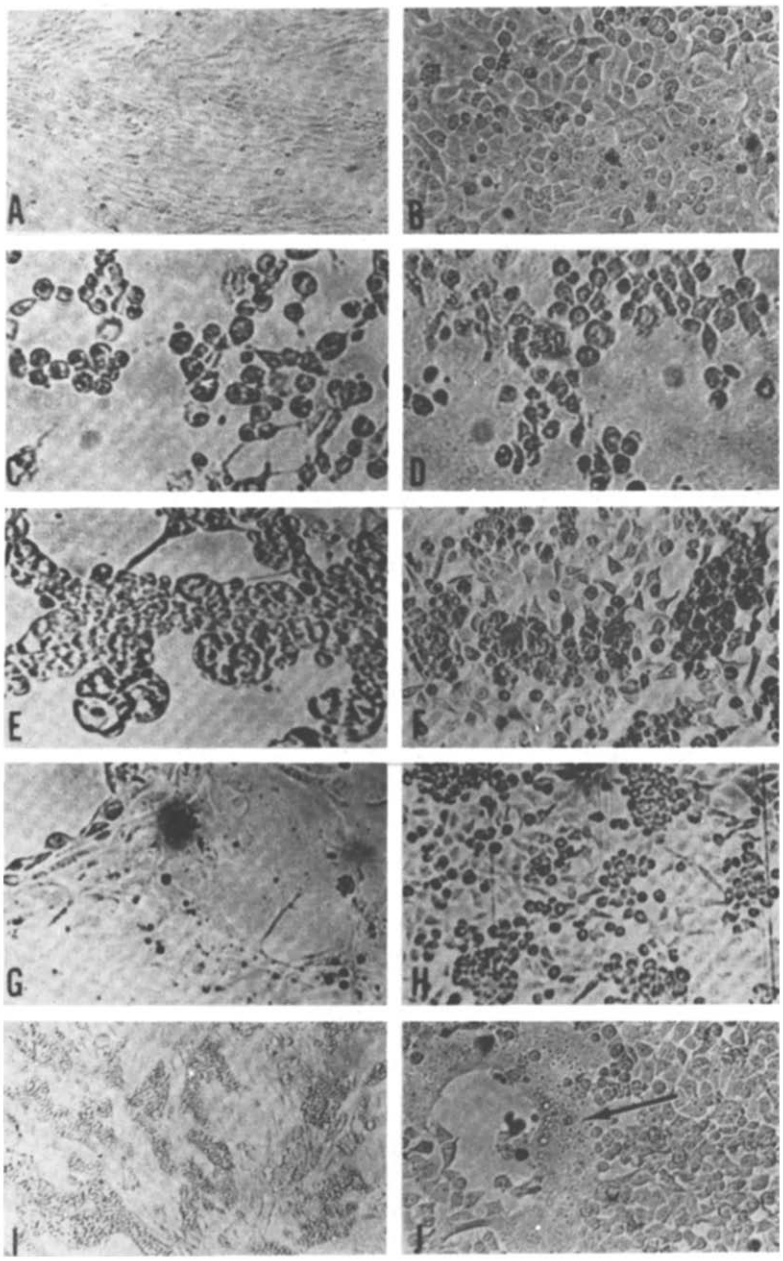

FIG. 1. Virus-induced cellular changes in primary monkey kidney cell cultures and HEp-2 cell cultures.

guinea-pig red blood cells is added to the infected monolayer of cells, allowed to adsorb at 4°C, then washed off. If influenza or parainfluenza is present, the red cells will adhere to the infected cell monolayer (Fig. 1, I). For rubella virus, the interference test has traditionally been used. By this method, cell cultures infected with suspected rubella virus are superinfected with an echovirus, a virus that readily produces CPE. In the presence of rubella, however, the expected virus-induced CPE does not develop but is interfered with.

The speed of appearance and progression of CPE can also be helpful in distinguishing viruses; however, this is also dependent upon the concentration of virus in the inoculum and the sensitivity of the particular cell culture used. Preliminary identification of a virus isolate can be made based upon the type of cell culture the virus is growing in and the character of the virus-induced cellular changes. For example, cytomegalovirus induces CPE only in human fibroblast cells, whereas herpes simplex virus (HSV) induces CPE in

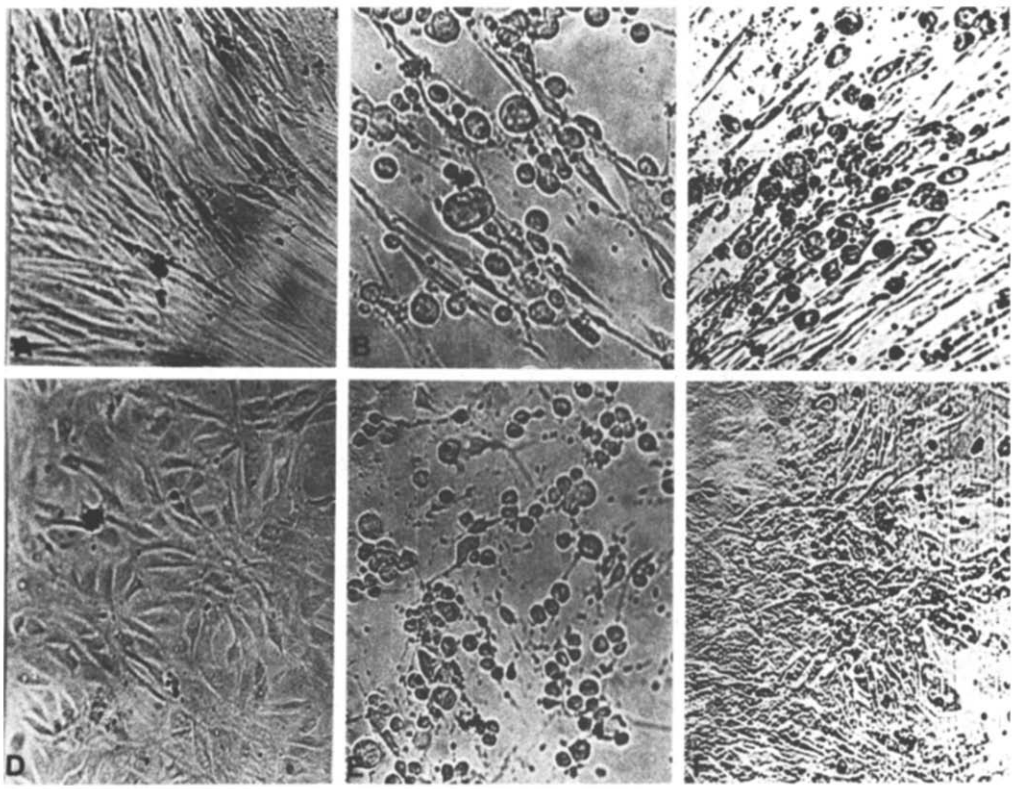

FIG. 2. Cytopathic effect induced by cytomegalovirus (CMV) and herpes simplex virus (HSV) in human diploid fibroblast (HDF) and rabbit kidney (RK) cells.

both human fibroblast and rabbit kidney (RK) cells (Fig. 2). Final identification usually requires a neutralization test using type-specific antiserum; however, in many laboratories more rapid methods of identification are now being applied, such as immunofluorescence (IF) with monoclonal antibodies (see Section 2.6).

2.4. THE USE OF MINI-LABS AND REFERENCE LABORATORIES

Several reports have demonstrated the feasibility of using mini or satellite laboratories, whose services are tailored to both the facilities of the laboratory and to the needs of the patient population they serve (Herrmann and Herrmann, 1977; Peterson *et al.*, 1980; Landry and Hsiung, 1981). For example, the cost of virus isolation can be significantly reduced by the use of microtiter plates containing different types of cell cultures. Virus isolation using the latter system compares favorably with standard techniques (Herrmann and Herrmann, 1977).

Peterson *et al.* (1980) reported the advantage of using satellite laboratories. Time in reporting results was reduced when primary virus isolation was performed in a local, small, hospital-based laboratory when compared with sending specimens to a state-wide reference laboratory. The number of virus isolations by the satellite laboratory was slightly greater than from the reference laboratory and the cost was comparable to that of routine bacteriological specimens.

In laboratories where the nature of the patient population is such that HSV is most frequently encountered, the most sensitive cell systems appear to be nonprimate cells, i.e. rabbit kidney or guinea-pig embryo (GPE) cells (Landry *et al.*, 1982c; Hsiung *et al.*, 1984). Since other human viruses generally do not grow well in nonprimate cells, presumptive identification can be made according to cell susceptibility and characteristic CPE. As shown in Table 2, HSV induces a characteristic CPE in both human diploid fibroblast and RK cells. Cytomegalovirus and varicella-zoster virus (VZV) only replicate in human fibroblasts. Enteroviruses grow best in primary MK, whereas adenoviruses

TABLE 2. *Examples of Cell Cultures to be Used for Primary Virus Isolation and Presumptive Diagnosis*

Virus Type Expected	Cytopathic Effect in Cell Cultures		
	HDF*	MK	RK
Herpes simplex virus	++	+/−	+++
Cytomegalovirus	++	−	−
Varicella-zoster virus	++	+/−	−
Enterovirus	+/−	++	−
Adenovirus	++†	+/−	−
Influenza-Parainfluenza viruses	−	−(HAds ++)	−

*HDF = human diploid fibroblasts.
MK = monkey kidney cells.
RK = rabbit kidney cells.
HAds = hemadsorption.
†Human epithelial cells are preferable, especially human kidney cells.

induce characteristic CPE best in continuous cell lines. On the other hand, influenza and parainfluenza usually do not induce CPE in cell cultures but show hemadsorption on MK cells when they are infected with these viruses.

Advantage has been taken of selective cell-culture systems for presumptive identification of enteroviruses (Hsiung, 1961, 1962; Landry *et al.*, 1982b) and more recently for typing for HSV types 1 and 2 (Nordlund *et al.*, 1977). Because HSV-2 produces plaques in both GPE and chick embryo (CE) cell cultures whereas HSV-1 induces plaques in GPE cells but not in CE cells, the two virus types can be easily identified when these two cell systems are used. However, IF with type-specific monoclonal antibodies is now available, is more rapid and as accurate as selective cell systems (Balkovic and Hsiung, 1985). Those viruses not identifiable by cell susceptibility or characteristic CPE can be referred to larger reference laboratories for final identification.

Although the number of community hospitals with virology laboratories is increasing yearly, the majority still do not have in-house viral cultures available. Therefore, if an accurate viral diagnosis is to be made, specimens must be sent out to reference laboratories or commercial laboratories for virus isolation. With the ready availability of overnight rapid delivery services, specimens can now be processed promptly with results comparable to in-house processing (Ray and Minnich, 1982). In addition, with a new specimen transport device using human fibroblast cell cultures, virus may replicate during transport (Warford *et al.*, 1984).

2.5. Recent Advances in Virus Isolation

With the increased utilization of virus isolation comes a demand for improved isolation rates and more rapid results. Therefore, common diagnostic procedures are being re-evaluated in an attempt to optimize the collection and transport of specimens, specimen processing, the conditions of culture incubation and the selection of the most sensitive cell culture system for each virus.

2.5.1. *Specimen Processing*

Different processing methods have been examined to determine the optimum for detection of enterovirus viremia (Prather *et al.*, 1984), and the factors influencing recovery of varicella-zoster virus (VZV) have also been studied (Levin *et al.*, 1984). A number of studies have determined the importance of centrifugation of specimens onto the monolayer. Improved isolation rates and more rapid results for HSV and CMV have been reported (Gleaves *et al.*, 1984, 1985a,b; Salmon *et al.*, 1986). Fractionation of semen with inoculation of the pellet fraction into culture has been associated with elimination of monolayer toxicity and enhanced CMV detection in AIDS patients (Howell *et al.*, 1986).

2.5.2. *Cell Culture Selection and Conditions of Incubation*

A continued search for better culture systems for each virus remains an important task of the diagnostic virologist. Recent reports have indicated that a mink lung cell line is highly sensitive to infection with HSV (Fayram *et al.*, 1985; Smith *et al.*, 1985). In another study MRC-5 cells were found to be more sensitive than WI-38 for CMV isolation (Gregory and Menegus, 1983a).

Incubation temperature has long been recognized as important in the isolation of respiratory viruses, for which 33°C is optimal. Recently, it has been reported that 36°C when used for isolation of cytomegalovirus (CMV) results in doubled isolation rates and more rapid onset of CMV CPE, by an average of 4 days (Gregory and Menegus, 1983b).

2.5.3. *Cultivation of Fastidious Viruses*

Perhaps the most important development in virus isolation has been the cultivation of several viruses previously considered not amenable to isolation in cell culture. Hepatitis A virus (HAV) has now been isolated directly from fecal extracts in several cell culture types (Provost *et al.*, 1981; Daemer *et al.*, 1981; Siegl *et al.*, 1984). Since the virus is not cytopathic, immunologic assays such as radioimmunoassay (RIA) or IF are necessary to detect its presence. Human rotavirus was able to be cultivated in cell cultures when trypsin was added to the media and now has been successfully isolated and propagated in several different cell cultures (Graham and Estes, 1980; Naguib *et al.*, 1984); IF was the most reliable method for detection and identification of rotavirus in culture. Several enteric adenoviruses, first detected by electron microscopy and considered fastidious, have now been isolated and propagated in several cell systems, with 293 cells considered the most sensitive (Brown *et al.*, 1984). Ability to isolate these viruses in cell culture greatly facilitates the study of these viruses, allows antigen production and makes way for the development of vaccine(s). However, for diagnostic use, other methodologies, such as serology for detection of HAV IgM antibody and ELISA for detection of rotavirus antigen, remain the methods of choice.

The initial isolation of the human immunodeficiency virus (HIV) was a discovery central to the identification of the causative agent of AIDS and to the development of simpler screening tests for this virus (Barre-Sinoussi *et al.*, 1983; Gallo *et al.*, 1984; Levy *et al.*, 1984). The mainstay for diagnosis of human immunodeficiency virus (HIV) is detection of viral antibody by ELISA, with positive serum samples retested with supplementary tests such as the Western blot, IF and radioimmunoprecipitation (Schupbach *et al.*, 1986). However, detection of viral antibody alone does not determine whether the individual is currently infected with the virus. In addition, antibody may not develop for six to twelve months after infection or may become undetectable late in the course of AIDS. The isolation of virus from the infected individual can serve this purpose. However, it remains an elaborate, labor intensive, and lengthy process, that is currently performed primarily in specialized research centers. The procedures for isolation have recently been reviewed elsewhere (Schupbach *et al.*, 1986; Griffith, 1987) (Fig. 3). Briefly, human mononuclear cells are separated from the peripheral blood of normal donors, and suspended in growth media containing a mitogen such as phytohemagglutinin and a T-cell growth factor such as interleukin 2. Several days after lymphocyte cultures have been initiated, patients' specimens are inoculated and are then observed for 3 to 4 weeks for viral cytopathic effect (Fig. 4) and the supernatants are assayed weekly for the products of viral replication such as reverse transcriptase or viral antigen. Freshly prepared stimulated lymphocyte cultures are added once a week. Although continuous cell lines are available that support the growth of HIV, these lines are not as sensitive as primary lymphocyte cultures for isolation of virus from patients' specimens (Falk *et al.*, 1987). Continuous cell lines have the advantage however of showing little CPE and producing large amounts of virus and thus are essential for the production of viral antigens for diagnostic tests. Other human

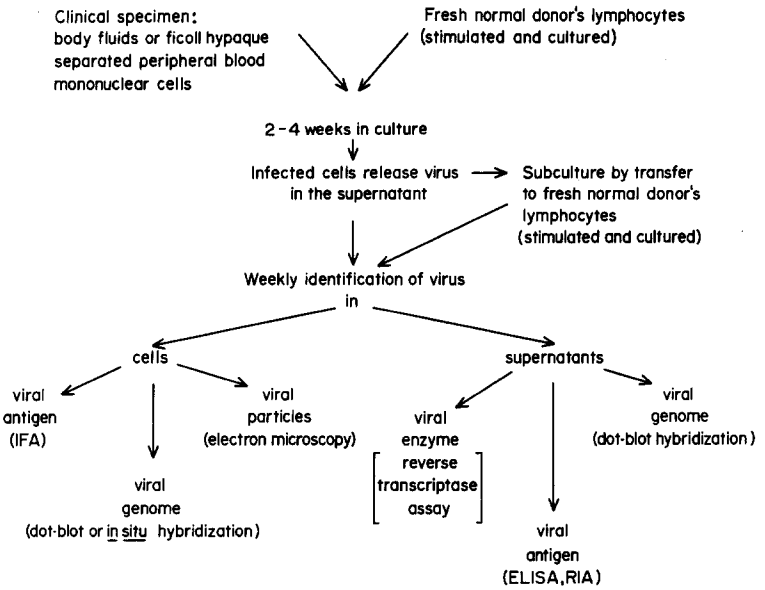

FIG. 3. Flow chart for isolation and identification of human immunodeficiency virus (HIV). (Reproduced with permission from Griffith, B. P., *Yale J. Biol. Med.* **60:** 578 (1988).)

retroviruses have also been isolated in culture and linked to disease in humans, such as human T-cell leukemia/lymphoma virus type 1 (HTLV-1; Poiesz *et al.*, 1980, 1981) and HIV type 2, which can cause an AIDS-like illness (Kanki *et al.*, 1986, 1987; Clavel *et al.*, 1987).

Recently, convenient ELISA tests for detection of HIV antigens (Goudsmit *et al.*, 1986; McDougal *et al.*, 1985) have been developed, but have not been as sensitive as culture since the latter serves to amplify the low titers of infectious virus present in patients' specimens. Although isolation of HIV is currently too tedious and expensive for routine diagnostic use, with anticipated methodologic improvements it will certainly play a larger role in the future.

2.6. Advances in Virus Identification

After preliminary identification of virus isolates by CPE in cell culture, final identification has required labor-intensive neutralization, hemagglutination inhibition or complement fixation tests. Despite the time invested, the results of these tests are not always clear-cut. In addition, with the increasing importance of viral diagnosis in patient care, more rapid specific identification is needed, as mistakes or delays in identification can adversely affect treatment and patient management.

2.6.1. *Monoclonal Antibodies*

A significant advance for diagnostic laboratories has been the development of monoclonal antibodies, allowing the production of reproducible, well-characterized, specific reagents for use in immunologic assays. As a result, fluorescein labelled monoclonal antibodies are now available for the rapid identification of viral isolates, including influenza A and B (Schmidt *et al.*, 1982; Walls *et al.*, 1986), HSV types 1 and 2 (Balachandran *et al.*, 1982; Balkovic and Hsiung, 1985), CMV (Fiacco *et al.*, 1984), and adenovirus (Cepko *et al.*, 1983; August and Warford, 1987), as well as polyclonal fluorescein labelled antisera for adenovirus and RSV (Kim *et al.*, 1983; Cheeseman *et al.*, 1986; Freymuth *et al.*, 1986). Thus, specific identification can now be made within hours of detection of CPE, or even before CPE is detected (see Section 3.1).

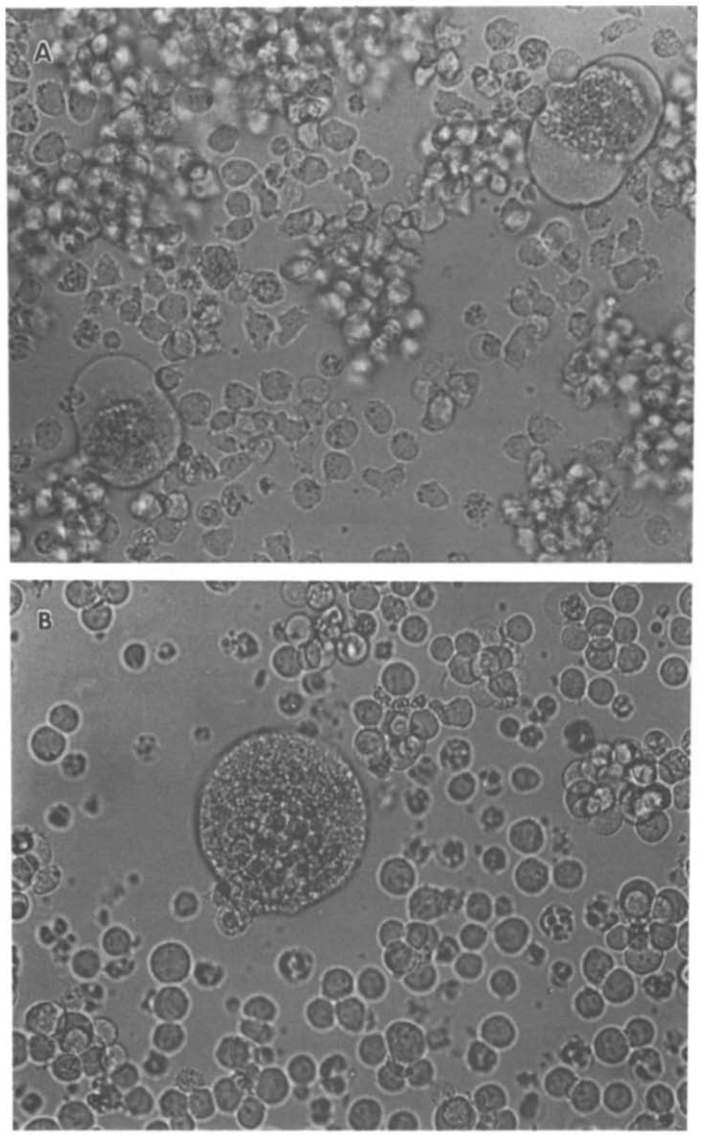

FIG. 4. Cytopathic changes in H9 cells, seven days post-inoculation with HIV (Strain HTLV-III_B). A. Cells with balloon-like formation (×950). B. Multinucleated giant cell (×950). (Reproduced with permission from Griffith, B. P., *Yale J. Biol. Med.* **60:** 579 (1988).)

2.6.2. *RNA Genome Analysis*

In recent years, a number of the tools of molecular geneticists have been used for the identification and fingerprinting of RNA and DNA viruses. Oligonucleotide mapping and polyacrylamide gel electrophoresis of viral proteins have been used to determine genetic epidemiologic relationships between polioviruses and have been useful in determining the relations between cases of paralytic polio and vaccine strains of polio (Minor, 1980; Nottay *et al.*, 1981). Oligonucleotide mapping has also been used to study the evolution of influenza A virus strains in nature (Nakajima *et al.*, 1978; Nakajima *et al.*, 1980; Young and Palese, 1979). Electropherotyping of human rotavirus strains has been used to identify strains involved in disease outbreaks within hospital settings and in different parts of the world (Albert *et al.*, 1982; Chiba *et al.*, 1984; Rodger, *et al.*, 1981; Rodriguez *et al.*, 1983; Spencer *et al.*, 1983). This technique has been useful in confirming the difference in rotavirus strains isolated in China from previously recognized rotavirus strains (Hung *et al.*, 1984).

2.6.3. *Restriction Endonuclease Analysis*

In recent years, restriction enzyme analysis has been used to identify and classify DNA viruses of the herpes-, adeno- and papovavirus groups. By this technique, viral DNA is incubated with a specific endonuclease resulting in cleavage of all susceptible DNA sequences. Then the fragments are separated by gel electrophoresis and a characteristic 'fingerprint' for that virus is obtained (Summers, 1980). The application of restriction endonuclease analysis has been particularly useful in the study of HSV. HSV-1 and HSV-2 can be readily distinguished by this technique and it is considered the gold standard for typing isolates (Mayo *et al.*, 1985b). In addition, strain-specific differences are evident, allowing further subclassification of isolates within an HSV type (Fig. 5). As a

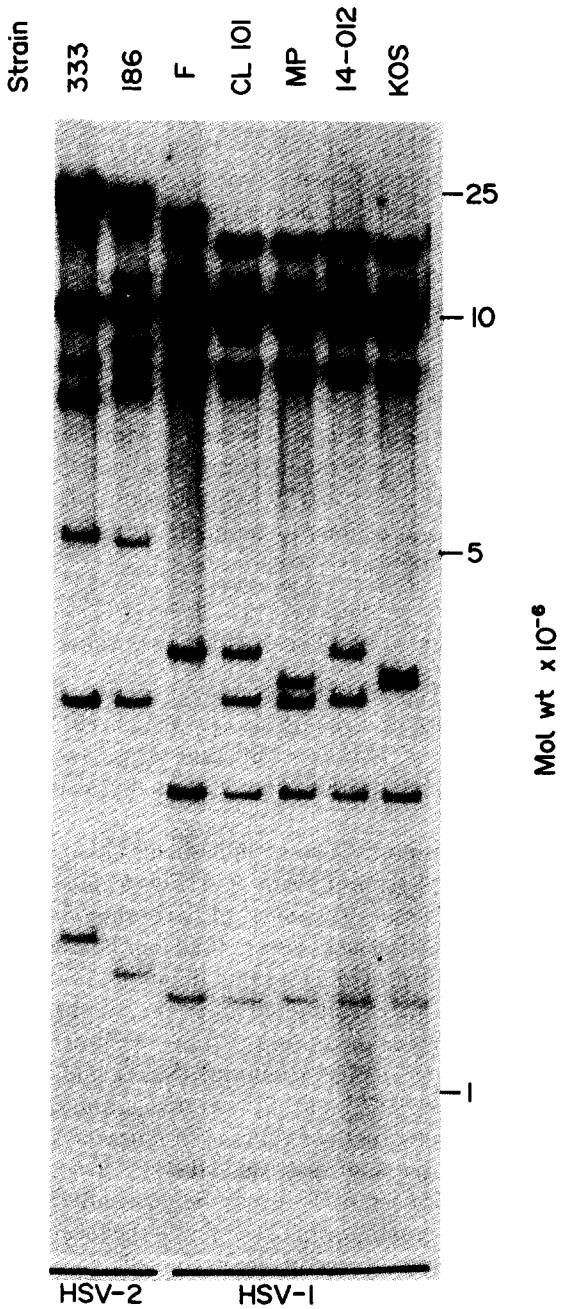

FIG. 5. Autoradiograph of EcoRI digestion products of ^{32}P labeled DNA isolated from five HSV-1 and two HSV-2 strains.

result, restriction endonuclease analysis has proved useful in the typing of HSV-1 and HSV-2 isolates on a large scale (Lonsdale, 1978), in tracing nosocomial outbreaks of HSV (Buchman *et al.*, 1978; Linneman *et al.*, 1978), and in dispelling concern that a clustering of cases of herpes encephalitis was due to circulation of a single neurovirulent strain of virus (Landry *et al.*, 1983). The same methodology has now been applied to tracing sources of CMV infection (Wilfert *et al.*, 1982; Yow *et al.*, 1982; Handsfield *et al.*, 1985), as well as studying the molecular epidemiology of VZV (Martin *et al.*, 1982) and adenoviruses (Kemp *et al.*, 1983; Wadell *et al.*, 1985). Restriction enzyme analysis has also been shown to be more reliable and specific than neutralization and hemagglutination tests for the identification adenoviruses (Fife *et al.*, 1985a,b; Hammond *et al.*, 1985). Thus, restriction endonuclease fingerprinting provides a useful additional method for virus identification.

2.7. Light Microscopy

Direct smears from skin lesions have long been useful in the rapid diagnosis of HSV, VZV and poxvirus infections. HSV and VZV both induce multinucleated cells and characteristic intranuclear inclusions (Cowdry type A), whereas poxvirus infections induce typical cytoplasmic inclusions (Guarnieri bodies) in infected cells. Where no viral culture facilities are available, Pap smears have also been used to detect the presence of HSV infection of the cervix. Characteristic CMV-induced intranuclear inclusions in both Pap smears and infected tissues have been used as markers for diagnosis of CMV infections. For certain virus infections, the cellular changes themselves in the affected organs are sufficiently characteristic to permit a presumptive diagnosis. Examples are the spongiform degeneration in the brains of patients with Creutzfeld–Jacob disease (Gibbs and Gajdusek, 1969) and the balloon degeneration of liver cells seen in viral hepatitis (Ishak, 1976). The recent commercial availability of high-quality immunologic staining reagents and non-radioactively labelled viral probes for *in situ* hybridization has allowed a more specific and sensitive diagnosis of viral infections to be made using tissue sections in a routine pathology laboratory (see Section 3).

2.8. Electron Microscopy and Immune Electron Microscopy

The electron microscope (EM) has been used in the diagnosis of viral diseases for several decades. Only by this method can a virus be directly visualized. Virus size and shape can be easily identified (Figs 6 and 7). However, different viruses with the same morphology cannot be distinguished by routine examination (e.g. smallpox and vaccinia, or HSV and VZV).

The EM techniques most commonly used include the negative staining of virus particles with the electron-dense salts of phosphotungstic acid (Figs 6 and 7, top row) or preparation of ultrathin sections of cells or tissues suspected of harboring virus (Figs 6 and 7, middle row). Clinical specimens or virus-infected culture fluid can be examined directly using the negative staining technique, thus providing a rapid diagnosis of virus infection (Hsiung *et al.*, 1979). However, difficulties are encountered when the number of virus particles in the sample examined is low. A number of techniques have been developed to enhance virus visualization, including the pseudoreplica technique (Smith and Melnick, 1962), agar gel diffusion (Anderson and Doane, 1972) and ultracentrifugation (Smith and Gehle, 1969). Although thin sectioning of tissues usually requires 3 or more days of specimen preparation for EM, a more reliable diagnosis may result since the fine structure of the virus particles and cells is more likely to be preserved. This may be especially important in cases where very few virus particles may be present or in determining the location of the virus particles. The recognition of a human papovavirus in the brain cells of a patient with progressive multifocal leukoencephalopathy (ZuRhein and Chou, 1965) and the identification of Epstein–Barr virus in cultured lymphoblastic cells derived from a Burkitt's lymphoma patient (Epstein *et al.*, 1964) would have been missed had not this EM technique been used.

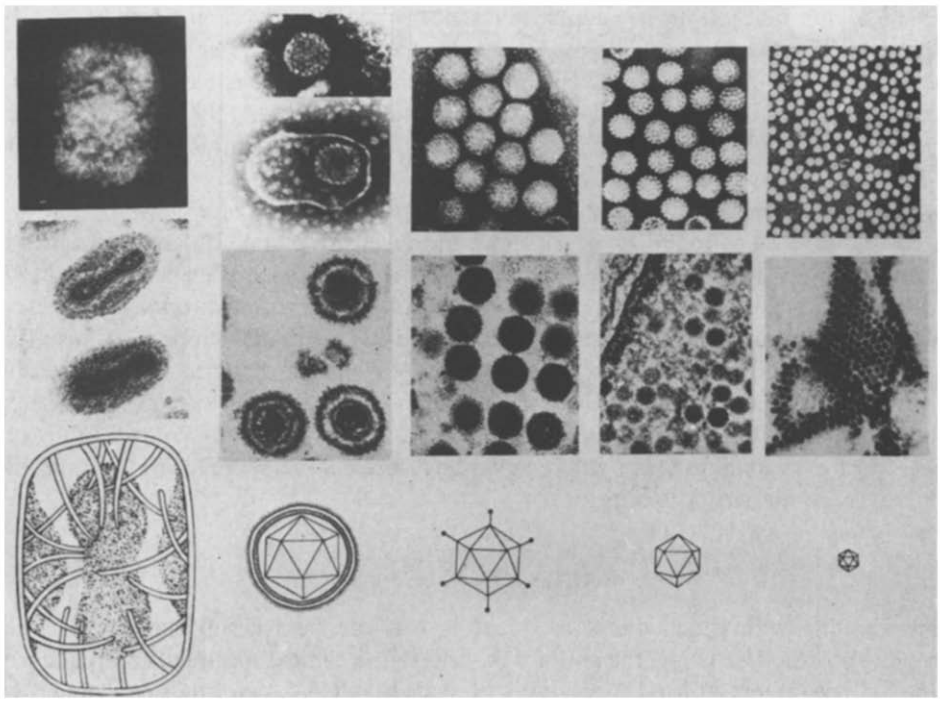

FIG. 6. Relative size and shape of DNA viruses.

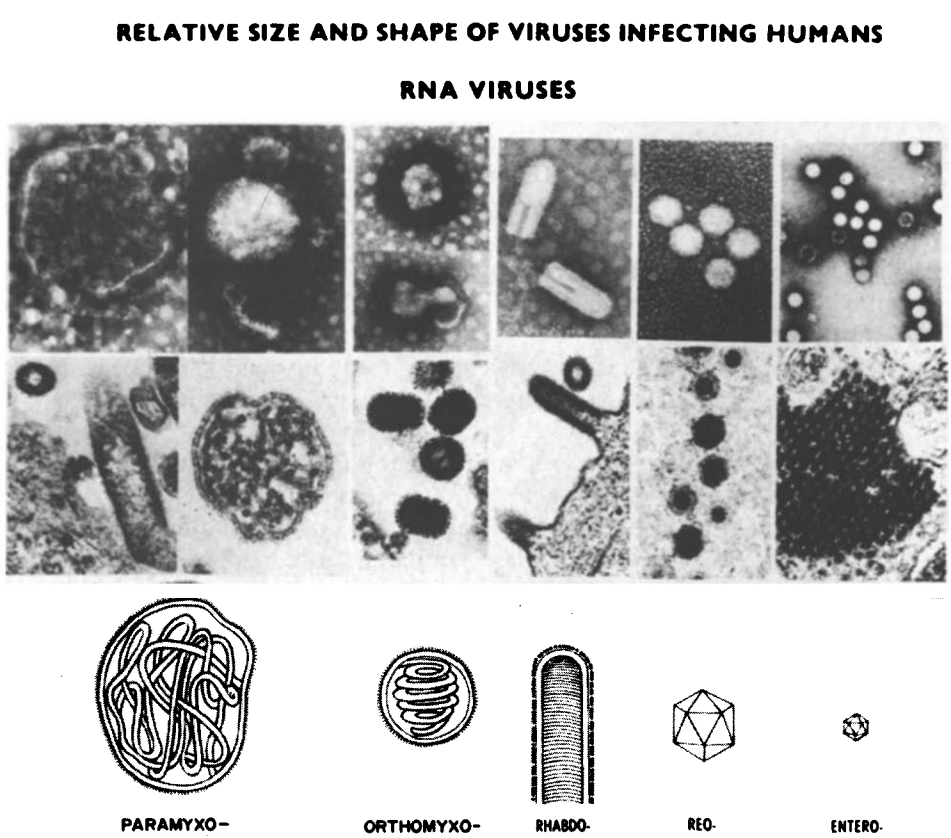

FIG. 7. Relative size and shape of RNA viruses.

In the 1970s, the application of EM techniques uncovered a number of new viruses which could not be isolated in culture. These included hepatitis B virus (Dane *et al.*, 1970), enteric adenoviruses in the stools of children with gastroenteritis (Flewett *et al.*, 1975), and, with the use of immune electron microscopy (IEM), hepatitis A (Feinstone *et al.*, 1973), rotavirus (Flewett *et al.*, 1973) and Norwalk agent (Kapikian *et al.*, 1972) were first visualized in stool contents. IEM, which involves the mixing of the patient's specimen with immune serum resulting in aggregation of viral particles rendering them readily visible, has also been useful in the rapid diagnosis of respiratory viruses in clinical specimens (Doane *et al.*, 1967; Joncas *et al.*, 1969).

Recent innovations have included the development solid-phase IEM by the use of protein A, which was found to be 30 times more sensitive than EM and 10 times more sensitive than ELISA for the detection of rotavirus in stools (Svensson *et al.*, 1983). Another modification is the use of the solid-phase IEM double-antibody technique, by which formvar carbon-coated grids are treated with diluted antibody, resulting in approximately 30-fold increase in virus particles. Viewing of the virus is facilitated by the addition of a second 'decorator' antibody. This has been used with success in the detection of papovaviruses (Giraldo *et al.*, 1982). However, despite the many contributions of EM and IEM to virus diagnosis, it is still too expensive and cumbersome for routine application in the average diagnostic laboratory.

3. RAPID VIRAL DIAGNOSIS

The rapidity with which the isolation of a virus can be accomplished is variable and depends upon the virus type, the amount of virus in the clinical specimen and the sensitivity of the culture system utilized. Certain viruses, such as HSV, can often be isolated within 24 hr of inoculation into cell culture, whereas other viruses require 7 or more days for isolation and some have not been amenable to culture by routinely employed methods. The delay encountered in the diagnosis of many common virus diseases has been a source of frustration to both physicians and laboratory personnel. With the advent of antiviral chemotherapy, this dilemma has become more acute. In order to have a beneficial effect on the outcome of an illness, therapy must be instituted early. This has led to tremendous interest in the development of so-called 'rapid viral diagnostic methods'. The formation of both European and Pan American groups for rapid viral diagnosis with regular symposia to keep members abreast of recent advances in the field is a direct result of this interest (McIntosh *et al.*, 1978, 1980; Richman *et al.*, 1984a,b). Ideally, rapid diagnostic methods should be capable of yielding results within a few hours of a patient's admission to the hospital with testing performed directly on clinical material. However, test results obtained within 1–2 days of admission would render viral diagnostic methods comparable to those routinely used in microbiology laboratories. Such 'rapid' techniques would include those used to identify viral antigens or nucleic acid directly in clinical specimens or after amplification of virus in cell cultures before cellular changes occur or in cases where no changes occur.

Many of the techniques to be discussed in this section have an immunologic basis, i.e. they depend upon the specific reaction between antigen and antibody. The reaction must be labeled with a marker to render it detectable. The marker can be a fluorescent dye, a radioisotope or an enzyme such as peroxidase. An important development leading to the increased utilization of immunologic detection techniques have included the availability of high-quality commercial reagents including monoclonal antibodies.

Another significant and very recent change in the field of rapid viral diagnosis has been the introduction of nucleic acid hybridization technology into the field of clinical virology. Recent advances that have allowed the application of these techniques include: first, molecular cloning, resulting in the production of well-characterized and specific reagents for use as probes; second, the recognition of the ability of nucleic acid to bind to nitrocellulose, which allows screening of large numbers of samples; and, third, the development of non-radioactive biotinylated probes suitable for use in clinical

laboratories. Hybridization techniques in clinical diagnosis remain experimental at this time; however, owing to the tremendous interest that exists in this area and the proliferation of studies published in the last few years, an overview will be presented.

The immunologic and hybridization techniques will be reviewed in terms of their application to both direct detection of viral antigens or genomes in clinical specimens and detection of virus infection after amplification in cell culture. In general, for viruses that replicate well in cell culture, direct detection methods ae less sensitive though more rapid than virus isolation. However, application of these methods to infected cell cultures can significantly shorten the time to reporting positive results and, in addition, confirm the identification of the virus.

It must be emphasized however, that all of the techniques discussed in this section are directed at specific viruses that are 'suspected'. They are not 'open minded', only virus isolation and EM will lead to the discovery of 'unsuspected' or 'new' viral agents.

3.1. Viral Antigen Detection

3.1.1. *Immunofluorescence*

Immunofluorescence (IF) techniques, which include the direct fluorescent antibody (FA) procedure and the indirect fluorescent antibody (IFA) procedure, have long been used in the diagnosis of viral diseases. First introduced in 1941, IF was developed specifically to detect antigens in animal tissues (Coons *et al.*, 1941). By this technique, specific antibody is tagged with a fluorescent dye, allowed to react with the antigen and, after a short incubation, the site of the antigen–antibody reaction can be visualized using a microscope with a u.v. light source.

Direct IF is used to detect antigen by utilizing a specific antibody which is conjugated directly with a fluorescent dye. It is quicker, simpler and exhibits less nonspecific staining than the indirect method. The indirect method utilizes specific antibody that is not conjugated but is allowed to react with the test antigen. Then, conjugated antibody is added which is directed against the animal species from which the primary antibody is made. This test can be used to detect antigen or antibody, and has the advantage of requiring only a single conjugate for the detection of many antigen–antibody reactions provided that all antisera are made in a single species. Although the indirect test is slightly more sensitive, it also gives more nonspecific results.

Many difficulties have been encountered since the introduction of IF techniques, but in recent years many of the problems have been overcome. For example, an adequate number of infected respiratory epithelial cells are essential for respiratory specimens. It is necessary to see labeled intracellular antigen in a distribution (intranuclear or intracytoplasmic) and in the cell type expected for the particular virus. Also, experience is required in distinguishing the nonspecific fluorescence seen with bacteria, fungi and mucus commonly present in respiratory specimens. Proper specimen collection and sample preparation are important in minimizing these problems. For skin lesions, there is little problem in the vesicular stage but once lesions have become crusted, nonspecific fluorescence becomes a problem. In brain biopsies, nonspecific fluorescence is not usually problematic, but in autopsy specimens, if bacterial overgrowth has occurred, again experience is required in distinguishing nonspecific fluorescence (Gardner, 1977). Owing to difficulties in obtaining specific sensitive antisera, it has been difficult to reproduce results outside of the research setting, until now. The availability of quality reagents and the demand for rapid diagnosis have contributed to this change.

IF was first applied to the direct detection of virus in clinical specimens with the identification of influenza A in nasal smears (Liu, 1956). Subsequently, rabies was detected in mouse brains utilizing this technique and quickly became the method of choice for rapid diagnosis of rabies virus infection (Goldwasser and Kissling, 1958). In addition, IF has been used to detect HSV in skin lesions (Biegeleisen *et al.*, 1959). More recently IF has been applied to the detection of a number of viruses including HSV (Schmidt *et al.*, 1980,

1983), VZV (Schmidt *et al.*, 1980), RSV (Bell *et al.*, 1983), and parainfluenza (Wong *et al.*, 1982; Waner *et al.*, 1985) in clinical specimens with varied results. It was also by IF that the delta hepatitis virus (HDV) was detected in liver cell nuclei and in serum of hepatitis B virus (HBV) carriers (Rizzetto *et al.*, 1977). The application of IF using monoclonal antibodies to direct detection of influenza (Shalit *et al.*, 1985) and CMV (Martin and Smith, 1986) in clinical specimens has produced promising results. Perhaps RSV has generated the greatest enthusiasm due to the difficulties encountered with culture and the benefits of rapid diagnosis with the availability of ribavirin treatment (Bell *et al.*, 1983; Lauer, 1982). Numerous investigators have found IF examination of nasopharyngeal aspirates using either polyclonal or monoclonal antibodies more sensitive than culture (Cheeseman *et al.*, 1986; Freymuth *et al.*, 1986; Swenson and Kaplan, 1986).

The advantages of immunofluorescent procedures performed directly on clinical specimens include speed, simplicity, low cost, and the ability to make a diagnosis in convalescence in some viral infections where virus is rendered non-infectious by the presence of antibody but is still visible by fluorescence. The ability to make a diagnosis when specimens have been delayed in their arrival in the laboratory is a great advantage. However, IF is highly dependent on proper collection of specimens. Even under study conditions, a significant percentage of specimens are unacceptable due to inadequate numbers of epithelial cells, which makes the specimen untestable.

IF techniques were also first used years ago for the rapid detection and identification of viruses after amplification in cell cultures. Examples include the rapid detection and identification of measles (Cohen *et al.*, 1955), VZV (Weller and Coons, 1954) and poliovirus (Kalter *et al.*, 1959) and subsequently rubella (Schmidt *et al.*, 1966). For this application, there have been a number of exciting and potentially useful innovations within the last two or three years. One group has used centrifugation of specimens onto monolayers in shell vials, followed by application of IF at 36 hr (Gleaves *et al.*, 1984) and then 16 hr post inoculation (Gleaves *et al.*, 1985a) for the rapid detection of CMV in urine. All CMV isolates were detected by IF at 36 or 16 hr respectively whereas an average of 9 days was required for detection of CMV CPE using standard virus isolation without centrifugation or IF staining. When BAL and blood specimens are tested for CMV by this technique, some false negative results are obtained (Paya *et al.*, 1987). It is also important to inoculate two or, for blood samples, three shell vials per specimen for optimal results (Paya *et al.*, 1988). The same methodology was applied to the early detection of HSV with excellent results (Gleaves *et al.*, 1985b). Centrifugation was shown to be important in early detection. However, when this same methodology was applied to rapid detection of influenza virus using monoclonal antibodies, only 56% of influenza isolates were detected at 24 hr post inoculation by IF, compared with an average of 4 days for conventional isolation (Espy *et al.*, 1986). Another study compared short term (24 hr) tissue culture followed by IF with standard virus isolation and found complete agreement between the two methods. However, when the same reagents were applied directly to clinical specimens, both false-negative and false-positive results were obtained (Nerurkar *et al.*, 1984a).

3.1.2. *Immunoperoxidase*

Immunoperoxidase (IP) techniques follow the same principles as IF techniques, however, the conjugate is an enzyme, most often horseradish peroxidase. The enzyme is coupled to specific antibody in the direct method, and to an antianimal species globulin in the indirect test. The presence of the enzyme conjugate bound to the virus-infected cells is detected by adding a substrate, diaminobenzidine or aminoethylcarbazole, then oxidizing it in the presence of hydrogen peroxide resulting in a reddish-brown color which is permanent. The test has the same potential applications as IF and it has a number of advantages over IF: the reaction can be detected with the naked eye or with a light microscope, which is important for laboratories with limited budgets; many of the products are electron dense and thus can be visualized with the electron microscope; most preparations are permanent; the reagents are more readily standardized and are more

stable; there are less nonspecific reactions; and IP has been more successful than IF on processed tissue. However, this procedure was first described in the early 1970s (Avrameas and Ternynck, 1971) and experience with it is much less extensive than with IF. A major problem has been the endogenous peroxidase present in leukocytes in clinical specimens, especially from the respiratory tract, which leads to nonspecific staining. Techniques have been developed to remove the endogenous peroxidase (Straus, 1971; Weir *et al.*, 1977), but they can also result in removal of unstable virus antigen, and if there is only a small amount of virus present, a false-negative result can be obtained.

The application of IP techniques to clinical material includes the identification of rabies (Atanasiu, 1975), HSV in a variety of clinical specimens (Morisset *et al.*, 1974; Schmidt *et al.*, 1983) including brain tissue (Benjamin and Ray, 1975), measles in the brains of patients with SSPE (Brown and Thormar, 1976), and hepatitis B in fixed liver sections (Burns, 1975). It has also been compared to IF for the detection of influenza A and respiratory syncytial virus (RSV) in respiratory specimens (Gardner *et al.*, 1978). The two techniques were in excellent agreement, but removal of endogenous peroxidase was a significant problem in specimens containing RSV, where removal of peroxidase resulted in loss of RSV antigen. Recent modifications that have resulted in more sensitive assays include the peroxidase–anti-peroxidase (PAP) (Sternberger and Joseph, 1979) and avidin–biotin complex (ABC) techniques (Hsu *et al.*, 1981).

IP methods were used early on to detect viral antigen in cell culture to obtain a more rapid diagnosis (Benjamin and Ray, 1974) and it is for this purpose that it has received much wider application recently. IP has been used to identify rubella isolates in cell culture (Schmidt *et al.*, 1981). More importantly, commercial kits for HSV cultivation and identification have been developed using Vero cell culture, followed in 48 hr by staining with the PAP technique. Although these kits provide a valuable introduction to virus isolation for those laboratories without virology expertise (see Fig. 8), numerous studies have not found them to be as sensitive as standard virus isolation. The sensitivity of the kits has ranged from 73 to 79% when compared with standard tissue culture (Fayram *et al.*, 1983; Hayden *et al.*, 1983; Rubin and Rogers, 1984; Sewell *et al.*, 1984). However the problem may well lie in the kits' use of Vero cells which are fairly insensitive to HSV

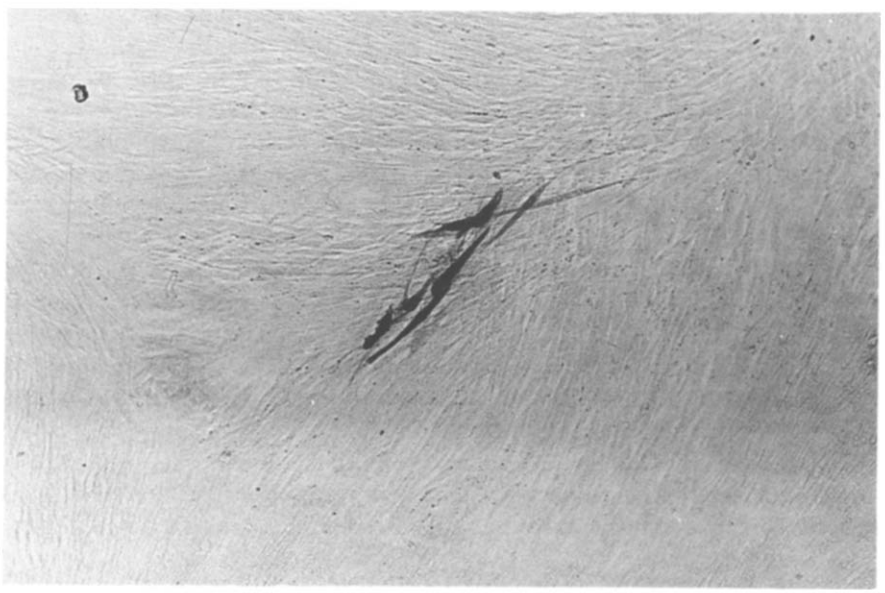

FIG. 8. Detection of herpes simplex virus (HSV) infected human diploid fibroblast (HDF) cells by immunoperoxidase staining. HSV infected HDF cells fixed and stained with the avidin–biotin complex (ABC) immunoperoxidase technique revealing a focus of darkly stained HSV infected cells (24 hr after inoculation).

infection when compared with more widely used HDF or primary RK cells. When other workers used HDF cell culture followed by IP staining at 24 hr, all HSV isolates were detected at 24 hr by IP staining that were eventually detected by standard culture (Miller and Howell, 1983). An additional study demonstrated that it is possible to significantly shorten the time involved in maintaining and observing cell cultures by application of the PAP technique for early detection of HSV in HDF cell culture. Over 16,000 specimens were processed for HSV; essentially all cultures positive for HSV were detected by 72 hr (two-thirds by 24 hr) by PAP staining, resulting in significant savings in time and materials (Mayo *et al.*, 1985a). The combination of centrifugation of specimens onto cell monolayers followed by overnight incubation and IP staining was found to be more sensitive as well as more rapid than standard cell culture for diagnosis of HSV (Salmon *et al.*, 1986). Thus this technique has much potential in rapid viral diagnosis, especially for laboratories without a fluorescence microscope.

3.1.3. *Enzyme-Linked Immunosorbent Assay*

In 1971, Engvall and Perlmann introduced the enzyme-linked immunosorbent assay (ELISA) for the quantitation of rabbit IgG (Engvall and Perlmann, 1971), a technique as sensitive as the radioimmunoassay (RIA), but with many advantages over the RIA. ELISA is similar to RIA except that an enzyme is used as the immunoglobulin marker instead of a radioactive isotope. When substrate is added to the enzyme-labelled immunoglobulin, a visible color reaction occurs which can be read visually or quantitated using a spectrophotometer. The ELISA can be used either for detection of antigen or antibody and has several variations modelled after the RIA. For detection of antigen, either the antibody sandwich or the competitive assay can be used. In the antibody sandwich method, specific antibody to the antigen to be detected is used to coat the surface of a solid phase support (such as polystyrene beads, microtiter plates, test tubes, etc.). Then the test sample (e.g. stool, body fluid) is added and allowed to react. For the direct or single antibody sandwich test, enzyme conjugated to specific antibody is then added and allowed to react. For the indirect or double-antibody sandwich test, unlabelled specific antibody is first added, then enzyme conjugated antiglobulin is added. As a final step, the amount of enzyme bound is detected by the addition of a substrate. The intensity of the subsequent color reaction is proportional to the amount of antigen in the test sample. In the competitive assay, specific antibody is adsorbed to the solid phase and the test specimen is added as above, in addition to a known amount of labeled antigen. The unlabeled antigen in the test specimen competes with the labeled antigen for antibody binding sites. Then substrate is added. The bound enzyme, and resultant color change, is less if antigen is contained in the material. The amount of antigen in the test sample is determined quantitatively by comparing the color obtained to known standards. The two enzymes most widely used in ELISA are horseradish peroxidase (Avrameas and Ternynck, 1971) and alkaline phosphatase (Engvall and Perlmann, 1971), but a number of others have also been used, each with advantages and disadvantages (Avrameas *et al.*, 1978; Hosli *et al.*, 1978; Watanabe *et al.*, 1979). The problems in ELISA are similar to those in other immunologic tests. The purity, the sensitivity and specificity of the reagents must be carefully controlled. Nonspecific binding is a problem that can be diminished by careful washing, addition of 1–4% species specific serum to the reaction mixture, and the use of high quality specific reagents. The introduction of monoclonal antibodies should also reduce this problem. In addition, the optimal conditions for the assay vary depending on the antigen, enzyme, substrate etc., and must be carefully monitored. Because of the variables, a number of control specimens with known amounts of antigen should always be included in every test.

Since its introduction, the ELISA has been used for the detection of a variety of antigens, antibodies and other biologic substances (Yolken, 1980). It has been widely applied to viral antibody detection with great success, most notably hepatitis B virus, for which it has supplanted the RIA, and human immunodeficiency virus (HIV). The ELISA has also been

used for the detection of viral antigens of viruses which are difficult to propagate in culture, such as a group A coxsackieviruses (Yolken and Torsch, 1980), human coronaviruses (Macnaughton *et al.*, 1983), enteric adenoviruses (Anderson *et al.*, 1983), Norwalk agent (Gary *et al.*, 1985) and hepatitis A (Mathieson *et al.*, 1977; Coulepis *et al.*, 1985). ELISA, for detection of these viruses, remains a research tool, since there has not been sufficient demand for these tests in clinical laboratories.

To date, ELISA has been especially useful in the diagnosis of rotavirus infections (Yolken *et al.*, 1977). ELISA kits for rotavirus antigen detection have been available commercially for a number of years now and have been found comparable to EM (Cheung *et al.*, 1982; Rubenstein and Miller, 1982). Recent modifications have resulted in an even more sensitive rotavirus ELISA kit (Doern *et al.*, 1986). However, group B rotaviruses recently detected in China (Hung *et al.*, 1984) are not detected by the current commercial ELISA kits.

Hepatitis B virus has not yet been propagated in cell culture which limits laboratory methods to serologic detection of HBV antibodies and antigens and more recently, hybridization for detection of viral DNA. Tests for at least six serologic markers for HBV are available commercially. Determining the pattern of these markers in the individual patient will help to establish the stage of the disease, the infectivity, immune status and prognosis of the patient. The application and interpretation of these tests has been reviewed in detail elsewhere (Chernesky *et al.*, 1984). The ELISA has also been applied to detection of delta virus antigen and antibody in serum (Crivelli *et al.*, 1981; Shattock and Morgan, 1983; Buti *et al.*, 1986), which should result in less need for diagnostic liver biopsy in these patients.

ELISA has also been used to detect a number of routinely cultured viruses in clinical specimens such as RSV (Hornsleth *et al.*, 1982; McIntosh *et al.*, 1982; Freymuth *et al.*, 1986; Swenson and Kaplan, 1986), influenza A, adenovirus (Harmon and Pawlick, 1982), HSV in lesion swabs (Morgan and Smith, 1984; Nerurkar *et al.*, 1984b; Warford *et al.*, 1984) and HSV in cerebrospinal fluid of patients with encephalitis (Coleman *et al.*, 1983). When used for direct detection of HSV in clinical specimens, ELISA was not sufficiently sensitive when compared to cell culture results (Sewell and Horn, 1985). However, when applied to HSV infected cell lysates, results were significantly improved (Morgan and Smith, 1984). ELISA could prove useful for the rapid and early identification of HSV when large numbers of cultures are processed. The most recent innovation has been an HSV ELISA spin amplification technique, in which samples are centrifuged onto monolayers and incubated for 2 days. The cell cultures are then lysed and assayed by ELISA for HSV antigen. This test was found to be highly sensitive and specific (Michalski *et al.*, 1986).

A significant recent application has been the development of ELISAs to detect the core protein (p24) of the AIDS virus, HIV (Higgins *et al.*, 1986; McDougal *et al.*, 1985). Although current techniques for the isolation of HIV are more sensitive than antigen detection, they are highly specialized and beyond the capabilities of a routine viral diagnostic laboratory. The ELISA has been used to detect HIV core antigen in serum and cerebrospinal fluid (Goudsmit *et al.*, 1986; Allain *et al.*, 1986). The presence of HIV antigen in blood has been found as early as two weeks after infection (Lange *et al.*, 1986), whereas development of HIV antibodies may require six months or more. Antigenemia, with a decline in HIV core antibodies, has also been found to precede the onset of AIDS (Lange *et al.*, 1986; Paul *et al.*, 1987). Direct detection of viral antigen also is useful in following patients on antiviral therapy, where a decline in core antigen in serum has been demonstrated in patients receiving azidothymidine (AZT) (Chaisson *et al.*, 1986). The availability of ELISA for detection of HIV antigen, therefore, could provide a useful additional diagnostic test for AIDS virus infections.

The advantages of ELISA include low cost, less specialized equipment, stability of reagents, avoidance of use of hazardous radioisotopes, wide applicability, and the ability to automate the test or read it visually. Its greatest potential is for the testing of large numbers of specimens for the same virus.

3.1.4. *Radioimmunoassay*

RIA was developed in 1960 and first applied to the detection of insulin levels in plasma (Yalow and Berson, 1960). Since that time RIA has been utilized to detect a wide variety of biologic substances in clinical chemistry laboratories. It combines the high sensitivity of radioisotope labelling with the specificity and broad applicability of the antigen–antibody reaction. In addition, large numbers of specimens are readily tested. The sensitivity and specificity also depend upon the quality of the reagents before and after labeling and adherence to rigid test procedures. Both a direct and indirect assay can be used, as in IF, IP and ELISA.

RIA has been utilized in the detection of hepatitis B antibody since 1971 (Lander *et al.*, 1971) and hepatitis B antigen since 1972 (Ling and Overby, 1972). However, in many laboratories, it has now been replaced by ELISA. Besides hepatitis B, RIA has been used to detect viral antigens in infected cells, generally in cell culture (Hayashi *et al.*, 1972, 1973; Joseph *et al.*, 1976; Laush *et al.*, 1974), but also in clinical specimens (Forghani *et al.*, 1974, 1978; Halonen *et al.*, 1980), and to detect viral antibody (Rosenthal *et al.*, 1972). The localization of antigen within cells is not possible by this method. RIA has less nonspecific reactivity than the enzymatic methods and its sensitivity could be useful in detecting small amounts of antigen in clinical specimens. However, it has the disadvantage of the deterioration of radioactive isotopes, requiring new reagents and standardization every few months, the hazards associated with the use of radioisotopes, and the expensive equipment required which limits its use to large centers. Owing to increasing concerns about the potential hazards to personnel, the disposal problems associated with radioactive isotopes, and the availability of alternatives of equal sensitivity, utilization of RIA can be expected to decrease.

3.1.5. *Latex agglutination*

In the past few years, the use of the simple latex agglutination test for the detection of rotavirus has been reported (Cevenini *et al.*, 1983; Haikala *et al.*, 1983). The sensitivity and specificity compare favorably with ELISA (Hughes *et al.*, 1984; Sambourg *et al.*, 1985; Doern *et al.*, 1986). By this technique, latex beads are sensitized to a specific antigen by incubation with immune serum or specific IgG. In the case of rotavirus, the test is performed by mixing clarified stool suspensions with the sensitized latex beads, than after a short incubation, examining macroscopically for clumping (agglutination) of the latex beads. Clumping should occur if the rotavirus antigen is present in the stool. The test is not sensitive for detection of small amounts of antigen, but during rotavirus gastroenteritis large quantities of antigen are usually excreted. This test has several potential advantages: it can be performed by unskilled personnel, it is rapid, relatively cheap and may prove useful for screening in doctors' offices or developing countries.

Latex agglutination has also been applied recently to detection of HSV in clinical specimens but it was not found to be sensitive. However, it was very sensitive and specific for positive identification of HSV after the appearance of viral CPE in cell culture (Ignotofsky *et al.*, 1985).

3.2. VIRAL GENOME DETECTION

Nucleic acid hybridization techniques have only recently been introduced into the field of clinical virology and to date they have been applied to studies of viral pathogenesis and to rapid viral diagnosis using clinical specimens (Landry and Fong, 1985). The principle of hybridization is simple. In its natural state, the DNA molecule is made up of two strands with each base specifically linked by hydrogen bonds to a complementary base on the other strand. The bonds between the bases can be broken by heating, or treatment with alkali, so that the two strands of DNA are dissociated from each other (denatured). However, under proper conditions, the dissociated strands will reassociate with complementary

partners. Under test conditions, a labeled single-stranded nucleic acid probe containing the specific sequences being sought is mixed with denatured (dissociated) sample DNA or RNA. If complementary nucleic acid sequences are present in the sample, labeled probe will reanneal with these sequences forming double-stranded 'hybrids' which now contain label. The labeled hybrids can be detected by a variety of methods and quantitated. Current techniques largely involve the hybridization of labeled probe to nucleic acid immobilized on a solid support, such as nitrocellulose. The technique most widely used in research, including studies of viral pathogenesis, has been the Southern blot. By this method, purified DNA samples are first cleaved with restriction endonucleases, the fragments separated by gel electrophoresis and then the DNA is transferred out of the gel and onto a nitrocellulose filter by the method of E. M. Southern (Southern, 1975). The nitrocellulose is then immersed in a hybridization solution containing labeled probe. After adequate time has elapsed for reannealing to occur, the nitrocellulose filter is removed from the solution and subjected to a series of washes, which can vary in stringency, to remove untreated probe and unstable hybrids. The binding of the labeled probe is confined to distinct bands, corresponding to nucleic acid fragments separated by electrophoresis; therefore it is possible to identify even weak signals as specific.

For detection of viral nucleic acid in clinical specimens, the most widely used technique to date has been the spot or dot–blot. By this method, nucleic acid or cell suspensions are spotted directly onto nitrocellulose filters, in a grid pattern, with or without suction filtration. The obvious advantages are the speed and simplicity (avoiding restriction enzyme analysis, gel electrophoresis and DNA transfer) and it does not require the laborious extraction and purification of DNA that is necessary for the Southern blot. In addition, large numbers of specimens can be processed simultaneously. However, since visually, only a spot is identified, it is of utmost importance to guard against non-specific results. False positive results in spot hybridizations have been reported due to reactions of residual bacterial plasmid vector sequences in the probe with patients' samples (Diegutis *et al.*, 1986). Careful attention to stringency of conditions, probe specificity, and positive and negative controls is essential. Spot hybridization has been used to detect a number of viruses in clinical specimens. When applied to detection of less readily isolated viruses, such as VZV, spot hybridization had a greater sensitivity than culture (Seidlin *et al.*, 1984). For CMV, the time to detection was greatly shortened, but 10^3–10^5 tissue culture infectious doses (50%) ($TCID_{50}$) per ml were necessary for a positive result (Chou and Merigan, 1982). In another study spot hybridization was found to be more sensitive than culture for detection of CMV in buffy coats (Spector *et al.*, 1984). When applied to viruses not routinely isolated, such as rotavirus (Flores *et al.*, 1983), enteric adenoviruses (Stalhandske *et al.*, 1983, 1985; Takiff *et al.*, 1985), parvoviruses (Clewley, 1985; Anderson *et al.*, 1985); papovaviruses (Gibson *et al.*, 1985; Wickenden *et al.*, 1985) and Epstein–Barr virus (Andiman *et al.*, 1983), spot hybridization could prove useful. Detection of HBV-DNA in serum by spot hybridization correlates with active virus replication (Carloni *et al.*, 1987). HBV-DNA has been detected in the absence of other serologic markers for HBV infection (Brechot *et al.*, 1985) and thus provides a new diagnostic tool that may be useful in prognosis and therapy (Bonino *et al.*, 1981; Hadziyannis *et al.*, 1983; Bonino, 1986). However, this technique was not found to be sensitive for the direct detection of viruses readily isolated in culture, such as enteroviruses (Hyypia *et al.*, 1984) and HSV (Redfield *et al.*, 1983).

A recently reported modification is the 'sandwich hybridization', which is based on the use of two separate nucleic acid fragments, one of which is attached to the filter and the other is labeled. The nucleic acid sequences of both fragments are complementary to that of the nucleic acid sought in the sample, but the two reagents have no sequences in common and therefore do not hybridize to each other. Thus a positive sample attaches to the reagent bound to the filter and then results in a three component DNA 'sandwich' by mediating the attachment of the labeled probe to the filter. Since the sample is kept in solution throughout the process, as opposed to being spotted onto the filter, components contained in crude samples, such as lipids, mucopolysaccharides, proteins etc., which can

non-specifically bind nucleic acids, are not fixed to the filter. This allows the processing of crude samples and the assay of either RNA or DNA, but the sandwich method has not been as sensitive as spot hybridization (Ranki *et al.*, 1983; Virtanen *et al.*, 1984). In addition ^{32}P or ^{125}I were used as labels in most reports to date which is a disadvantage for a clinical laboratory.

Biotinylated probes have now been used for spot hybridization (Hyypia, 1985) and *in situ* hybridization, in which intact cells, such as paraffin embedded tissues, frozen tissues or touch preps are examined for viral genomes (Brigati *et al.*, 1983; Forghani *et al.*, 1985; Beckmann *et al.*, 1985). When used for detection of CMV in lung tissue, *in situ* hybridization was found to be similar in sensitivity to culture and IF with monoclonal antibody and more sensitive than routine histology (Myerson *et al.*, 1984a; Myerson *et al.*, 1984b) (Fig. 9). *In situ* hybridization has proven useful in the detection of human papillomavirus (HPV) in genital tract tissues. HPV has not yet been propagated in cell culture, but over 40 types have been identified by restriction enzyme analysis and hybridization studies. Certain types, such as types 6 and 11, are commonly associated with genital warts, but are rarely associated with cervical cancer, whereas genital infection with other types, such as types 16 and 18, are considered high risk for progression to malignancy (Campion *et al.*, 1986; Crum *et al.*, 1984). One recent report on detection of HPV infection in clinical specimens, found *in situ* hybridization with radiolabeled probe inferior to Southern blot and spot hybridization (Caussy *et al.*, 1988). Yet others have found *in situ* techniques with biotinylated probes highly sensitive (Beckmann *et al.*, 1985). Biotinylated DNA probes are now available commercially to distinguish infection with types 6 and 11, from infection with 'high risk' type 16. This should have an impact on management of patients with cervical dysplasia. *In situ* hybridization has the advantage that histology can be evaluated at the same time, it gives information about the localization of sequences within a tissue and what cell type is infected, and it can be more sensitive if only a few sequences are present but are concentrated in one area. However, procedures are labor intensive and sampling can be a problem.

In addition to direct detection of viruses in clinical specimens, recent studies have also applied spot hybridization with radioactive probes to the detection of HSV (Stalhandske

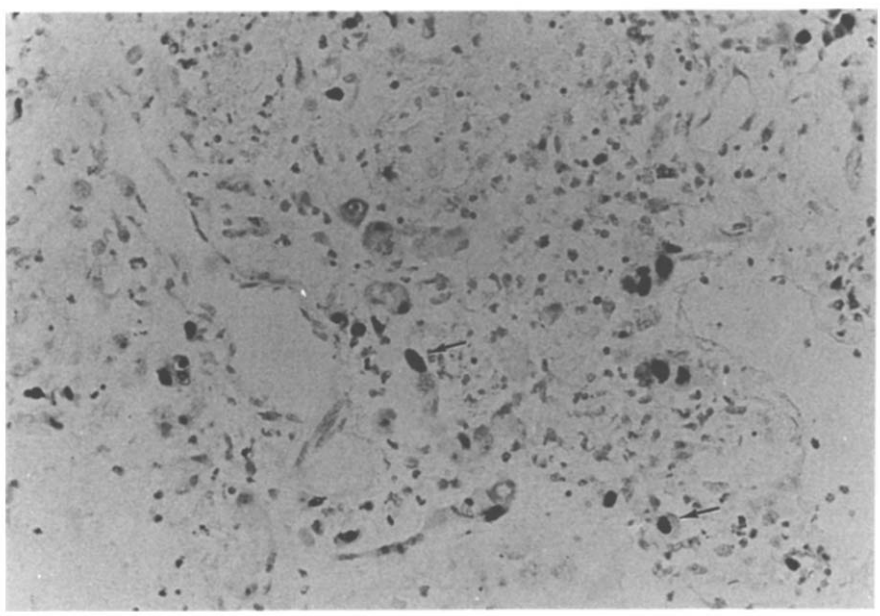

FIG. 9. Detection of cytomegalovirus (CMV) infected cells in lung tissue using *in situ* hybridization with a biotinylated CMV DNA probe. *In situ* hybridization was performed using a biotinylated CMV DNA probe (Myerson *et al.*, 1984a) and formalin fixed, paraffin embedded lung tissue from a bone marrow transplant patient with pneumonia. CMV infected cells were rendered readily visible by dark nuclear and cytoplasmic staining. (Photograph courtesy of Dr D. Myerson.)

and Petterson, 1982) and enteroviruses (Rotbart *et al.*, 1984) in cell culture lysates. An infectivity titer for enteroviruses of 10^6–10^7 $TCID_{50}$ per ml in the lysate was necessary for positive results. When *in situ* hybridization with a biotinylated cloned DNA probe was compared with avidin-biotin IP staining for detection of HSV infected cells in two different cell systems, IP staining was found to be more sensitive (Landry *et al.*, 1986). Significantly, when a highly sensitive cell system was used, CPE alone was comparable in rapidity and sensitivity to viral antigen or DNA detection methods applied in a less sensitive cell system.

4. ANTIVIRAL AGENTS

As knowledge of the biology and biochemistry of viral functions increases, the potential for the discovery of new specific antiviral agents increases accordingly. The current need for accurate, reliable diagnosis of viral infections is to a great extent the result of the discovery and availability of new antiviral agents. Although it is beyond the scope of this review to present a comprehensive report of antiviral chemotherapy, several of the currently available antiviral agents and some of the most promising new antivirals will be discussed.

4.1. Drugs Currently Available

4.1.1. *Amantadine*

The precise mechanism of action of this compound is not clear although early events of virus penetration and uncoating are almost certainly involved. *In vitro*, several viruses are sensitive to the antiviral activity of amantadine, a cyclic primary amine, but influenza type A is particularly sensitive. Inhibition of influenza A virus replication occurs with 25 μg/ml or less. One study using a plaque reduction assay, reported that most clinical isolates were sensitive to 0.4 μg/ml or less (LaMontagne and Galasso, 1978). Early animal studies demonstrated the effectiveness of amantadine in protection of animals from influenza A virus infection. Doses of 0.6–40 mg/kg protected mice against subsequent influenza A challenge. Protection was observed when the drug was started as late as 72 hr after infection but no protection was afforded when administered after 72 hr (Davies *et al.*, 1964). Amantadine is considered effective for both prophylactic and therapeutic use in humans against all strains of influenza A viruses. Studies have demonstrated that amantadine was approximately 70% effective in preventing influenza and was also effective in treating the disease (LaMontagne and Galasso, 1978). Signs and symptoms of disease disappeared more rapidly in patients receiving drug when compared with a placebo group. There was also a decrease in duration and quantity of virus shedding in the treatment group. Side-effects, primarily central nervous system symptoms, occurred in 2–5% of patients. More recent studies again have demonstrated the effectiveness of amantadine prophylaxis of influenza A (Pettersson *et al.*, 1980; Younkin *et al.*, 1983) and it is recommended particularly for unvaccinated persons at high risk.

4.1.2. *Iododeoxyuridine*

5-Iodo-2′-deoxyuridine (IDU) is incorporated into viral DNA in place of thymidine resulting in essentially nonfunctional viral DNA. The nucleotide of IDU may also interfere with various enzyme systems involved in viral DNA synthesis. This mechanism of action is similar to that of other halogenated deoxypyrimidine nucleosides such as bromodeoxyuridine and fluorodeoxyuridine (DeClercq and Torrence, 1978). Concentrations of IDU which inhibit replication of vaccinia virus by 95% (2.8 μM) have no effect on noninfected cells (Prusoff and Goz, 1975).

The antiherpetic effect of IDU *in vivo* was demonstrated in rabbits soon after the discovery of the effects in cell culture (Kaufman, 1962). Controlled studies in humans

followed quickly and confirmed that IDU was effective in treating herpes keratoconjunctivitis (Kaufman *et al.*, 1962; Burns, 1963; Laibson and Leopold, 1964). Toxicity or allergic reactions may occur with prolonged use of IDU and alternative therapy may therefore be necessary (McGill *et al.*, 1974; Amon *et al.*, 1975). IDU-resistant HSV strains can occur experimentally (Underwood *et al.*, 1965) and such resistant mutants have been isolated from patients (Hirano *et al.*, 1979). IDU was the first effective antiherpetic drug approved for human use; however, it is too toxic for systemic administration and is not effective topically on skin or mucous membranes.

4.1.3. *Trifluorothymidine*

5-Trifluoromethyl-2′-deoxyuridine (TFT) exerts the highest antiviral activity of any of the fluorinated pyrimidines (Heidelberger, 1975). Its mechanism of action (Kalman, 1975) is similar but not identical to that of other pyrimidine nucleoside analogs (see above).

TFT specifically inhibits herpesvirus replication *in vitro* (Umeda and Heidelberger, 1969) and has been shown to be effective in treatment of herpes simplex virus and vaccinia virus keratitis in rabbits (Kaufman and Heidelberger, 1964). In clinical trials of TFT treatment of herpes keratitis, it has been shown to be at least as effective as IDU or adenine arabinoside (ara-A) and its use has been associated with fewer side-effects. One trial has shown TFT to be more effective than IDU (Pavan-Langston and Foster, 1977). Another trial compared TFT to ara-A in the treatment of herpetic ameboid ulcers and found that healing of TFT-treated ulcers was slightly more rapid than that of ara-A-treated ulcers (Coster *et al.*, 1979). However, TFT is also too toxic for systemic administration and, like IDU, its use is limited to eye infections.

4.1.4. *Adenine Arabinoside*

The primary mechanism of action of adenine arabinoside (9-B-D-arabinofuranosyladenine, ara-A or vidarabine) is inhibition of DNA synthesis by inhibition of virus DNA polymerase and incorporation into viral DNA. Both cellular and viral DNA inhibition occurs but inhibition of cellular DNA synthesis is less marked (Muller *et al.*, 1977). In cell cultures, vidarabine exhibits a broad range of antiviral activity against DNA viruses including HSV 1 and 2, VZV, human CMV as well as other animal herpesviruses and poxviruses (Shannon, 1975). Topical vidarabine therapy is effective in treating HSV keratitis (see above), but more important is its use in treatment of systemic diseases. An early study demonstrated the efficacy of treatment of HSV encephalitis in mice (Sloan *et al.*, 1968) and a similar more recent study found decreased titers of HSV in the brain and prolonged survival of vidarabine-treated mice (Griffith *et al.*, 1975). Topical treatment of mice inoculated cutaneously with HSV reduced mortality and decreased establishment of latency in sensory ganglia of vidarabine-treated mice if treatment was begun soon after infection (Klein and Freidman-Kien, 1977). Vidarabine had only a minimal effect on CMV in a murine model (Overall *et al.*, 1976) and resulted in decreased urinary excretion in a human study, but no clinical improvement was apparent (Ch'ien *et al.*, 1974). Treatment of VZV infections in man with vidarabine has demonstrated some antiviral effect (Walden *et al.*, 1977). Some of the most encouraging results utilizing vidarabine have come from the study of HSV encephalitis victims. In 1977, the results of a collaborative encephalitis study demonstrated the efficacy of the drug. Mortality due to biopsy-proven HSV encephalitis was 70% whereas treatment with vidarabine reduced it to 28% (Whitley *et al.*, 1977).

A follow-up study has confirmed the original observations and established that age and level of consciousness at the start of therapy are two important factors that influence outcome (Whitley *et al.*, 1981). A beneficial effect of vidarabine treatment on neonatal HSV infection has been reported. It was also suggested that very early institution of therapy might improve outcome of the disease (Whitley *et al.*, 1980a), but increasing

the dose of drug did not further decrease morbidity or mortality (Whitley *et al.*, 1983). Thus, vidarabine was the first drug approved for systemic use in serious herpesvirus infections. However, it is not absorbed well after topical administration.

4.1.5. *Acyclovir*

Acyclovir (ACV), also known as acycloguanosine or 9-(2-hydroxyethoxy-methyl)-guanine, is phosphorylated in herpesvirus-infected cells by a virus-coded enzyme, thymidine kinase (TK). The resulting ACV monophosphate is further phosphorylated by cellular kinases to ACV triphosphate. ACV triphosphate is a competitive inhibitor of viral DNA polymerase and may further inhibit viral DNA synthesis by being incorporated into the DNA thereby causing termination of the DNA chain (Elion *et al.*, 1977). *In vitro*, ACV inhibits HSV 1 and 2, varicella-zoster and Epstein–Barr viruses. Human CMV has been reported to be sensitive to high levels of ACV *in vitro* but clinical isolates are usually resistant at levels of drug attainable in patients (Crumpacker *et al.*, 1979).

Animal HSV experiments using rabbits (Pavan-Langston *et al.*, 1978), mice (Mayo *et al.*, 1979), hairless mice (Klein *et al.*, 1979) and guinea-pigs (Landry *et al.*, 1982a) demonstrated the effectiveness and low toxicity of ACV. Human trials followed rapidly. One study demonstrated effectiveness of topical ACV administration in ocular disease (Jones *et al.*, 1979).

Another uncontrolled study of patients with neoplastic disease or bone marrow transplants noted improvement in cutaneous or systemic HSV or VZV infections (Selby *et al.*, 1979). A randomized, double-blind study in bone marrow transplant recipients demonstrated the effectiveness of intravenously administered ACV in preventing the appearance of culture positive HSV lesions. ACV did not cure latent infection as evidenced by appearance of HSV lesions after the cessation of therapy (Saral *et al.*, 1981). A preliminary report comparing vidarabine with ACV for treatment of neonatal HSV infections suggests that ACV is at least as effective as vidarabine for treatment of these severe infections (Whitley *et al.*, 1983).

Importantly, topical treatment of human primary genital HSV lesions with a 5% ACV ointment shortened the mean duration of virus shedding and also the time to complete crusting of lesions (Corey *et al.*, 1982). In addition, short term, oral therapy of both primary and recurrent genital HSV infections significantly reduced virus shedding and time to healing of lesions (Nilsen *et al.*, 1982; Bryson *et al.*, 1983). Long-term, oral therapy prevents recurrences of genital lesions in most ACV-treated patients as long as therapy is maintained. However, when treatments are discontinued, the recurrence rates are similar to placebo-treated groups (Straus *et al.*, 1984). In addition, acyclovir has been reported to be more effective than vidarabine in the treatment of HSV encephalitis (Whitley *et al.*, 1986).

4.1.6. *Ribavirin*

Ribavirin (virazole) is a purine analog resembling guanosine with a wide range of activity against both RNA and DNA viruses. The drug interferes with the synthesis of guanosine monophosphate, with resultant inhibition of both RNA and DNA synthesis. Influenza viruses are among the most sensitive to inhibition (Sidwell *et al.*, 1979).

Ribavirin has been shown to inhibit RSV replication *in vitro* (Hruska *et al.*, 1980) and in an animal model (Hruska *et al.*, 1982). Several double-blind studies have shown that aerosol administration of ribavirin to infected infants resulted in more rapid improvement in overall severity of illness and increased disappearance of RSV from respiratory secretions. There was no evidence of intolerance or toxicity in the treated babies (Hall *et al.*, 1983; Taber *et al.*, 1983). This drug has been approved for aerosol treatment of infants and young children with severe lower respiratory infections due to RSV.

4.2. DRUGS CURRENTLY UNDER INVESTIGATION

4.2.1. *Phosphonoformate*

The trisodium salt of phosphonoformate (PFA) inhibits herpesvirus DNA polymerase at levels of drug which do not appreciably affect cellular polymerase. In cell culture, 100 mM PFA inhibits herpesvirus replication by 59–96% depending on the virus (Helgstrand *et al.*, 1978; Reno *et al.*, 1978; Larsson and Oberg, 1981). This mechanism of action is the same as that of phosphonoacetate (PAA) but PFA is preferred because of the dermal toxicity associated with topical PAA application (Harris and Boyd, 1977; Alenius and Oberg, 1978). Recent *in vitro* studies have demonstrated greater activity against HSV-1 and HSV-2 when PFA was used in combination with 5-methoxymethyldeoxyuridine than when either drug was used alone (Ayisi *et al.*, 1985).

In animal models, PFA is effective in treating cutaneous herpes in guinea-pigs (Alenius and Oberg, 1978), herpes keratitis in rabbits (Alenius *et al.*, 1980), and genital herpes in guinea-pigs (Alenius and Nordlinder, 1979). In the latter genital herpes model in guinea-pigs, treatment was effective only if begun within 24 hr after infection.

A more recent investigation has found that PFA treatment can also be effective in the treatment of guinea-pig genital herpes when begun near the time of appearance of symptoms (Mayo *et al.*, 1983; Lucia *et al.*, 1983). A double-blind controlled study on cutaneous labial herpes in humans has similarly demonstrated a beneficial effect of PFA treatment on duration of HSV-induced lesions (Wallin *et al.*, 1980). There have been some concerns, however, about long-term deposition of the drug in bone.

4.2.2. *Bromovinyldeoxyuridine*

Bromovinyldeoxyuridine (BVDU) is a nucleoside analog which is preferentially incorporated into viral DNA. HSV TK is involved in this preferential incorporation because TK mutants of HSV-1 are resistant to the effects of BVDU. Although active against both HSV-1 and HSV-2 *in vitro*, BVDU inhibits HSV-2 at a concentration that is 100 times greater than that necessary to inhibit HSV-1 (DeClercq *et al.*, 1980b). The preferential inhibition of HSV-1 may be due to the different rates at which the virus-associated kinases catalyze the second step of BVDU phosphorylation from the mono- to the diphosphate (Fyfe, 1982). BVDU has been found to be nontoxic and effective in topical treatment of experimental herpes keratitis in rabbits (Maudgal *et al.*, 1980), orofacial herpes in mice (Park *et al.*, 1982) and cutaneous herpes in guinea-pigs (Freeman *et al.*, 1985). Oral administration has been used in humans to treat herpes zoster (DeClercq *et al.*, 1980a). No drug-induced toxicity was found in the patients studied while progression of lesion formation was arrested within 24 hr after the start of therapy. Topical treatment of ocular HSV and VZV infections has been shown to be very effective (Maudgal *et al.*, 1984).

4.2.3. *Fluoropyrimidines*

The fluoropyrimidines, FIAC (1-(2′-deoxy-2′-fluoro-B-D-arabinofuranosyl)-5-iodocytosine), FIAU (1-(2′-deoxy-2′-fluoro-B-D-arabinofuranosyl)-5-iodouracil) and FMAU (1-(2′-deoxy-2′-fluoro-B-D-arabinofuranosyl)-5-methyluracil) inhibit HSV-1 and HSV-2 replication in cell culture. FIAC and FMAU are equally active against HSV-1 and HSV-2 strains and have about the same potency as ACV when assayed in rabbit kidney cells (DeClercq *et al.*, 1980b; Trousdale *et al.*, 1983). The mechanism of action is believed similar to ACV in that triphosphate nucleotide analogs bind to virus DNA polymerase, may act as chain terminator for viral DNA replication and HSV TK-negative mutants are many fold less susceptible to inhibition.

Animal studies have shown FIAU and FMAU to be more active than ACV in treatment of HSV encephalitis in mice (Schinazi *et al.*, 1983). In rabbits, topical application of FIAC and FMAU was effective in the treatment of eye infections (Trousdale *et al.*, 1981, 1983).

A guinea-pig model of genital HSV infection compared FIAC, FIAU, FMAU, ACV and PFA and found that the three fluoropyrimidines were all more effective than either ACV or PFA for treatment of primary genital HSV-2 infections. FMAU was the most effective of all the drugs tested (Mayo and Hsiung, 1984). In humans, FIAC was reported to be therapeutically superior to ara-A for treatment of VZV and HSV infections in immunosuppressed patients (Fox *et al.*, 1982).

4.2.4. *Dihydroxypropoxymethylguanine*

The compound 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) is also known as BIOLF-62, 2'NDG and BW759. This acyclic nucleoside is structurally related to ACV and has a similar mode of action against the herpes group of viruses *in vitro* (Ashton *et al.*, 1982; Cheng *et al.*, 1983; Martin *et al.*, 1983). *In vivo*, mouse models have shown DHPG to be very effective, more so than ACV, for the treatment of encephalitis and vaginitis due to HSV-2 (Smee *et al.*, 1983). DHPG is also effective against HSV-2 in a guinea-pig model of primary and recrudescent genital herpes (Fraser-Smith *et al.*, 1983). When compared with ACV, however, DHPG is more toxic, but it has increased activity against both Epstein–Barr virus and CMV. This increased activity against CMV makes DHPG unique, although there are variable reports as to the degree of such activity (Cheng *et al.*, 1983; Smith *et al.*, 1982; Freitas *et al.*, 1985; Shanley *et al.*, 1985). DHPG appears to be effective in controlling CMV associated retinitis and colitis as long as treatment is continued (Masur *et al.*, 1986).

4.2.5. *Azidothymidine*

Azidothymidine (3'-azido-3'-deoxythymidine or AZT) is a nucleoside analog which competitively inhibits the reverse transcriptase of HIV in cell culture and also inhibits infectivity and cytopathic effect *in vitro*. Concentrations which effectively block *in vitro* infectivity and CPE of HIV do not affect *in vitro* immune functions of normal human T-cells (Mitsuya *et al.*, 1985). In clinical trials with AIDS and ARC (AIDS-related complex) patients, there were 19 deaths among the 137 patients receiving placebo and one death among the 145 patients receiving AZT. There also appeared to be fewer opportunistic infections in the AZT group (Fischl *et al.*, 1987). Additional trials are underway (Yarchoan and Broder, 1987).

In another study, AZT treatment was associated with a significant decrease in HIV core antigen in the serum of AZT treated patients compared with untreated controls (Chaisson *et al.*, 1986). As a result, AZT has been made available on an investigational basis to AIDS patients who have had *Pneumocystis carinii* pneumonia and who satisfy certain other criteria. AZT also has excellent penetration across the blood–brain barrier, which hopefully will benefit patients with HIV-associated neurologic disease (Yarchoan *et al.*, 1987). Unfortunately, bone marrow toxicity can be a significant problem.

4.3. Drug Sensitivity Testing

Drug sensitivity testing of clinical isolates is an important function of microbiology laboratories and is essential for the administration of appropriate and effective drugs. Antiviral susceptibility testing will also be necessary and is within the capability of the virus laboratory, but performance standards need to be established. Two methods are commonly used in the laboratory for testing drug sensitivity of a given virus. One of the methods is to determine the virus yield in liquid culture medium. Basically this is done by adding varying concentrations of drug to the culture medium of virus-infected cells and assaying aliquots of the medium for the yield of virus. The resulting reduction of virus yield can be plotted against virus yield without drug. The second and perhaps the simplest method of antiviral assay which can be performed by a routine laboratory is a plaque reduction assay. Plaque formation in the absence of the test drug is compared to plaque formation

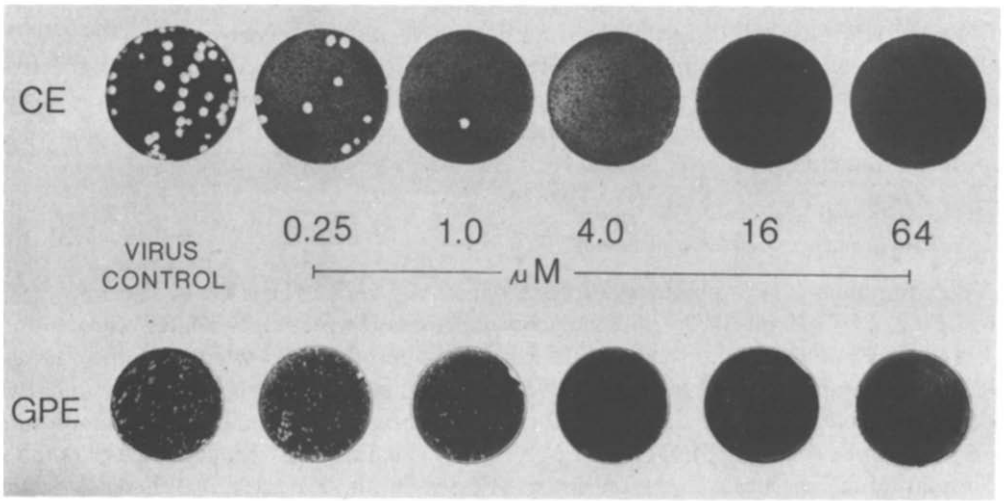

FIG. 10. The effect of acyclovir on herpes simplex virus type 2 (HSV-2) plaque formation.

in the presence of the drug at different concentrations (Fig. 10). It should be noted that different results are obtained when different cell culture systems are used for the plaque reduction assay. As illustrated in Fig. 10, a 0.25 μM concentration of ACV is necessary to inhibit 80% of HSV-2 induced plaque formation when CE cells are used for the assay, whereas 4 μM of the same drug is needed to inhibit the same amount of virus when GPE cells are used. Thus, the importance of selection of the cell culture system used for drug sensitivity tests is apparent.

Rapid techniques such as nucleic acid hybridization screening (Gadler *et al.*, 1984) and automated CPE inhibition assays (Moran *et al.*, 1985) are now being applied to drug sensitivity testing and can significantly facilitate the ease with which large numbers of antiviral agents can be tested for effectiveness against virus isolates.

5. THE IMPORTANCE OF ACCURATE VIRAL DIAGNOSIS

To the practising physician, in the absence of specific treatment, there seems to be little to be gained from diagnosing viral diseases. However, for the following reasons, an accurate viral diagnosis can benefit both the individual patient and the public at large.

5.1. PATIENT MANAGEMENT

Although no treatment is available for the majority of viral illnesses, obtaining an accurate diagnosis still has important implications for patient management. When the exact etiology of an illness is known, unnecessary and often uncomfortable diagnostic procedures, as well as unwarranted antibiotics, can be avoided, and in addition, the physician can more effectively manage any problems that may arise.

5.2. PROGNOSIS

In addition to aiding the management of the acute illness, an accurate viral diagnosis allows for prognostication. The expected course of the illness can be described. This would be particularly important in congenital infections such as rubella and CMV. In genital herpes simplex infection, the patient and contacts should be advised about risk of recurrency, especially in relation to pregnancy, infections of newborns, as well as the increased risk of cervical cancer. In genital HPV infections, detection of low risk or high risk HPV types would be critical in determining potential for progression to cervical cancer.

5.3. Prophylactic and Therapeutic Intervention

In certain situations, prophylactic intervention is critical. Pregnant women with a history of genital herpes, infection with herpes below the waist or a sexual contact with genital herpes should be monitored frequently with cervicovaginal cultures for HSV the last 4–8 weeks of pregnancy (Visintine *et al.*, 1978). If HSV is isolated with the week prior to delivery, caesarean section should be performed within 4 hr of the rupture of the membranes to prevent infection of the fetus. Knowledge of the immune status to CMV of kidney transplant recipients and donors is critical for a successful outcome. Seronegative recipients receiving kidneys from seropositive donors have a significant risk of contracting CMV infection and of rejecting the kidney (Lopez *et al.*, 1974; Ho *et al.*, 1975). Passive immunization with immunoglobulin is available for certain serious infections, such as hepatitis contacts, immunosuppressed children exposed to VZV, and is combined with vaccination in persons exposed to rabies. Amantadine, as discussed above, can prevent or lessen the severity of infection with influenza A and has been useful in protecting unvaccinated, high risk populations.

As described in the preceding section, specific antiviral therapy is now also possible for serious herpes infections such as herpes simplex encephalitis, neonatal infection with HSV, and VZV infections in the compromised host with acyclovir or adenine arabinoside. HSV keratitis can be treated with topical IDU, vidarabine, ACV, or TFT. Acyclovir has also proved of benefit in treatment of genital herpes infections. Ribavirin therapy is effective in treatment of lower respiratory RSV infection in young children. In addition, newer and more promising drugs are being developed.

5.4. Control of Nosocomial Infections

Nosocomial viral infections, an important cause of morbidity and mortality in hospitalized patients, can be best prevented when an accurate viral diagnosis is obtained and the medical staff are educated as to the proper precautions to prevent spread of the disease. In-hospital transmission of numerous virus infections has been documented. These include influenza (Blumenfeld *et al.*, 1959), respiratory syncytial (Hall *et al.*, 1975), parainfluenza (Mufson *et al.*, 1973), enteroviruses (Gear and Measroch, 1973), rotaviruses (Ryder *et al.*, 1977), varicella-zoster (Meyers *et al.*, 1979), herpes simplex (Linneman *et al.*, 1978), hepatitis viruses (Matthew *et al.*, 1973; Postic *et al.*, 1978), rubella (Carne *et al.*, 1973), and adenoviruses (Barr *et al.*, 1958). The newborn infant and the compromised host suffer the most serious consequences. When the offending agent is identified, proper precautions can be instituted.

5.5. Public Health Measures

The importance of viral diagnosis in public health has long been recognized, as illustrated by the control of hepatitis, arbovirus and rabies infections. It has been the major impetus behind effective vaccination programs and allows for the continued evaluation of the efficacy of current vaccines. Continued surveillance is particularly important in determining the antigenic composition of influenza vaccines.

5.6. Advancement of Medical Science

Since 1970, we have witnessed the discovery of rotaviruses (Flewett *et al.*, 1973), Norwalk agent (Kapikian *et al.*, 1972), JC and BK papovaviruses (Padgett *et al.*, 1971; Gardner *et al.*, 1971), delta agent (Rizzetto *et al.*, 1977), and the recognition that non-A, non-B hepatitis viruses account for the majority of transfusion associated hepatitis (Hoofnagle *et al.*, 1977). The most dramatic discovery however, has been that of HIV as the etiologic agent of AIDS (Barre-Sinoussi *et al.*, 1983; Gallo *et al.*, 1984; Levy *et al.*, 1984). Viruses have been implicated in many well known diseases, such as Paget's,

polymyositis, chronic neurologic syndromes, autoimmune diseases, diabetes, and cardiomyopathy. Although perhaps not of immediate benefit to the patient, enlarging our knowledge and understanding of the pathogenesis and spectrum of virus-induced diseases will lead to improvement in medical care in the future.

5.7. PHYSICIAN EDUCATION

A final and very important reason for obtaining an accurate viral diagnosis is the education of physicians. Because of the lack of therapy, it has not been important for physicians to be well versed on the specifics of many viral diseases. It has been adequate to diagnose a 'viral syndrome'. When specific diagnoses are obtained, the physician is stimulated to learn more. As we approach an age of chemotherapy, the increased clinical acumen of the physician in diagnosing viral disease will be decidedly more important.

6. CONCLUDING REMARKS

Since the discovery of tissue culture over 40 years ago, many changes have occurred in the field of diagnostic virology. Interest in different virus groups has fluctuated tremendously (Hsiung, 1980), there have been significant technological advances and many 'new' viruses have been discovered (Hsiung, 1984) of which HIV and other human retroviruses are the most striking example. Nothing, however, will have a greater impact on diagnostic virology than the availability of effective chemotherapy. Until recently, virus laboratories have existed either as part of health departments or university research laboratories and their services have not been readily available to community hospitals or practising physicians. However, over the next decade, with the expected progress in antiviral therapy, significant changes can be anticipated. Since minimal amounts of virus may be present in clinical samples, transporting them to a reference laboratory can result in loss of infectious virus and even negative findings. With facilities close by, time to virus isolation and numbers of isolations can be optimized. If significant numbers of specimens are processed, cost will be favorably affected. In addition, communication between the laboratory and physician will be facilitated. Several recent reports have demonstrated the feasibility of establishing satellite or mini laboratories (Herrmann and Herrmann, 1977; Peterson *et al.*, 1980) or laboratories operated on a small scale (Landry and Hsiung, 1981) whose services are tailored to the needs of the patient populations they serve. High-quality commercial reagents are now becoming available for many rapid diagnostic methods. Continued progress in this area can be anticipated in the near future as the need increases. As we become more optimistic about our ability to intervene in the course of viral diseases a greater need to obtain an accurate viral diagnosis is evident.

REFERENCES

ALBERT, M. J., SOENARTO, Y. and BISHOP, R. F. (1982) Epidemiology of rotavirus diarrhea in Yogyakarta, Indonesia, as revealed by electrophoresis of genome RNA. *J. Clin. Microbiol.* **16:** 731–733.

ALENIUS, S. and OBERG, B. (1978) Comparison of therapeutic effects of five antiviral agents on cutaneous herpesvirus infection in guinea pigs. *Archs Virol.* **58:** 277–288.

ALENIUS, S. and NORDLINDER, H. (1979) Effect of trisodium phosphonoformate in genital infection of female guinea pigs with herpes simplex virus type 2. *Archs Virol.* **60:** 197–206.

ALENIUS, S., EKLIND, K. and OBERG, B. (1980) Effect of trisodium phosphonoformate and idoxuridine on experimental herpes simplex keratitis in immunized and non-immunized rabbits. *Acta Ophthalmol.* **58:** 167–173.

ALLAIN, J. P., LAURIAN, Y., PAUL, D. A. and SENN, D. (1986) Serological markers in early stages of human immunodeficiency virus infection in hemophiliacs. *Lancet* **II:** 1233–1236.

AMON, R. B., LIS, A. W. and HANIFIN, J. M. (1975) Allergic contract dermatitis caused by idoxuridine. *Archs Derm.* **111:** 1581–1584.

ANDERSON, L. J., GODFREY, E., MCINTOSH, K. and HIERHOLZER, J. C. (1983) Comparison of a monoclonal antibody with a polyclonal serum in an enzyme-linked immunosorbent assay for detecting adenovirus. *J. Clin. Microbiol.* **18:** 463–478.

ANDERSON, M. J., JONES, S. E. and MINSON, A. C. (1985) Diagnosis of human parvovirus infection by dot-blot hybridization using cloned viral DNA. *J. Med. Virol.* **15:** 163–172.

ANDERSON, N. and DOANE, F. W. (1972) Agar diffusion method for negative staining of microbial suspensions in salt solutions. *Appl. Microbiol.* **24:** 495–496.

ANDIMAN, W., GRADOVILLE, L., HESTON, L., NEYDORFF, R., SAVAGE, M. E., KITCHINGMAN, G., SHEDD, D. and MILLER, G. (1983) Use of cloned probes to detect Epstein–Barr viral DNA in tissues of patients with neoplastic and lymphoproliferative diseases. *J. Infect. Dis.* **148:** 967–977.

ASHTON, W. T., KARKAS, J. D., FIELD, A. K. and TOLMAN, R. L. (1982) Activation by thymidine kinase and potent antiherpetic activity of 2′nor-2′-deoxyguanosine (2′NDG). *Biochem. Biophys. Res. Commun.* **108:** 1716–1721.

ATANASIU, P. (1975) Immunofluorescent and immunoperoxidase techniques for the rapid diagnosis of rabies. *Ann. Microbiol. (Institut Pasteur)* **126B:** 69.

AUGUST, M. J. and WARFORD, A. L. (1987) Evaluation of a commercial monoclonal antibody for detection of adenovirus antigen. *J. Clin. Microbiol.* **25:** 2233–2235.

AVRAMEAS, S. and TERNYNCK, T. (1971) Peroxidase-labelled antibody and Fab conjugates with enhanced intracellular penetration. *Immunochemistry* **8:** 1175–1179.

AVRAMEAS, S., TERNYNCK, T. and GULSDON, J. L. (1978) Coupling of enzymes to antibodies and antigens. *Scand. J. Immunol.* **8** (Suppl. 7): 7–23.

AYISI, N. K., GUPTA S. V. and BABIUK, L. A. (1985) Combination chemotherapy: interaction of 5-methoxymethyldeoxyuridine with trifluorothymidine, phosphonoformate and acycloguanosine against herpes simplex viruses. *Antiviral Res.* **5:** 13–27.

BALACHANDRAN, N., FRAME, B., CHERNECKY, M., KRAISELBURD, E., KOURI, Y., GARCIA D., LAVERY, C. and RAWLS, W. E. (1982) Identification and typing of herpes simplex viruses with monoclonal antibodies. *J. Clin. Microbiol.* **16:** 205–208.

BALKOVIC, E. B. and HSIUNG, G. D. (1985) Comparison of immunofluorescence with commercial monoclonal antibodies to biochemical and biological techniques for typing clinical herpes simplex virus isolates. *J. Clin. Microbiol.* **22:** 870–872.

BARR J., KJE LEN, L. and SVEDMYR, A. (1958) Hospital outbreaks of adenovirus type 3 infections. *Acta Paediatr.* **47:** 365–382.

BARRE-SINOUSSI, F., CHERMANN, J. C., REY, R., NUGEYRE, M. T., CHAMARET, S., GRUEST, J., DAUGUET, C., AXLER-BLIN, C., VEZINET-BRUN, F., ROUZIOUX, C., ROZENBAUM, W. and MONTAGNIER, L. (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome. *Science* **220:** 868–871.

BECKMANN, A. M., MYERSON, D., DALING, J. R., KIVIAT, N. B., FENOGLIO, C. M. and MCDOUGALL, J. K. (1985) Detection and localization of human papillomavirus DNA in human genital condylomas by *in situ* hybridization with biotinylated probes. *J. Med. Virol.* **16:** 265–273.

BELL, D. M., WALSH, E. E., HRUSKA, J. F., SCHNABEL, K. C. and HALL, C. B. (1983) Rapid detection of respiratory syncytial virus with a monoclonal antibody. *J. Clin. Microbiol.* **17:** 1099–1101.

BENJAMIN, D. R. and RAY, C. G. (1974) Use of immunoperoxidase for the rapid identification of human myxoviruses and paramyxoviruses in tissue culture. *Appl. Microbiol.* **28:** 47–51.

BENJAMIN, D. R. and RAY, C. G. (1975) Use of immunoperoxidase on brain tissue for the rapid diagnosis of herpes encephalitis. *Am. J. Clin. Pathol.* **64:** 472–476.

BIEGELEISEN, J. Z. JR, SCOTT, L. V. and LEWIS, V. JR (1959) Rapid diagnosis of herpes simplex virus infections with fluorescent antibody. *Science* **129:** 640–641.

BLUMENFELD, H. L., KILBOURNE, E. D., LOURIA, D. B. and ROBERTS, D. E. (1959) Studies on influenza in the pandemic of 1957–1958. I. An epidemiologic, clinical and serologic investigation of an intrahospital epidemic with a note on vaccine efficacy. *J. Clin. Invest.* **38:** 199–212.

BONINO, F. (1986) The importance of hepatitis B viral DNA in serum and liver. *J. Hepatol.* **3:** 136–141.

BONINO, E., HOYER, B., NELSON, J., ENGLE, R., VERME, G. and GERIN, J. (1981) Hepatitis B virus DNA in the sera of HBsAg carriers—A marker of active hepatitis B virus replication in the liver. *Hepatology* **4:** 386–391.

BRECHOT, C., DEGOS, F., LUGASSY, C., THIERS, V., ZAFRANI, S., FRANCO, D., BISMUTH, H., TRÉPO, C., BENHAMOU, J.-P., WANDS, J., ISSELBACHER, K., TIOLLAIS, P. and BERTHELOT, P. (1985) Hepatitis B virus DNA in patients with chronic liver disease and negative tests for hepatitis B surface antigen. *New Engl. J. Med.* **312:** 270–276.

BRIGATI, D. J., MYERSON, D., LEARY, J. J., SPALHOLZ, B., TRAVIS, S. Z., FONG, C. K. Y., HSIUNG, G. D. and WARD, D. C. (1983) Detection of viral genomes in cultured cells and paraffin-embedded tissue sections using biotin-labeled hybridization probes. *Virology* **126:** 32–50.

BROMBERG, K., DAIDONE, B., CLARKE, L. and SIERRA, M. F. (1984) Comparison of immediate and delayed inoculation of HEp-2 cells for isolation of respiratory syncytial virus. *J. Clin. Microbiol.* **20:** 123–124.

BROWN, H. R. and THORMAR, H. (1976) Immunoperoxidase staining of simple nuclear bodies in subacute sclerosing panencephalitis (SSPE) by antiserum to measles nucleocapsids. *Acta Neuropathol.* **36:** 259–264.

BROWN, M., PETRIC, M. and MIDDLETON, P. J. (1984) Diagnosis of fastidious enteric adenoviruses 40 and 41 in stool specimens. *J. Clin. Microbiol.* **20:** 334–338.

BRYSON, Y. J., DILLON, M., LOVETT, M., ACURA, G., TAYLOR, S., CHERRY, J. D., JOHNSON, B. L., WIESMEIER, E., GROWDON, W., CREAGH-KIRK, T. and KEENEY, R. (1983) Treatment of first episodes of genital herpes simplex virus infection with oral acyclovir. *New Engl. J. Med.* **308:** 916–921.

BUCHMAN, T. C., ROIZMAN, B., ADAMS, G. and STOVER, B. H. (1978) Restriction endonuclease fingerprinting of herpes simplex virus DNA: A novel epidemiological tool applied to a nosocomial outbreak. *J. Infect. Dis.* **138:** 488–498.

BURNS, J. (1975) Immunoperoxidase localization of hepatitis B antigen in formalin–paraffin processed liver tissue. *Histochemistry* **44:** 133–135.

BURNS, R. P. (1963) A double-blind study of IDU in human herpes simplex keratitis. *Archs Ophthalmol.* **70:** 381–384.

BUTI, M., ESTEBAN, R., JARDI, R., ESTEBAN, J. I. and GUARDIA, J. (1986) Serological diagnosis of acute delta hepatitis. *J. Med. Virol.* **18:** 81–85.

CAMPION, M. J., MCCANCE, D. J., CUZICK, J. and SINGER, A. (1986) Progressive potential of mild cervical atypia: Prospective cytologic, colposcopic and virologic study. *Lancet* **II:** 237–240.

CARLONI, G., COLLOCA, S., DELFINI, C., MANZIN, A., CLEMENTI, M. and GALIBERT, F. (1987) Detection of HBV infectivity by spot hybridization in HBeAg-negative chronic carriers: HBV DNA in sera from asymptomatic and symptomatic subjects. *J. Med. Virol.* **21:** 15–23.

CARNE, S., DEWHURST, C. J. and HURLEY, R. (1973) Rubella epidemic in a maternity unit. *Br. Med. J.* **i:** 444–446.

CAUSSY, D., ORR, W., DAYA, A. D., ROTH, P., REEVES, W. and RAWLS, W. (1988) Evaluation of number of shell vial cultures per clinical specimen for rapid diagnosis of cytomegalovirus infection. *J. Clin. Microbiol.* **26:** 755–757.

CEPKO, C. L., WHETSTONE, C. A. and SHARP, P. A. (1983) Adenovirus hexon monoclonal antibody that is group specific and potentially useful as a diagnostic reagent. *J. Clin. Microbiol.* **17:** 360–364.

CEVENINI, R., RUMPIANESI, F., MAZZARACCHIO, R., DONATI, M., FAICIERI, E. and LAZZARI, R. (1983) Evaluation of a new latex agglutination test for detecting human rotavirus in faeces. *J. Infect.* **7:** 130–133.

CHAISSON, R. E., ALLAIN, J. P. and VOLBERDING, P. A. (1986) Significant changes in HIV antigen level in the serum of patients treated with azidothymidine. *New Engl. J. Med.* **315:** 1610–1611.

CHEESEMAN, S. H., PIERIK, L. T., LEOMBRUNO, D., SPINOS, K. E. and MCINTOSH, K. (1986) Evaluation of a commercially available direct immunofluorescent staining reagent for the detection of respiratory syncytial virus in respiratory secretions. *J. Clin. Microbiol.* **24:** 155–156.

CHENG, Y. C., HUANG, E. S., LIN, J. C., MAR, E. C., PAGANO, J. S., DUTSCHMAN, G. E. and GRILL, S. P. (1983) Unique spectrum of activity of 9-1(1,3-dihydroxy-2-propoxy) methyl guanine against herpesvirus *in vitro* and its mode of action against herpes simplex virus type 1. *Proc. Natn. Acad. Sci. U.S.A.* **80:** 2767–2770.

CHERNESKY, M. A., ESCOBAR, M. R., SWENSON, P. D. and SPECTER, S. C. (1984) Laboratory diagnosis of hepatitis viruses. Washington D.C.: American Society of Microbiologists, Cumitech 18, pp. 1–12.

CHEUNG, E. Y., HNATKO, S. I., GUNNING, H. and WILSON, J. (1982) Comparison of rotazyme and direct electron microscopy for detection of rotavirus in human stools. *J. Clin. Microbiol.* **16:** 562–563.

CHIBA, Y., MIYAZAKI, C., MAKINO, Y., MUTANDA, L. N., KIBUE, A., LICHENGA, E. O. and TUKEI, P. M. (1984) Rotavirus infection of young children in two districts of Kenya from 1982 to 1983 as analyzed by electrophoresis of genomic RNA. *J. Clin. Microbiol.* **19:** 579–582.

CHIEN, L. T., CANNON, N. J., WHITLEY, R. J., DIETHELM, A. G., DISMUKES, W. E., SCOTT, C. W., BUCHANAN, R. A. and ALFORD, C. A. JR (1974) Effect of adenine arabinoside on cytomegalovirus infections. *J. Infect. Dis.* **130:** 32–39.

CHOU, S. and MERIGAN, T. C. (1982) Rapid detection and quantitation of human cytomegalovirus in urine through DNA hybridization. *New Engl. J. Med.* **308:** 921–925.

CLAVEL, F., MANSINHO, K., CHAMARET, S., GUETARD, D., FAVIER, V., NINA, J., SANTOS-FERREIRA, M. O., CHAMPALIMAUD, J. L. and MONTAGNIER, L. (1987) Human immunodeficiency virus type 2 infection associated with AIDS in West Africa. *New Engl. J. Med.* **316:** 1180–1185.

CLEWLEY, J. P. (1985) Detection of human parvovirus using a molecularly cloned probe. *J. Med. Virol.* **15:** 173–181.

COHEN, S. M., GORDON, I., RAPP, F., MACAULAY, J. C. and BUCKLEY, S. M. (1955) Fluorescent antibody and complement-fixation tests of agents isolated in tissue culture from measles patients. *Proc. Soc. Exp. Biol. Med.* **90:** 118–122.

COLEMAN, R. M., BAILEY, P. D., WHITLEY, R. J., KEYSERLING, H. and NAHMIAS, A. J. (1983) ELISA for the detection of herpes simplex virus antigens in the cerebrospinal fluid of patients with encephalitis. *J. Virol. Methods* **7:** 117–125.

COONS, A. H., CREECH, H. J. and JONES, R. N. (1941) Immunological properties of an antibody containing a fluorescent group. *Proc. Soc. Exp. Biol. Med.* **47:** 200–202.

COREY, L., NAHMIAS, A. J., GUINAN, M. E., BENEDETTI, J. K., CRITCHLOW, C. W. and HOLMES, K. K. (1982) A trial of topical acyclovir in genital herpes simplex virus infections. *New Engl. J. Med.* **306:** 1313–1319.

COSTER, D. J., JONES, B. R. and MCGILL, J. I. (1979) Treatment of amoeboid herpetic ulcers with adenine arabinoside or trifluorothymidine. *Br. J. Ophthalmol.* **63:** 418–421.

COULEPIS, A. G., VEALE, M. F., MACGREGOR, A., KORNITSCHUK, M. and GUST, I. D. (1985) Detection of hepatitis A virus and antibody by solid-phase radioimmunoassay and enzyme-linked immunosorbent assay with monoclonal antibodies. *J. Clin. Microbiol.* **22:** 119–124.

CRIVELLI, O., RIZZETTO, M., LAVARINI, C., SMEDILE, A. and GERIN, J. L. (1981) Enzyme-linked immunosorbent assay for detection of antibody to the HBsAg-associated delta antigen. *J. Clin. Microbiol.* **14:** 173–177.

CRUM, C. P., IKENBERG, H., RICHART, R. M. and GISSMAN, L. (1984) Human papillomavirus type 16 and early cervical neoplasia. *New Engl. J. Med.* **310:** 880–883.

CRUMPACKER, C. S., SCHNIPPER, L. E., ZAIA, J. A. and LEVIN, M. J. (1979) Growth inhibition by acycloguanosine of herpesviruses isolated from human infections. *Antimicrob. Agents Chemother.* **15:** 642–645.

DAEMER, R. J., FEINSTONE, S. M., GUST, I. D. and PURCELL, R. H. (1981) Propagation of human hepatitis A virus in African green monkey kidney cell culture: primary isolation and serial passage. *Infect. Immun.* **32:** 338–393.

DANE, D. S., CAMERON, C. H. and BIGGS, M. (1970) Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. *Lancet* **i:** 695–698.

DAVIES, W. L., GRUNERT, R. R., HOFF, R. F., MCGAHAN, J. W., NEUMAYER, E. M., PAULSHOCK, M., WATTS, J. C., WOOD, T. R., HERRMANN, E. C. and HOFFMAN, C. E. (1964) Antiviral activity of l-adamantanamine (amantadine). *Science* **144:** 862–863.

DECLERCQ, E. and TORRENCE, R. V. (1978) Nucleoside analogs with selective antiviral activity. *J. Carbohydrates Nucleosides, Nucleotides* **5:** 187–224.

DECLERCQ, E., DEGREEF, H., WILDIERS, J., DEJONGE, G., DROCHMANS, A., DESCAMPS, J. and DESOMER, P. (1980a) (E)-5-(2-bromovinyl)-2-deoxyuridine in severe herpes zoster. *Br. Med. J.* **281:** 1178.

DECLERCQ, E., DESCAMPS, J., VERHELST, G., WALKER, R. T., JONES, A. S., TORRENCE, P. F. and SHUGAR, D. (1980b) Comparative efficacy of anti-herpes drugs against different strains of herpes simplex virus. *J. Infect. Dis.* **141:** 563–574.

DIEGUTIS, P. S., KEIRNAN, E., BURNETT, L., NIGHTINGALE, B. N. and COSSART, Y. E. (1986) False-positive results with hepatitis B virus DNA dot-hybridization in hepatitis B surface antigen-negative specimens. *J. Clin. Microbiol.* **23:** 797–799.

DOANE, F. W., CHATIYANONDA, K., MCLEAN, D. M., ANDERSON, N., BANNATYNE, R.M. and RHODES, A. J. (1967) Rapid laboratory diagnosis of paramyxovirus infection by electron microscopy. *Lancet* **ii:** 751–753.

DOERN, G. V., HERRMANN, J. E., HENDERSON, P., STOBBS-WALRO, D., PERRON, D. M. and BLACKLOW, N. R. (1986) Detection of rotavirus with a new polyclonal antibody enzyme immunoassay (Rotazyme II) and a commercial latex agglutination test (Rotalex): comparison with a monoclonal antibody enzyme immunoassay. *J. Clin. Microbiol.* **23:** 226–229.

DURST, M., GISSMAN, L., IKENBERG, H. and ZUR HAUSEN, H. (1983) A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc. Natn. Acad. Sci. U.S.A.* **80:** 3812–3815.

ELION, G. B., FURMAN, P. A., FYFE, J. A., DE MIRANDA, P., BEAUCHAMP, L. and SCHAEFFER, H. J. (1977) Selectivity of action of an antiherpetic agent. 9-(2-hydroxyethoxymethyl)guanine. *Proc. Natn. Acad. Sci., U.S.A.* **74:** 5716–5720.

ENDERS, J. F., WELLER, T. H. and ROBBINS, F. C. (1949) Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. *Science* **109:** 85–87.

ENGVALL, E. and PERLMANN, P. (1971) Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. *Immunochemistry* **8:** 871–874.

EPSTEIN, M. A., ACHONG, B. G. and BARR, Y. M. (1964) Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet* **i:** 702–703.

ESPY, M. J., SMITH, T. F., HARMON, M. W. and KENDAL, A. P. (1986) Rapid detection of influenza virus by shell vial assay with monoclonal antibodies. *J. Clin. Microbiol.* **24:** 677–679.

FALK, L., PAUL, D. and KNIGGE, M. (1987) Comparative sensitivity of H9 vs. PHA-stimulated human mononuclear leukocytes for HTLV-III isolation from clinical specimens. Abstracts of the III International Conference on AIDS, Number 248, Washington DC, USDHHS and WHO, 1987.

FAYRAM, S. L., AARNAES, S. and DE LA MAZA, L. M. (1983) Comparison of Cultureset to a conventional tissue culture fluorescent antibody technique for isolation and identification of herpes simplex virus. *J. Clin. Microbiol.* **18:** 215–216.

FAYRAM, S. L., AARNAES, S. L. and DE LA MAZA, L. M. (1985) Evaluation of three cell lines for the isolation of herpes simplex virus. Abstracts of the Annual Meeting of the American Society for Microbiology, Las Vegas.

FEINSTONE, S. M., KAPIKIAN, A. Z. and PURCELL, R. H. (1973) Hepatitis A: detection by immune electron microscopy of a virus-like antigen associated with acute illness. *Science* **182:** 1026–1028.

FIACCO, V., BRYSON, Y. J. and BRUCKNER, D. A. (1984) Comparison of monoclonal and polyclonal antibody for confirmation of cytomegalovirus isolates by fluorescent staining. *J. Clin. Microbiol.* **19:** 928–930.

FIFE, K. H., ASHLEY, R. and COREY, L. (1985a) Isolation and characterization of six new genome types of human adenovirus types 1 and 2. *J. Clin. Microbiol.* **21:** 20–23.

FIFE, K. H., ASHLEY, R., SHIELDS, A. F., SLATER, D., MEYERS, J. D. and COREY, L. (1985b) Comparison of neutralization and DNA restriction enzyme methods for typing clinical isolates of human adenovirus. *J. Clin. Microbiol.* **22:** 95–100.

FISCHL, M. A., RICHMAN, D. D., GRIECO, M. H., GOTTLIEB, M. S., VOLBERDING, P. A., LASKIN, O. L., LEEDOM, J. M., GROOPMAN, J. E., MILVAN, D., SCHOOLEY, R. T., JACKSON, G. G., DURACK, D. T., KING, D. and THE AZT COLLABORATIVE WORKING GROUP (1987) The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. *New Engl. J. Med.* **317:** 185–191.

FLEWETT, T. H., BRYDEN, A. S. and DAVIES, H. (1973) Virus particles in gastroenteritis (Letter). *Lancet* **ii:** 1497.

FLEWETT, T. H., BRYDEN, A. S., DAVIES H. and MORRIS, C. A. (1975) Epidemic viral enteritis in a long-stay children's ward. *Lancet* **i:** 4–5.

FLORES, J., PURCELL, R. H., PEREZ, J., WYATT, R. G., BOEGGEMAN, E., SERENO, M., WHITE, L., CHANOCK, R. M. and KAPIKIAN, A. Z. (1983) A dot hybridization assay for detection of rotavirus. *Lancet* **1:** 555–558.

FORGHANI, B., SCHMIDT, N. J. and LENNETTE, E. H. (1974) Solid-phase radioimmunoassay for identification of herpesvirus hominis types 1 and 2 from clinical materials. *Appl. Microbiol.* **28:** 661–667.

FORGHANI B., SCHMIDT, N. J. and LENNETTE, E. H. (1978) Radioimmunoassay of measles virus antigen and antibody in SSPE brain tissue. *Proc. Soc. Exp. Biol. Med.* **157:** 268–272.

FORGHANI, B., DUPUIS, K. W. and SCHMIDT, N. J. (1985) Rapid detection of herpes simplex virus DNA in human brain tissue by *in situ* hybridization. *J. Clin. Microbiol.* **22:** 656–658.

FOX, J. J., WATANABE, K. A., LOPEZ, C., PHILIPS, F. S. and LEYLAND-JONES, B. (1982) Chemistry and potent antiviral activity of 2′-fluoro-5-substituted-arabinosyl-pyrimidine nucleosides. In: *Herpesvirus. Clinical, pharmacological and basic aspects.* SHITOA, H., CHENG, Y. C. and PRUSOFF, W. H. (eds) Excerpta Medica, Amsterdam, Oxford-Princeton.

FRASER-SMITH, E. B., SMEE, D. F. and MATTHEWS, T. R. (1983) Efficacy of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl) guanine against primary and recrudescent genital herpes simplex virus type 2 infections in guinea pigs. *Antimicrob. Agents Chemother.* **24:** 883–887.

FREEMAN, D. J., SACKS, S. L., DECLERCQ, E. and SPRUANCE, S. L. (1985) Preclinical assessment of topical treatments of herpes simplex virus infection: 5% (E)-5-(2-bromovinyl)-2′-deoxyuridine cream. *Antiviral Res.* **5:** 169–177.

FREITAS, V. R., SMEE, D. F., CHERNOW, M., BOEHME, R. and MATTHEWS, T. R. (1985) Activity of 9-(1,3-dihydroxy-2-propoxymethyl) guanine compared with that of acyclovir against human, monkey and rodent cytomegaloviruses. *Antimicrob. Agents Chemother.* **28:** 240–245.

FREYMUTH, F., QUIBRIAC, M., PETITJEAN, J., AMIEL, M. L., POITHIER, P., DENIS, A. and DUHAMEL, J. F. (1986) Comparison of two new tests for rapid diagnosis of respiratory syncytial virus infections by enzyme-linked immunosorbent assay and immunofluorescence techniques. *J. Clin. Microbiol.* **24:** 1013–1016.

FYFE, J. A. (1982) Differential phosphorylation of (E)-5-(2-bromovinyl)-2′-deoxyuridine monophosphate by thymidylate kinases from herpes simplex virus types 1 and 2 and varicella zoster viruses. *Molec. Pharmac.* **21:** 432–437.

GADLER, H., LARSSON, A. and SOLVER, E. (1984) Nucleic acid hybridization of antiviral compounds on herpes simplex virus type 1 DNA synthesis. *Antiviral Res.* **4:** 63–70.

GALLO, R. C., SALAHUDDIN, S. Z., POPOVIC, M., SHEARER, G. M., KAPLAN, M., HAYNES, B. F., PALKER, T. J., REDFIELD, R., OLESKE, J., SAFAI, B., WHITE, G., FOSTER, P. and MARKHAM, P. D. (1984) Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk of AIDS. *Science* **224:** 500–503.

GARDNER, P. S. (1977) Rapid virus diagnosis. *J. Gen. Virol.* **36:** 1–29.

GARDNER, P. S., GRANDIES, M. and MCQUILLIN, J. (1978) Comparison of immunofluorescence and immunoperoxidase methods for viral diagnosis at a distance: a WHO collaborative study. *Bull. WHO* **56:** 105–110.

GARDNER, S. D., FIELD, A. M., COLEMAN, D. V. and HULME, B. (1971) New human papovavirus (BK) isolated from urine after renal transplantation. *Lancet* **i:** 1253–1257.

GARY, G. W., JR, KAPLAN, J. E., STINE, S. E. and ANDERSON, L. J. (1985) Detection of Norwalk virus antibodies and antigen with a biotin-avidin immunoassay. *J. Clin. Microbiol.* **22:** 274–278.

GEAR, J. H. S. and MEASROCH, V. (1973) Coxsackievirus infections of the newborn. *Prog. Med. Virol.* **15:** 42–62.

GIBSON, P. E., GARDNER, S. D. and PORTER, A. A. (1985) Detection of human polyomavirus DNA in urine specimens by hybridot assay. *Archs Virol.* **84:** 233–240.

GIBBS, C. J., JR and GAJDUSEK, D. C. (1969) Infection as the etiology of spongiform encephalopathy (Creutzfeld–Jakob Disease). *Science* **165:** 1023–1025.

GIRALDO, G., BETH, E., LEE, J., DEHARVEN, E. and CHERNESKY, M. (1982) Solid phase immune electron microscopy-double-antibody technique for rapid detection of papovaviruses. *J. Clin. Microbiol.* **15:** 517–521.

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:** 917–919.

GLEAVES, C. A., SMITH, T. F., SHUSTER, E. A. and PEARSON, G. R. (1985a) Comparison of standard tube and shell vial cell culture techniques for the detection of cytomegalovirus in clinical specimens. *J. Clin. Microbiol.* **21:** 217–221.

GLEAVES, C. A., WILSON, D. J., WOLD, A. D. and SMITH, T. F. (1985b) Detection and serotyping of herpes simplex virus in MRC-5 cells by use of centrifugation and monoclonal antibodies 16 hr postinoculation. *J. Clin. Microbiol.* **21:** 29–32.

GOLDWASSER, R. A. and KISSLING, R. E. (1958) Fluorescent antibody staining of street and fixed rabies virus antigens in mouse brains. *Proc. Soc. Exp. Biol. Med.* **98:** 219–223.

GOUDSMIT, J., DE WOLF, F., PAUL, D. A., EPSTEIN, L. G., LANGE, J. M. A., KRONE, W. J. A., SPEELMAN, H., WOLTERS, E. C., VAN DER NOORDAA, J., OLESKE, J. M., VAN DER HELM, H. J. and COUTINHO, R. A. (1986) Expression of human immunodeficiency virus antigen (HIV-Ag) in serum and cerebrospinal fluid during acute and chronic infection. *Lancet* **ii:** 177–180.

GRAHAM, D. Y. and ESTES, M. K. (1980) Proteolytic enhancement of rotavirus infectivity: biologic mechanisms. *Virology* **101:** 432–439.

GREGORY, W. W. and MENEGUS, M. A. (1983a) Practical protocol for cytomegalovirus isolation: use of MRC-5 cell monolayers incubated for 2 weeks. *J. Clin. Microbiol.* **17:** 605–609.

GREGORY, W. W. and MENEGUS, M. A. (1983b) Effect of incubation temperature on isolation of cytomegalovirus from fresh clinical specimens. *J. Clin. Microbiol.* **18:** 1003–1005.

GRIFFITH, B.P. (1987) Principles of laboratory isolation and identification of the human immunodeficiency virus (HIV). *Yale. J. Biol. Med.* **60:** 575–587.

GRIFFITH, J. F., FITZWILLIAM, J. F., CASAGRANDE, S. and BUTLER, S. R. (1975) Experimental herpes simplex virus encephalitis: comparative effects of treatment with cytosine arabinoside and adenine arabinoside. *J. Infect. Dis.* **132:** 506–510.

HADZIYANNIS, S. J., LIEBERMAN, H. M., KARVOUNTZIS, G. G. and SHAFRITZ, D. A. (1983) Analysis of liver disease, nuclear HBcAg, viral replication and hepatitis B virus DNA in liver and serum of HBeAg vs. anti-HBe positive carriers of hepatitis B virus. *Hepatology* **3:** 656–662.

HAIKALA, O. J., KOKKONEN, J. O., LEINONEN, M. K., NURMI, T., MANTYJARVI, P. and SARKKINEN, H. K. (1983) Rapid detection of rotavirus in stool by latex agglutination: Comparison with radioimmunoassay and electron microscopy and clinical evaluation of the test. *J. Med. Virol.* **11:** 91–97.

HALONEN, P., SARKKINEN, H., ARSTILA, P., HJERTSSON, E. and TORFASON, E. (1980) Four-layer radioimmunoassay for detection of adenovirus in stool. *J. Clin. Microbiol.* **11:** 614–617.

HALL, C. B., DOUGLAS, R. G., JR, GEIMAN, J. M. and MESSNER, M. K. (1975) Nosocomial respiratory syncytial virus infections. *New Engl. J. Med.* **293:** 1343–1346.

HALL, C. B., MCBRIDE, J. T., WALSH, E. E., BELL, D. M., GALA, C. L., HILDRETH, S., TEN EYCK, L. G. and HALL, W. J. (1983) Aerosolized ribavirin treatment of infants with respiratory syncytial virus infection. A randomized double-blind study. *New Engl. J. Med.* **308:** 1443–1447.

HAMMOND, G. W., MAUTHE, G., JOSHUE, J. and HANNAN, C. K. E. (1985) Examination of uncommon clinical isolates of human adenoviruses by restriction endonuclease analysis. *J. Clin. Microbiol.* **21:** 611–616.

HANDSFIELD, H. H., CHANDLER, S. H., CAINE, V. A., MEYERS, J. D., COREY, L., MEDEIROS, E. and MCDOUGALL, J. K. (1985) Cytomegalovirus infection in sex partners: evidence for sexual transmission. *J. Infect. Dis.* **151:** 344–348.

HANTO, D. W., FRIZZERIA, G., PURTILO, D. T., SAKAMOTO, K., SULLIVAN, J. L., SAEMUNDSEN, A. J., KLEIN, G., SIMMONS, R. L. and NAJARIAN, J. S. (1981) Clinical spectrum of lymphoproliferative disorders in renal transplant recipients and evidence for the role of Epstein–Barr virus. *Cancer Res.* **41:** 4253–4261.

HARMON, M. W. and PAWLIK, K. M. (1982) Enzyme immunoassay for direct detection of influenza type A and adenovirus antigens in clinical specimens. *J. Clin. Microbiol.* **15:** 5–11.

HARRIS, S. R. B. and BOYD, M. R. (1977) The activity of iododeoxyuridine, adenine, arabinoside, cytosine arabinoside, ribavirin and phosphonoacetic acid against herpes virus in the hairless mouse model. *J. Antimicrob. Chemother.* **3:** 91–98.

HAYASHI, K., ROSENTHAL, J. and NOTKINS, A. L. (1972) Iodine-125-labeled antibody to viral antigens binding to the surface of virus-infected cells. *Science* **176:** 516–518.

HAYASHI, K., LODMELL, D., ROSENTHAL, J. and NOTKINS, A. L. (1973) Binding of ^{125}I-labeled anti-IgG, rheumatoid factor and anti-C3 to immune complexes on the surface of virus-infected cells. *J. Immun.* **110:** 316–319.

HAYDEN, F. G., SORENSEN, A. S. and BATEMAN, J. A. (1983) Comparison of the Immulok cultureset kit and virus isolation for detection of herpes simplex virus in clinical specimens. *J. Clin. Microbiol.* **18:** 222–224.

HEIDELBERGER, C. (1975) On the molecular mechanism of the antiviral activity of trifluorothymidine. *Ann. N.Y. Acad. Sci.* **255:** 317–325.

HELGSTRAND, E., ERICKSSON, B., JOHANSSON, N. G., LANNERO, B., LARASSON, A., MISIORNY, A., NOREN, J. O., SJOBERG, B., STENBERG, K., STENING, G., OBERG, B., ALENIUS, S. and PHILIPSON, L. (1978) Trisodium phosphonoformate, a new antiviral compound. *Science* **201:** 819–821.

HENLE, G., HENLE, W., CLIFFORD, P., DIEHLE, V., KAFUKO, G. W., KIRYA, B. G., KLEIN, G., MORROW, R. H., MUNUBE, G. M. R., PIKE, P., TUKEL, P. M. and ZIEGLER, J. L. (1969) Antibodies to Epstein–Barr virus in Burkitt's lymphoma and control groups. *J. Natn. Cancer Inst.* **43:** 1147–1157.

HERRMANN, E. C. JR (1974) New concepts and developments in applied diagnostic virology. *Prog. Med. Virol.* **17:** 221–289.

HERRMANN, E. C., JR and HERRMANN, J. A. (1976) A survey of virus diagnostic facilities in medical centers. *J. Infect. Dis.* **133:** 359–362.

HERRMANN, J. A. and HERRMANN, E. C. JR (1977) The mini viral diagnostic laboratory—a necessary adjunct to the use of antiviral drugs. *Ann. N.Y. Acad. Sci.* **284:** 122–127.

HIGGINS, J. R., PEDERSEN, N. C. and CARLSON, J. R. (1986) Detection and differentiation by sandwich enzyme-linked immunosorbent assay of human T-cell lymphotropic virus type III/lymphadenopathy-associated virus and acquired immunodeficiency syndrome-associated retrovirus like clinical isolates. *J. Clin. Microbiol.* **24:** 424–430.

HIRANO, A., YUMURA, K., KURIMURA, T., KATSUMOTO, T., MORIYAMA, H. and MANABE, R. (1979) Analysis of herpes simplex virus isolated from patients with recurrent herpes keratitis exhibiting 'treatment-resistance' to 5-iodo-2-deoxyuridine. *Acta Virol.* **23:** 226–230.

HO, M. (1977) Virus infections after transplantation in man. *Archs Virol.* **55:** 1–24.

HO, M., SUWANSIRIKUL, S., DOWLING, J. N., YOUNGBLOOD, L. A. and ARMSTRONG, J. A. (1975) The transplanted kidney as a source of cytomegalovirus infection. *New Engl. J. Med.* **293:** 1109–1112.

HOOFNAGLE, J. H., GERETY, R. J., TABOR, E. and FEINSTONE, S. M. (1977) Transmission of non-A and non-B hepatitis. *Ann. Intern. Med.* **87:** 14–20.

HORNSLETH, A., FRILS, B., ANDERSON, P. and BRENOE, E. (1982) Detection of respiratory syncytial virus in nasopharyngeal secretions by ELISA: Comparison with fluorescent antibody technique. *J. Med. Virol.* **10:** 273–281.

HOSLI, P., AVRAMEAS, S., ULLMANN, A., VOGT, E. and RODRIGOT, M. (1978) Quantitative ultramicro-scale immunoenzymatic method for measuring Ig antigenic determinants in single cells. *Clin. Chem.* **24:** 1325–1330.

HOWELL, C. L., MILLER, M. J. and BRUCKNER, D. A. (1986) Elimination of toxicity and enhanced cytomegalovirus detection in cell cultures inoculated with semen from patients with acquired immunodeficiency syndrome. *J. Clin. Microbiol.* **24:** 657–660.

HRUSKA, J. F., BERNSTEIN, J. M., DOULGLAS, R. G. and HALL, C. B. (1980) Effects of ribavirin on respiratory syncytial virus *in vitro*. *Antimicrob. Agents Chemother.* **17:** 770–775.

HRUSKA, J. F., MORROW, P. W., SUFFIN, S. C. and DOUGLAS, R. G. (1982) *In vitro* inhibition of respiratory syncytial virus by ribavirin. *Antimicrob. Agents Chemother.* **21:** 125–130.

HSIUNG, G. D. (1961) Application of primary cell cultures in the study of animal viruses III. Biological and genetic study of enteric viruses of man (enteroviruses). *Yale J. Biol. Med.* **33:** 359–371.

HSIUNG, G. D. (1962) Further studies on characterization and grouping ECHO viruses. *Ann. N.Y. Acad. Sci.* **101:** 413–422.

HSIUNG, G. D. (1977) Laboratory diagnosis of viral infections. General principles and recent developments. *Mt Sinai J. Med.* **44:** 1–26.

HSIUNG, G. D. (1980) Progress in clinical virology—1960 to 1980. A recollection of twenty years. *Yale J. Biol. Med.* **53:** 1–4.

HSIUNG, G. D. (1984) Diagnostic virology from animals to automation. *Yale J. Biol. Med.* **57:** 727–733.

HSIUNG, G. D., FONG, C. K. Y. and AUGUST, M. (1979) The use of electron microscopy in diagnosis of virus infection: an overview. *Prog. Med. Virol.* **25:** 133–159.

HSIUNG, G. D., LANDRY, M. L., MAYO, D. R. and FONG, C. K. Y. (1984) Laboratory diagnosis of herpes simplex virus type 1 and type 2 infections. *Clinics Derm.* **2:** 67–82.

HSU, S. M., RAINE, L. and FANGER, H. (1981) A comparative study of the PAP method and an avidin-biotin complex method for studying polypeptide hormones with radio-immunoassay antibodies. *Am. J. Clin. Pathol.* **75:** 734–738.

HUGHES, J. H., TUOMARI, A. V., MANN, D. R. and HAMPARIAN, V. V. (1984) Latex immunoassay for rapid detection of rotavirus. *J. Clin. Microbiol.* **20:** 441–447.

HUNG, T., WANG, C., FANG, Z., CHOU, Z., CHANG, X., LIONG, X., CHEN, G., YAO, H., CHAO, T., YE, W., DEN, S. and CHANG, W. (1984) Waterborne outbreak of rotavirus diarrhea in adults in China caused by a novel rotavirus. *Lancet* **i:** 1139–1142.

HYYPIA, T. (1985) Detection of adenovirus in nasopharyngeal specimens by radioactive and nonradioactive DNA probes. *J. Clin. Microbiol.* **21:** 730–733.

HYYPIA, T., STALHANDSKE, P., VAINIONPAA, R. and PETTERSSON, U. (1984) Detection of enteroviruses by spot hybridization. *J. Clin. Microbiol.* **19:** 436–438.

IGNOTOFSKY, G., MAYO, D., SIRPENSKI, S., SENTERFIR, L., WALLEN, W. and ILTIS, J. (1985) A rapid latex particle assay for the detection of herpes simplex viral (HSV) antigens for confirmation of cell culture and direct detection in clinical isolates. Abstracts of the American Society for Microbiology, V8, p. 390, Las Vegas.

ISHAK, K. G. (1976) Light microscopic morphology of viral hepatitis. *Am. J. Clin. Pathol.* **65:** 787–827.

JOHNSON, F. B., LEAVITT, R. W. and RICHARDS, D. F. (1984) Evaluation of the virocult transport tube for isolation of herpes simplex virus from clinical specimens. *J. Clin. Microbiol.* **20:** 120–122.

JONCAS, J., BERTHIAUME, L., WILLIAMS, R., BEAUDRY, P. and PAVILANIS, V. (1969) Diagnosis of viral respiratory infection by electron microscopy. *Lancet* **i:** 956–959.

JONES, B. R., COSTER, D. J., VISION, P. N., THOMPSON, G. M., COBO, L. M. and FALCON, M. G. (1979) Efficacy of acycloguanosine (Wellcome 248U) against herpes-simplex corneal ulcers. *Lancet* **i:** 243–244.

JORDAN, M. C., ROUSSEAU, W. E., NOBLE, G. R., STEWART, J. A. and CHIN, T. D. Y. (1973) Association of cervical cytomegaloviruses with venereal disease. *New Engl. J. Med.* **288:** 932–934.

JOSEPH, B. S., PERRIN, L. H. and OLDSTONE, M. B. A. (1976) Measurement of virus antigens on the surface of HeLa cells persistently infected with wild type and vaccine strains of measles virus by radioimmune assay. *J. Gen. Virol.* **30:** 329–337.

KALMAN, T. L. (1975) Molecular aspects of the mechanism of action of 5-fluorodeoxyuridine. *Ann. N.Y. Acad. Sci.* **255:** 326–331.

KALTER, S. S., HATCH, M. H. and AJELLO, G. W. (1959) The laboratory diagnosis of poliomyelitis with fluorescent antibodies. *Bact. Proc.* 89–90.

KANKI, P. J., BARIN, F., M'BOUP, S., ALLAN, J. S., ROMET-LEMONNE, J. L., MARLINK, R., MCLANE, M. F., LEE, T. H., ARBEILLE, B., DENIS, F. and ESSEX, M. (1986) New human T-lymphotropic retrovirus related to simian T-lymphotropic virus type III (STLV-III AGM). *Science* **232:** 238–243.

KANKI, P. J., M'BOUP, S., RICARD, D., BARIN, F., DENIS, F., BOYE, C., SANGARE, L., TRAVERS, K., ALBAUM, M., MARLINK, R., ROMET-LEMONNE, J. L. and ESSEX, M. (1986) Human T-lymphotropic virus type 4 and the human immunodeficiency virus in West Africa. *Science* **236:** 827–831.

KAPIKIAN, A. Z., WYATT, R. G., DOLIN, R., THORNBILL, T. S., KALICA, A. R. and CHANOCK, R. M. (1972) Visualization by immune electron microscopy of a 27 nm particle associated with acute infectious non bacterial gastroenteritis. *J. Virol.* **10:** 1075–1081.

KAUFMAN, H. E. (1962) Clinical cure of herpes simplex keratitis by 5-iodo-2-deoxyuridine. *Proc. Soc. Exp. Biol. Med.* **109:** 251–252.

KAUFMAN, H. E. and HEIDELBERGER, C. (1964) Therapeutic antiviral action of 5-trifluoromethyl-2-deoxyuridine in herpes simplex keratitis. *Science* **145:** 585–586.

KAUFMAN, H. E., MARTOLA, E. L. and DOHLMAN, C. (1962) Use of 5-iodo-2-deoxyuridine (IDU) in treatment of herpes simplex keratitis. *Archs Ophthalmol.* **68:** 235–239.

KEMP, M. C., HIERHOLZER, J. C., CABRADILLA, C. P. and OBIJESKI, J. F. (1983) The changing etiology of epidemic keratoconjunctivitis: Antigenic and restriction enzyme analysis of adenovirus types 19 and 37 over a 10-year period. *J. Infect. Dis.* **148:** 24–33.

KIM, H. W., WYATT, R. G., FERNIE, B. F., BRANDT, C. D., ARROBIO, J. O., JEFFRIES, B. C. and PARROTT, R. H. (1983) Respiratory syncytial virus detection by immunofluorescence in nasal secretions with monoclonal antibodies against selected surface and internal proteins. *J. Clin. Microbiol.* **18:** 1399–1404.

KLEIN, R. J. and FREIDMAN-KIEN, A. E. (1977) Latent herpes simplex virus infections in sensory ganglia of mice after topical treatment with adenine arabinoside and adenine arabinoside monophosphate. *Antimicrob. Agents Chemother.* **12:** 577–581.

KLEIN, R. J., FREIDMAN-KIEN, A. E. and DESTEFANO, E. (1979) Latent herpes simplex virus infections in sensory ganglia of hairless mice prevented by acycloguanosine. *Antimicrob. Agents Chemother.* **15:** 723–729.

LAIBSON, P. R. and LEOPOLD, I. H. (1964) An evaluation of double blind IDU therapy in 100 cases of herpetic keratitis. *Trans. Am. Acad. Ophthal.* **68:** 22–23.

LAMONTAGNE, J. R. and GALLASO, G. J. (1978) Amantadine and rimantadine. Clinical studies against influenza. *J. Infect. Dis.* **138:** 928–931.

LANDER, J. J., ALTER, H. J. and PURCELL, R. H. (1971) Frequency of antibody to hepatitis-associated antigen as measured by a new radioimmunoassay technique. *J. Immunol.* **106:** 1166–1170.

LANDRY, M. L. and FONG, C. K. Y. (1985) Nucleic acid hybridization in the diagnosis of viral infections. *Clin. Lab. Med.* **5:** 513–529.

LANDRY, M. L. and HSIUNG, G. D. (1981) The virus diagnostic laboratory: its function in a VA Medical Center. *Conn. Med.* **45:** 417–422.

LANDRY, M. L., MADORE, H. P., FONG, C. K. Y. and HSIUNG, G. D. (1982b) Use of guinea pig embryo cell cultures for isolation and propagation of group A coxsackieviruses. *J. Clin. Microbiol.* **13:** 588–593.

LANDRY, M. L., MAYO, D. R. and HSIUNG, G. D. (1982c) Comparison of guinea pig embryo cells, rabbit kidney cells, and human embryonic lung fibroblast cell strains for isolation of herpes simplex virus. *J. Clin. Microbiol.* **15:** 842–847.

LANDRY, M. L., LUCIA, H. L., HSIUNG, G. D., PRONOVOST, A. D., DANN, P. R., AUGUST, M. J. and MAYO, D. R. (1982a) Effect of acyclovir on genital infection with herpes simplex virus types 1 and 2 in the guinea pig model. *Am. J. Med.* **73**(1A): 143–150.

LANDRY, M. L., BERKOVITS, N., SUMMERS, W. P., BOOSS, J., HSIUNG, G. D. and SUMMERS, W. C. (1983) Herpes simplex encephalitis: analysis of a cluster of cases by restriction endonuclease mapping of virus isolates. *Neurology* **33:** 831–835.

LANDRY, M. L., ZIBELLO, T. A. and HSIUNG, G. D. (1986) Comparison of *in situ* hybridization and immunologic staining with cytopathology for detection and identification of herpes simplex virus infection in cultured cells. *J. Clin. Microbiol.* **24:** 968–971.

LANGE, J. M. A., PAUL, D. A., HUISMAN, H. G., DE WOLF, F., VAN DEN BERG, H., COUTINHO, R. A., DANNER, S. A., VAN DER NOORDAA, J. and GOUDSMIT, J. (1986) Persistent antigenemia and decline of HIV core antibodies associated with transition to AIDS. *Br. Med. J.* **293:** 1459–1462.

LARSSON, A. and OBERG, B. (1981) Selective inhibition of herpesvirus DNA synthesis by Foscarnet. *Antiviral Res.* **1:** 55–62.

LAUER, B. A. (1982) Comparison of virus culturing and immunofluorescence for rapid detection of respiratory syncytial virus in nasopharyngeal secretions: sensitivity and specificity. *J. Clin. Microbiol.* **16:** 411–412.

LAUSH, R. N., MURASKO, D. M., ALBRECHT, T. and RAPP, F. (1974) Detection of specific surface antigen on cells transformed by cytomegalovirus with the techniques of mixed hemagglutination and ^{125}I-labeled anti-globulin. *J. Immun.* **112:** 1680–1684.

LEVIN, M. J., LEVENTHAL, S. and MASTERS, H. A. (1984) Factors influencing quantitative isolation of varicella-zoster virus. *J. Clin. Microbiol.* **6:** 880–883.

LEVY, J. A., HOFFMAN, A. D., KRAMER, S. M., LANDIS, J. A., SHIMABUKURO, J. M. and OSHIRO, L. S. (1984) Isolation of lymphotropic retrovirus from San Francisco patients with AIDS. *Science* **225:** 840–842.

LING, C. M. and OVERBY, L. R. (1972) Prevalence of hepatitis B virus antigen as revealed by direct radioimmune assay with ^{125}I-antibody. *J. Immun.* **109:** 834–841.

LINNEMAN, C. C. JR, BUCHMAN, T. G., LIGHT, I. J., BALLARD, J. L. and ROIZMAN, B. (1978) Transmission of herpes-simplex virus type 1 in a nursery for the newborn: identification of viral isolates by DNA 'finger-printing'. *Lancet* **i:** 964–966.

LIU, C. (1956) Rapid diagnosis of human influenza infection from nasal smears by means of fluorescein-labeled antibody. *Proc. Soc. Exp. Biol. Med.* **92:** 883–887.

LONSDALE, D. M. (1978) A rapid technique for distinguishing herpes simplex virus type 1 from type 2 by restriction enzyme technology. *Lancet* **i:** 849.

LOPEZ, C., SIMMONS, R. L., MAUER, S. M., NAJARIAN, J. S., GOOD, R. A. and GENTRY, S. (1974) Association of renal allograft rejection with virus infections. *Am. J. Med.* **56:** 280–289.

LUCIA, H. L., MAYO, D. R. and HSIUNG, G. D. (1983) Herpes simplex virus induced changes in the vaginal cytology of the guinea pig. *Acta Cytol.* **27:** 365–370.

MACNAUGHTON, M. R., FLOWERS, D. and ISAACS, D. (1983) Diagnosis of human coronavirus infections in children using enzyme-linked immunosorbent assay. *J. Med. Virol.* **11:** 319–325.

MARTIN, J. C., DVORAK, C. A., SMEE, D. F., MATTHEWS, T. R. and VERHEYDEN, J. P. H. (1983) 9-(1,3-dihydroxy-2-propoxy) methylguanine; a new potent and selective antiherpes agent. *J. Med. Chem.* **26:** 759–761.

MARTIN, J. H., DOHNER, D. E., WELLINGHOFF, W. J. and GELB, L. D. (1982) Restriction endonuclease analysis of varicella-zoster vaccine virus and wild-type DNAs. *J. Med. Virol.* **9:** 69–76.

MARTIN, W. J. II and SMITH, T. F. (1986) Rapid detection of cytomegalovirus in bronchoalveolar lavage by a monoclonal antibody method. *J. Clin. Microbiol.* **23:** 1006–1008.

MASUR, H., LANE, H. C., PALESTINE, A., SMITH, P. D., MANISCHEWITZ, J., STEVENS, G., FUJIKAWA, L., MACHER, A. M., NUSSENBLATT, R., BAIRD, B., MEGILL, M., WITTEK, A., QUINNAN, G. V., PARRILLO, J. E., ROOK, A. H., ERON, L. J., PORETZ, D. M., GOLDENBERG, R. I., FAUCI, A. S. and GELMANN, E. P. (1986) Effect of 9-(1,3-dihydroxy-2-propoxymethyl)guanine on serious cytomegalovirus disease in eight immuno-suppressed homosexual men. *Ann. Intern. Med.* **104:** 41–44.

MATHIESON, L. R., FEINSTONE, S. M., SKINHOE, J. P. and PURCELL, R. H. (1977) *Abstracts of the 12th Meeting of the European Association for the Study of the Liver.* Kavouri, 8–10 September, p. 6.

MATTHEW, E. B., DIETZMAN, D. E., MADDEN, D. L., NEWMAN, S. J., SILVER, J. L., NAGLER, B., BOUTON, S. M. and KOSTAFINSKI, M. (1973) A major epidemic of infectious hepatitis in an institution for the mentally retarded. *Am. J. Epidemiol.* **98:** 199–215.

MAUDGAL, P. C., DECLERCQ, E., DESCAMPS, J., MISSOTTEN, L., DESOMER, P., BUSSON, R., VANDERHAEGHE, H., VERHELST, G., WALKER, R. T. and JONES, A. S. (1980) (E)-5-(2-Bromovinyl)-2-deoxyuridine in the treatment of experimental herpes simplex keratitis. *Antimicrob. Agents Chemother.* **17:** 8–12.

MAUDGAL, P. C., DECLERCQ, E. and MISSOTTEN, L. (1984) Efficacy of bromovinyldeoxyuridine in the treatment of herpes simplex virus and varicella-zoster virus eye infections. *Antiviral Res.* **4:** 281–291.

MAYO, D. R. and HSIUNG, G. D. (1984) Treatment of primary acute genital herpes in guinea pigs by intraperitoneal administration of fluoropyrimidines. *Antimicrob. Agents Chemother.* **26:** 354–357.

MAYO, D. R., RICHARDS, J. C. and RAPP, F. (1979) Acycloguanosine treatment of herpesvirus infections in footpads and nervous tissue of normal and immunosuppressed mice. *Intervirology* **12:** 345–348.

MAYO, D. R., LUCIA, H. L. and HSIUNG, G. D. (1983) Effect of phosphonoformate on symptomatic genital herpes simplex virus type 2 infection of guinea pigs. *Intervirology* **19:** 26–32.

MAYO, D. R., BRENNAN, T., EGBERTSON, S. H. and MOORE, D. F. (1985a) Rapid herpes simplex virus detection in clinical samples submitted to a State Virology Laboratory. *J. Clin. Microbiol.* **21:** 768–771.

MAYO, D., HILLIARD, J., LANDRY, M., EGBERTSON, S. and MIRKOVIC, R. (1985b) Comparison of four methods for typing low-passage herpes simplex virus isolates. *Intervirology* **23:** 112–115.

MCDOUGAL, J. S., CORT, S. P., KENNEDY, M. S., CABRIDILLA, C. D., FEORINO, P. M., FRANCIS, D. P., HICKS, D., KALYANARAMAN, V. S. and MARTIN, L. S. (1985) Immunoassay for the detection and quantitation of infectious human retrovirus, lymphadenopathy-associated virus (LAV). *J. Immun. Methods* **76:** 171–183.

MCGILL, J., WILLIAMS, H., MCKINNON, J., HOST-WILSON, A. D. and JONES, B. R. (1974) Reassessment of idoxuridine therapy of herpetic keratitis. *Trans. Ophthalmol. Soc. U.K.* **94:** 542–552.

MCINTOSH, K., WILFERT, C., CHERNESKY, M., PLOTKIN, S. and MATTHEIS, M. J. (1978) Summary of a workshop on new and useful methods in viral diagnosis. *J. Infect. Dis.* **138:** 414–419.

MCINTOSH, K., WILFERT, C., CHERNESKY, M., PLOTKIN, S. and MATTHEIS, M. J. (1980) Summary of a workshop on new and useful techniques in rapid viral dignosis. *J. Infect. Dis.* **142:** 793–802.

McINTOSH, K., HENDRY, R. M., FAHNESTOCK, M. L. and PIERIK, L. T. (1982) Enzyme-linked immunosorbent assay for detection of respiratory syncytial virus infection: application to clinical samples. *J. Clin. Microbiol.* **16:** 329–333.

MEYERS, J. D., MACQUARRIE, M. B., MERIGAN, T. C. and JENNISON, M. H. (1979) Nosocomial varicella—Part 1: Outbreak in oncology patients at a children's hospital. *West. J. Med.* **130:** 196–199.

MICHALSKI, F. J., SHAIKH, M., SAHRAIE, F., DESAI, S., VERANO, L. and VALLABHANENI, J. (1986) Enzyme-linked immunosorbent assay spin amplification technique for herpes simplex virus antigen detection. *J. Clin. Microbiol.* **24:** 310–311.

MILLER, M. J. and HOWELL, C. L. (1983) Rapid detection and identification of herpes simplex virus in cell culture by a direct immunoperoxidase staining procedure. *J. Clin. Microbiol.* **18:** 550–553.

MINOR, P. D. (1980) Comparative biochemical studies of type 3 poliovirus. *J. Virol.* **34:** 73–84.

MITSUYA, H., WEINHOLD, K. J., FURMAN, P. A., ST CLAIR, M. H., LEHRMAN, S. N., GALLO, R. E., BOLOGNESI, D., BARRY, D. W. and BRODER, S. (1985) 3′-Azido-3′-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus *in vitro*. *Proc. Natn. Acad. Sci., U.S.A.* **82:** 7096–7100.

MORAN, D. M., KERN, E. R. and OVERALL, J. E. (1985) Synergism between recombinant human interferon and antiviral agents against herpes simplex virus: Examination with an automated microtiter plate assay. *J. Infect. Dis.* **151:** 1116–1122.

MORGAN, M. A. and SMITH, T. F. (1984) Evaluation of an enzyme linked immunosorbent assay for the detection of herpes simplex virus antigen. *J. Clin. Microbiol.* **19:** 730–732.

MORISSET, R., KURSTAK, C. and KURSTAK, E. (eds) (1974) Diagnosis of herpes simplex virus infections with immunoperoxidase. In: *Viral Immunodiagnosis*, pp. 31–39. Academic Press, New York.

MUFSON, M. A., MOCEGA, H. E. and KRAUSE, H. E. (1973) Acquisition of parainfluenza 3 virus infection by hospitalized children. I. Frequencies, rates and temporal data. *J. Infect. Dis.* **128:** 141–147.

MULLER, S. A., HERRMANN, E. C. and WINKELMANN, R. K. (1972) Herpes simplex infections in hematologic malignancies. *Am. J. Med.* **52:** 102–114.

MULLER, W. E. G., ZAHN, R. K., BITTLINGMAIER, K. and FALKE, D. (1977) Inhibition of herpesvirus DNA synthesis by 9-B-D-arabinofuranosyladenine in cellular and cell-free systems. *Ann. N.Y. Acad. Sci.* **284:** 34–48.

MYERSON, D., HACKMAN, R. C. and MEYERS, J. D. (1984a) Diagnosis of cytomegaloviral pneumonia by *in situ* hybridization. *J. Infect. Dis.* **150:** 272–277.

MYERSON, D., HACKMAN, R. C., NELSON, J. A., WARD, D. C. and MCDOUGALL, J. K. (1984b) Widespread presence of histologically occult cytomegalovirus. *Hum. Path.* **15:** 430–439.

NAGUIB, T., WYATT, R. G., MOIELDIN, M. S., ZAKI, A. M., IMAM, I. Z. and DUPONT, H. L. (1984) Cultivation and subgroup determination of human rotaviruses from Egyptian infants and young children. *J. Clin. Microbiol.* **19:** 210–212.

NAKAJIMA, K., DESSELBERGER, U. and PALESE, P. (1978) Recent human influenza A (H1N1) viruses are closely related genetically to strains isolated in 1950. *Nature* **274:** 334–339.

NAKAJIMA, S., NAKAJIMA, K., TAKEUCHI, Y. and SUGIURA, A. (1980) Influenza surveillance based on oligonucleotide mapping of RNA of H1N1 viruses prevalent in Japan, 1978–1979. *J. Infect. Dis.* **142:** 492–502.

NERURKAR, L. S., NAMBA, M. and SEVER, J. L. (1984a) Comparison of standard tissue culture, tissue culture plus staining, and direct staining for detection of genital herpes simplex virus infection. *J. Clin. Microbiol.* **19:** 631–633.

NERURKAR, L. S., NAMBA, M., BRASHEARS, G., JACOB, A. J., LEE, Y. J. and SEVER, J. L. (1984b) Rapid detection of herpes simplex virus in clinical specimens by use of a capture biotin–streptavidin enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* **20:** 109–114.

NILSEN, A. E., AASEN, T., HALSOS, A. M., KINGE, B. R., TJOTTA, E. A., WIKSTROM, K. and FIDDIAN, A. P. (1982) Efficacy of oral acyclovir in the treatment of initial and recurrent genital herpes. *Lancet* **ii:** 571–573.

NG, A. B. P., REAGAN, J. W. and YEN, S. S. C. (1970) Herpes genitalis. *Obstet. Gynecol.* **36:** 645–651.

NORDLUND, J. J., ANDERSON, C., HSIUNG, G. D. and TENSER, R. B. (1977) The use of temperature sensitivity and selective cell culture for differentiation of herpes simplex virus types 1 and 2 in a clinical laboratory. *Proc. Soc. Exp. Biol. Med.* **155:** 118–123.

NOTTAY, B. K., KEW, O. M., HATCH, M. H., HEYWARD, J. T. and OBIJESKI, J. F. (1981) Molecular variation of type 1 vaccine-related and wild polioviruses during replication in humans. *Virology* **108:** 405–423.

OVERALL, J. C. JR, KERN, E. R. and GLASGOW, L. A. (1976) Effective antiviral chemotherapy in cytomegalovirus infection in mice. *J. Infect. Dis.* **133** (Suppl. A): A237–A244.

PADGETT, B. L., WALKER, D. L., ZURHEIN, G. M., ECKROADE, R. J. and DESSEL, B. H. (1971) Cultivation of papova-like virus from human brain with progressive multifocal leukoencephalopathy. *Lancet* **i:** 1257–1260.

PARK, N. H., PAVAN-LANGSTON, D., BOISJOLY, H. M. and DECLERQ, E. (1982) Chemotherapeutic efficacy of (E)-5-(2-bromovinyl)-2′-deoxyuridine for orofacial infection with herpes simplex type 1 in mice. *J. Infect. Dis.* **145:** 909–913.

PAUL, D. A., FALK, L. A., KESSLER, H. A., CHASE, R. M., BLAAUW, B., CHUDWIN, D. S. and LANDAY, A. L. (1987) Correlation of serum HIV antigen and antibody with clinical status in HIV-infected patients. *J. Med. Virol.* **22:** 357–363.

PAVAN-LANGSTON, D. and FOSTER, C. S. (1977) Trifluorothymidine and idoxyuridine therapy of herpetic keratitis. *Am. J. Ophthalmol.* **84:** 818–825.

PAVAN-LANGSTON, D., CAMPBELL, R. and LASS, J. (1978) Acyclic antimetabolite therapy of experimental herpes simplex keratitis. *Am. J. Ophthalmol.* **86:** 618–623.

PAYA, C., WOLD, A. D. and SMITH, T. F. (1987) Detection of cytomegalovirus infections in specimens other than urine by shell vial assay and conventional tube cultures. *J. Clin. Microbiol.* **25:** 755–757.

PAYA, C., WOLD, A. D., ILSTRUP, D. M. and SMITH, T. F. (1988) Evaluation of number of shell vial cultures per clinical specimen for rapid diagnosis of cytomegalovirus infection. *J. Clin. Microbiol.* **26:** 198–200.

PETERSON, L. R., MOORE, B. M., EDELMAN, C. K. and BALFOUR, H. H. JR (1980) Primary virus isolation by a satellite laboratory. *Archs Pathol. Lab. Med.* **104:** 9–10.

PETTERSSON, R. F., HELLSTROM, P. E., PENTTINEN, K., PYHALA, R., TOKOLA, O., VARTIO, T. and VISAKORPI, R. (1980) Evaluation of amantadine in the prophylaxis of influenza A (HINI) virus infection: A controlled field trial among young adults and high risk patients. *J. Infect. Dis.* **142:** 377–383.

POIESZ, B. J., RUSCETTI, F. W., GAZDAR, A. F., BUNN, P. A., MINNA, J. D. and GALLO, R. C. (1980) Detection and isolation of type-C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. Natn. Acad. Sci. U.S.A.* **77:** 7415–7419.

POIESZ, B. J., RUSCETTI, F. W., REITZ, M. S., KALYANARAMAN, V. S. and GALLO, R. C. (1981) Isolation of a new type-C retrovirus (HTLV) in primary uncultured cells of a patient with Sezary T-cell leukemia. *Nature* **294:** 268–271.

POPOVIC, M., SANGADHARAN, M. G., READ, E. and GALLO, R. C. (1984) Detection, isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224:** 495–500.

POSTIC, B., SHREINER, D. P., HANCHETT, J. E. and ATCHISON, R. W. (1978) Containment of hepatitis B virus infection in a hemodialysis unit. *J. Infect. Dis.* **138:** 884–889.

PRATHER, S. L., DAGAN, R., JENISTA, J. A. and MENEGUS, M. A. (1984) The isolation of enteroviruses from blood: comparison of four processing methods. *J. Med. Virol.* **14:** 221–227.

PROVOST, P. J., GIESA, P. A., MCALEER, W. J. and HILLEMAN, M. R. (1981) Isolation of hepatitis A virus *in vitro* in cell culture directly from human specimens (41149). *Proc. Soc. Exp. Biol. Med.* **167:** 201–206.

PRUSOFF, W. H. and GOZ, B. (1975) Halogenated pyrimidine and deoxyribonucleosides. In: *Handbook of Experimental Pharmacology*, Vol. 38(2), pp. 272–347. EICHLER, O., FARRAH, A., HERKEN, H. and WELCH, A. D. (eds) Springer-Verlag, Berlin.

RANKI, M., PALVA, A., VIRTANEN, M., LAAKSONEN, M. and SODERLUND, H. (1983) Sandwich hybridization as a convenient method for the detection of nucleic acids in crude samples. *Gene* **21:** 77–85.

RAWLS, W. E., TOMPKINS, W. A. F. and MELNICK, J. L. (1969) The association of herpes-virus type 2 and carcinoma of the uterine cervix. *Am. J. Epidemiol.* **89:** 547–554.

RAY, C. G. and MINNICH, L. L. (1982) Regional diagnostic virology services: Are satellite laboratories necessary? *JAMA* **247:** 1309–1310.

REDFIELD, D. C., RICHMAN, D. C., ALBANIL, S., OXMAN, M. N. and WAHL, G. M. (1983) Detection of herpes simplex virus in clinical specimens by DNA hybridization. *Diagn. Microbiol. Infect. Dis.* **1:** 117–128.

RENO, J. M., LEE, L. F. and BOEIZI, J. A. (1978) Inhibition of herpesvirus replication and herpes-virus-induced deoxyribonuclei and polymerase by phosphonoformate. *Antimicrob. Agents Chemother.* **13:** 188–192.

RICHMAN, D. D., CLEVLAND, P. H., REDFIELD, D. C., OXMAN, M. N. and WAHL, G. M. (1984a) Rapid viral diagnosis. *J. Infect. Dis.* **149:** 298–310.

RICHMAN, D., SCMIDT, N., PLOTKIN, S., YOLKEN, R., CHERNESKY, M., MCINTOSH, K. and MATTHEIS, M. (1984b) Summary of a workshop on new and useful methods in rapid viral diagnosis. *J. Infect. Dis.* **150:** 941–951.

RIZZETTO, M., CANESE, M. G., ARICO, S., CRIVELLI, O., TREPO, C., BONINO, F. and VERME, G. (1977) Immunofluorescence detection of new antigen–antibody system (delta/anti-delta) associated to hepatitis B virus in liver and in serum of HBsAg carriers. *Gut* **18:** 997–1003.

RODGER, S. M., BISHOP, R. F., BIRCH, C., MCLEAN, B. and HOLMES, I. H. (1981) Molecular epidemiology of human rotaviruses in Melbourne, Australia, from 1973 to 1979, as determined by electrophoresis of genome ribonucleic acid. *J. Clin. Microbiol.* **13:** 272–278.

RODRIQUEZ, W. J., KIM, H. W., BRANDT, C. D., GARDNER, M. K. and PARROTT, R. H. (1983) Use of electrophoresis of RNA from human rotavirus to establish the identity of strains involved in outbreaks in a tertiary care nursery. *J. Infect. Dis.* **148:** 34–40.

ROSENTHAL, J. D., HAYASHI, K. and NOTKINS, A. L. (1972) Rapid microimmunoassay for the measurement of antiviral antibody. *J. Immun.* **109:** 171–173.

ROTBART, H. A., LEVIN, M. J. and VILLARREAL, L. P. (1984) Use of subgenomic poliovirus DNA probes to detect the major subgroups of enteroviruses. *J. Clin. Microbiol.* **20:** 1105–1108.

RUBENSTEIN, A. S. and MILLER, M. F. (1982) Comparison of an enzyme immunoassay with electron microscopic procedures for detecting rotavirus. *J. Clin. Microbiol.* **15:** 938–944.

RUBIN, S. J. and ROGERS, S. (1984) Comparison of Cultureset and primary rabbit kidney cell culture for the detection of herpes simplex virus. *J. Clin. Microbiol.* **19:** 920–922.

RYDER, R. W., MCGOWAN, J. E., HATCH, M. H. and PALMER, E. L. (1977) Reovirus-like agents as a cause of nosocomial diarrhea in infants. *J. Pediatr.* **90:** 698–702.

SALMON, V. C., TURNER, R. B., SPERANZA, M. J. and OVERALL, J. C., JR (1986) Rapid detection of herpes simplex virus in clinical specimens by centrifugation and immunoperoxidase staining. *J. Clin. Microbiol.* **23:** 683–686.

SAMBOURG, M., GOUDEAU, A., COURANT, C., PINON, G. and DENIS, F. (1985) Direct appraisal of latex agglutination testing, a convenient alternative to enzyme immunoassay for the detection of rotavirus in childhood gastroenteritis, by comparison of two enzyme immunoassays and two latex tests. *J. Clin. Microbiol.* **21:** 622–625.

SARAL, R., BURNS, W. H., LASKIN, O. L., SANTAS, G. W. and LIFTMAN, P. S. (1981) Acyclovir prophylaxis of herpes-simplex-virus infections. A randomized, double blind, controlled trial in bone-marrow-transplant recipients. *New Engl. J. Med.* **305:** 63–67.

SCHINAZI, R. F., PETERS, J., SOKOL, M. and NAHMIAS, A. J. (1983) Therapeutic activities of 1-(2-fluoro-2-deoxy-B-D-arabinofuranosyl)-5-iodocytosine and thymine alone and in combination with acyclovir and vidarabine in mice infected intracerebrally with herpes simplex virus. *Antimicrob. Agents Chemother.* **24:** 95–103.

SCHMIDT, N. J., LENNETTE, E. H., WOODIE, J. D. and HO, H. H. (1966) Identification of rubella virus isolates by immunofluorescent staining, and a comparison of the sensitivity of three cell culture systems for recovery of virus. *J. Lab. Clin. Med.* **68:** 502–509.

SCHMIDT, N. J., GALLO, D., DEVLIN, V., WOODIE, J. D. and EMMONS, R. W. (1980) Direct immunofluorescence staining for detection of herpes simplex and varicella-zoster virus antigens in vesicular lesions and certain tissue specimens. *J. Clin. Microbiol.* **12:** 651–655.

SCHMIDT, N. J., HO, H. H. and CHIN, J. (1981) Application of immunoperoxidase staining to more rapid detection and identification of rubella virus isolates. *J. Clin. Microbiol.* **13:** 627–630.

SCHMIDT, N. J., OTA, M., GALLO, D. and FOX, V. L. (1982) Monoclonal antibodies for rapid, strain-specific identification of influenza virus isolates. *J. Clin. Microbiol.* **16:** 763–765.

SCHMIDT, N. J., DENNIS, J., DEVLIN, V., GALLO, D. and MILLS, J. (1983) Comparison of direct immunofluorescence and direct immunoperoxidase procedures for detection of herpes simplex virus antigen in lesion specimens. *J. Clin. Microbiol.* **18:** 445–448.

SCHUPBACH, J., DIMARZO VERONESE, F. and GALLO, R. C. (1986) Human Retroviruses. In: *Clinical Virology Manual*, SPECTER, S. and LANCZ, G. J. (ed.) Elsevier, New York.

SEIDLIN, M., TAKIFF, H. E., SMITH, H. A., HAY, J. and STRAUS, S. E. (1984) Detection of varicella-zoster virus by dot-blot hybridization using a molecularly cloned viral DNA probe. *J. Med. Virol.* **13:** 53–61.

SELBY, P. J., POWLES, R. L., JAMESON, B., KAY, H. E., WATSON, J. G., THORNTON, R., MORGNSTERN, G., CLINK, H., MCELWAIN, T., PRENTICE, H., ROSS, M., CORRINGHAM, R., HOFFBRAND, A. V. and BRIGDEN, D. (1979) Parenteral acyclovir therapy for herpesvirus infections in man. *Lancet* **ii:** 1267–1270.

SEWELL, D. L. and HORN, S. A. (1985) Evaluation of a commercial enzyme-linked immunosorbent assay for the detection of herpes simplex virus. *J. Clin. Microbiol.* **21:** 457–458.

SEWELL, D. L., HORN, S. A. and SILBECK, P. W. (1984) Comparison of Cultureset and Bartels Immunodiagnostics with conventional tissue culture for isolation and identification of herpes simplex virus. *J. Clin. Microbiol.* **19:** 705–706.

SHALIT, I., MCKEE, P. A., BEAUCHAMP, H. and WANER, J. L. (1985) Comparison of polyclonal antiserum versus monoclonal antibodies for the rapid diagnosis of influenza A virus infections by immunofluorescence in clinical specimens. *J. Clin. Microbiol.* **22:** 877–879.

SHANLEY, J. D., MORNINGSTAR, J. and JORDAN, M. C. (1985) Inhibition of murine cytomegalovirus lung infection and interstitial pneumonitis by acyclovir and 9-(1,3-dihydroxy-2-propoxymethyl) guanine. *Antimicrob. Agents Chemother.* **28:** 172–175.

SHANNON, W. M. (1975) Adenine arabinoside: Antiviral activity *in vitro*. In: *Adenine Arabinoside: An Antiviral Agent*, pp. 1–43. PAVAN-LANGSTON, D., BUCHANAN, R. A. and ALFORD, C. A. (eds) Raven Press, N.Y.

SHATTOCK, A. G. and MORGAN, B. M. (1983) Sensitive enzyme-immunoassay for the detection of delta antigen and anti-delta using serum as a delta antigen source. *J. Med. Virol.* **12:** 73–82.

SHAW, G. M., HAHN, B. H., ARYA, S. K., POPOVIC, M., GALLOW, R. C. and WONG-STAAL, F. (1984) Molecular characterization of human T cell leukemia (lymphotropic) virus type III in the acquired immune deficiency syndrome. *Science* **226:** 1165–1171.

SHIELDS, A. F., HACKMAN, R. C., FIFE, K.H., COREY, L. and MEYERS, J. D. (1985) Adenovirus infections in patients undergoing bone-marrow transplantation. *New Engl. J. Med.* **312:** 529–530.

SIDWELL, R. W., ROBINS, R. K. and HILLYARD, I. W. (1979) Ribavirin: an antiviral agent. *Pharmac. Ther.* **6:** 123–135.

SIEGL, G., DECHASTONAY, J. and KRONAUER, G. (1984) Propagation and assay of hepatitis A virus *in vitro*. *J. Virol. Methods* **9:** 53–67.

SLOAN, B. J., MILLER, F. A., EHRLICH, J., MCLEAN, I. W. and MACHAMER, H. E. (1968) Antiviral activity of 9-B-D-arabinofuranosyladenine. IV. Activity against intracerebral herpes simplex virus infections in mice. *Antimicrob. Agents Chemother.* 161–171.

SMEE, D. F., MARTIN, J. C., VERHEYDEN, J. P. H. and MATTHEWS, T. R. (1983) Anti-herpesvirus activity of the acyclic nucleoside 9-(1,3 dihydroxy-2-propoxymethyl) guanine. *Antimicrob. Agents Chemother.* **23:** 676–682.

SMITH, C., ROGERS, S. and RUBIN, S. J. (1985) Comparison of mink lung and primary rabbit kidney cell culture for herpes simplex virus isolation. Abstracts of the Annual Meeting of the American Society for Microbiology, Las Vegas, 1985.

SMITH, K. O. and GEHLE, W. D. (1969) Pelleting viruses and virus-infected cells for thin-section electron microscopy. *Proc. Soc. Exp. Biol. Med.* **130:** 1117–1119.

SMITH, K, O. and MELNICK, J. L. (1962) A method for staining virus particles and identifying their nucleic acid type in the electron microscope. *Virology* **17:** 480–490.

SMITH, K. O., GALLOWAY, K. S., KENNELL, W. L., OGILVIE, K. K. and RADATUS, B. K. (1982) A new nucleoside analog, 9-(2-hydroxy-1-(hydroxymethyl) guanine, highly active *in vitro* against herpes simplex virus types 1 and 2. *Antimicrob. Agents Chemother.* **22:** 55–61.

SOUTHERN, E. M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Molec. Biol.* **98:** 503–517.

SPECTOR, S., RAU, J. A., SPECTOR, D. H. and MCMILLAN, R. (1984) Detection of human cytomegalovirus in clinical specimens by DNA–DNA hybridization. *J. Infect. Dis.* **150:** 121–126.

SPENCER, E., AVENDANO, F. and ARAYA, M. (1983) Characteristics and analysis of electropherotypes of human rotavirus isolated in Chile. *J. Infect. Dis.* **148:** 41–48.

STAGNO, S., REYNOLDS, D. W., TSIANTOS, A., FUCCILLO, D. A., LONG, W. and ALFORD, C. A. (1975) Comparative serial virologic and serologic studies of symptomatic and subclinical congentially and natally acquired cytomegalovirus infections. *J. Infect. Dis.* **132:** 568–577.

STALHANDSKE, P. and PETTERSSON, U. (1982) Identification of DNA viruses by membrane filter hybridization. *J. Clin. Microbiol.* **15:** 744–747.

STALHANDSKE, P., HYYPIA, T., GADLER, H., HALONEN, P. and PETTERSSON, U. (1983) The use of molecular hybridization for demonstration of adenoviruses in human stools. *Curr. Topics Microbiol. Immun.* **104:** 287–298.

STALHANDSKE, P., HYYPIA, T., ALLARD, A., HALONEN, P. and PETTERSSON, U. (1985) Detection of adenoviruses in stool specimens by nucleic acid spot hybridization. *J. Med. Virol.* **16:** 213–218.

STERNBERGER, L. A. and JOSEPH, S. A. (1979) The unlabelled antibody method. Contrasting color staining of paried pituitary hormone without antibody removal. *J. Histochem. Cytochem.* **27:** 1424–1429.

STOVER, D. E., ZAMAN, M. B., HAJDU, S. I., LANGE, M., GOLD, J. and ARMSTRONG, D. (1984) Bronchoalveolar lavage in the diagnosis of diffuse pulmonary infiltrates in the immunosuppressed host. *Ann. Intern. Med.* **101:** 1–7.

STRAUS, S. E., TAKIFF, H. E., SEIDLIN, M., BACHRACH, S., LININGER, L., DIGIOVANNA, J. J., WESTERN, K. A., SMITH, H. A., LEHRMAN, S. N., CRIEGH-KIRK, T. and ALLING, D. W. (1984) Suppression of frequently recurring genital herpes. *New Engl. J. Med.* **310:** 1545–1550.

STRAUS, W. (1971) Inhibition of peroxidase by methanol and by methanol–nitroferricyanide for use in immunoperoxidase procedures. *J. Histochem. Cytochem.* **19:** 682–688.

SUMMERS, W. C. (1980) Molecular epidemiology of DNA viruses: applications of restriction endonuclease cleavage site analysis. *Yale J. Biol. Med.* **53:** 55–59.

SVENSSON, L., GRANDIEN, M. and PETTERSSON, C. A. (1983) Comparison of solid-phase immune electron microscopy by use of protein A with direct electron microscopy and enzyme linked immunosorbent assay for detection of rotavirus in stool. *J. Clin. Microbiol.* **18:** 1244–1249.

SWENSON, P. D. and KAPLAN, M. H. (1986) Rapid detection of respiratory syncytial virus in nasopharyngeal aspirates by a commercial enzyme immunoassay. *J. Clin. Microbiol.* **23:** 485–488.

TABER, L. H., KNIGHT, V., GILBERT, B. E., MCCLUNG, H. W., WILSON, S. Z., NORTON, H. J., THURSON, J. M., GORDON, W. H., ATMAR, R. L. and SCHLAUDT, W. R. (1983) Ribavirin aerosol treatment of bronchiolitis associated with respiratory syncytial virus infection in infants. *Pediatrics* **72:** 613–618.

TAKIFF, H. E., SEIDLIN, M., KRAUSE, P., ROONEY, J., BRANDT, C., RODRIQUEZ, W., YOLKEN, R. and STRAUSS, S. E. (1985) Detection of enteric adenoviruses by dot–blot hybridization using a molecularly cloned viral DNA probe. *J. Med. Virol.* **16:** 107–118.

TROUSDALE, M. D., NESBURN, A. B., WATANABE, K. A. and FOX, J. J. (1981) Evaluation of the antiherpetic activity of 2′-fluoro-5-iodo-ara-C in rabbit eyes and cell cultures. *Invest. Ophthalmol. Vis. Sci.* **21:** 826–832.

TROUSDALE, M. D., NESBURN, A. B., SU, T. L., LOPEZ, C., WATANABE, K. A. and FOX, J. J. (1983) Activity of 1-(2′-fluoro-2′-deoxy-B-D-arabinofuranosyl) thymine against herpes simplex virus in cell cultures and rabbit eyes. *Antimicrob. Agents Chemother.* **23:** 808–813.

UMEDA, M. and HEIDELBERGER, C. (1969) Fluorinated pyrimidines XXXI. *Proc. Soc. Exp. Biol. Med.* **130:** 24–29.

UNDERWOOD, G. E., ELLIOTT, G. A. and BUTHALA, D. A. (1965) Herpes keratitis in rabbits: pathogenesis and effect of antiviral nucleosides. *Ann. N.Y. Acad. Sci.* **130:** 151–167.

VIRTANEN, M., SYVANEN, A. C., ORAM, J., SODERLUND, H. and RANKI, M. (1984) Cytomegalovirus in urine: Detection of viral DNA by sandwich hybridization. *J. Clin. Microbiol.* **20:** 1083–1088.

VISINTINE, A. M., NAHMIAS, A. J. and JOSEY, W. E. (1978) Genital herpes. *Perinatal Care* **2:** 32–41.

WAART, M., SNELTING, A., CICHY, J., WOLTERS, G. and SCHUURS, A. (1978) Enzyme-immunoassay in the diagnosis of hepatitis with emphasis on the detection of 'e' antigen (HBeAg). *J. Med. Virol.* **3:** 43–49.

WADELL, G., COONEY, M. K., LINHARES, A. D. C., DE SILVA, L., KENNETH, M. L., KONO, R., REN, G. F., LINDMAN, K., NASCIMENTO, J. P., SCHOUB, B. D. and SMITH, C. D. (1985) Molecular epidemiology of adenoviruses: global distribution of adenovirus 7 genome types. *J. Clin. Microbiol.* **21:** 403–408.

WALDEN, P. A. M., NEWLANDS, E. S., COLEMAN, J. C., TATTERSALL, M. H. N. and BAGSHAWE, K. D. (1977) Chemotherapy for varicella-zoster infections. *Br. Med. J.* **i:** 378.

WALLIN, J., LERNESTEDT, J. O. and LYCKE, E. (1980) Treatment of recurrent herpes labialis with trisodium phosphonoformate. *Curr. Chemother. Infect. Dis.* **2:** 1361–1362.

WALLS, H.H., HARMON, M. W., SLAGLE, J. J., STOCKSDALE, C. and KENDAL, A. P. (1986) Characterization and evaluation of monoclonal antibodies developed for typing influenza A and influenza B viruses. *J. Clin. Microbiol.* **25:** 2233–2235.

WANER, J. L., WHITEHURST, N. J., DOWNS, T. and GRAVES, D. G. (1985) Production of monoclonal antibodies against parainfluenza 3 virus and their use in diagnosis by immunofluorescence. *J. Clin. Microbiol.* **22:** 535–538.

WARFORD, A. L., EVELAND, W. G., STRONG, C. A., LEVY, R. A. and REKRUT, K. A. (1984) Enhanced virus isolation by use of the transporter of a regional laboratory. *J. Clin. Microbiol.* **19:** 561–562.

WATANABE, N., NITSU, Y., OHTSUKA, S., KOSEKI, J., KOHGO, Y., URUSHIZAKI, I., KATO, K. and ISHIKAWA, E. (1979) Enzyme immunoassay for human ferritin. *Clin. Chem.* **25:** 80–82.

WEIR, E. E., PRETLOW, T. G., PITTS, A. and WILLIAMS, E. E. (1977) Destruction of endogenous peroxidase activity in order to locate cellular antigens by peroxidase labelled antibodies. *J. Histochem. Cytochem.* **22:** 51–54.

WELLER, T. H. and COONS, A. H. (1954) Fluorescent antibody studies with agents of varicella and herpes zoster propagated *in vitro*. *Proc. Soc. Exp. Biol. Med.* **86:** 789–794.

WHITLEY, R. J. and THE NIAID COLLABORATIVE ANTIVIRAL STUDY GROUP (1983) Interim summary of mortality in herpes simplex encephalitis and neonatal herpes simplex virus infections: vidarabine versus acyclovir. *J. Antimicrob. Chemother.* **12** (Suppl. B): 105–112.

WHITLEY, R. J., SOONG, S., DOLIN, R., GALASSO, G. J., CHIEN, L. T. and ALFORD, C. A., JR (1977) Adenine arabinoside therapy of biopsy proved herpes simplex encephalitis. *New Engl. J. Med.* **297:** 289–294.

WHITLEY, R. J., NAHMIAS, A. J., VISINTINE, A. M., FLEMING, C. L. and ALFORD, C. A. (1980b) The natural history of herpes simplex virus infection of mother and newborn. *Pediatrics* **66:** 489–494.

WHITLEY, R. J., NAHMIAS, A. J., SOONG, S. J., GALASSO, G. J., FLEMING, C. L. and ALFORD, C. A. (1980a) Vidarabine therapy of neonatal herpes simplex virus infection. *Pediatrics* **66:** 495–501.

WHITLEY, R. J., SOONG, S. J., HIRSCH, M. S., KARCHMER, A. W., DOLIN, R., GALASSO, G. J., DUNICK, J. K. and ALFORD, C. A. (1981) Herpes simplex encephalitis. Vidarabine therapy and diagnostic problems. *New Engl. J. Med.* **304:** 313–318.

WHITLEY, R. J., YEAGER, A., KARTUS, P., BRYSON, Y., CONNOR, J. D., ALFORD, C. A., NAHMIAS, A. and SOONG, S. J. (1983) Neonatal herpes simplex virus infection: Follow-up evaluation of vidarabine therapy. *Pediatrics* **72:** 778–785.

WHITLEY, R. J., ALFORD, C. A., HIRSCH, M. S., SCHOOLEY, R. T., LUBY, J. P., AOKI, F. Y., HENLEY, D., NAHMIAS, A. J., SOONG, S. J. and THE NIAID COLLABORATIVE ANTIVIRAL STUDY GROUP (1986) Vidarabine versus acyclovir therapy in herpes simplex encephalitis. *New Engl. J. Med.* **314:** 144–149.

WICKENDEN, C., MALCOLM, A. D. B., STEELE, A. and COLEMAN, D. V. (1985) Screening for wart virus infection in normal and abnormal cervices by DNA hybridization of cervical scrapes. *Lancet* **i:** 65–67.

WILFERT, C. M., HUANG, E. S. and STAGNO, S. (1982) Restriction endonuclease analysis of cytomegalovirus deoxyribonucleic acid as an epidemiologic tool. *Pediatrics* **70:** 717–721.

WOLTERS, G., KUIJPERS, L., KACAKI, J. and SCHUURS, A. (1976) Solid phase enzyme-immunoassay for detection of hepatitis B surface antigen. *J. Clin. Pathol.* **29:** 873–879.

WONG, D. T., WELLIVER, R. C., RIDDLESBERGER, K. R., SUN, M. S. and OGRA, P. L. (1982) Rapid diagnosis of parainfluenza virus infection in children. *J. Clin. Microbiol.* **16:** 164–167.

WONG-STAAL, F., HAHN, B., MANZARI, V., COLOMBINI, S., FRANCHINI, G., GELMAN, E. P. and GALLO, R. C. (1983) A survey of human leukaemias for sequences of a human retrovirus. *Nature* **302:** 626–628.

YALOW, R. W. and BERSON, S. A. (1960) Immunoassay of endogenous plasma insulin in man. *J. Clin. Invest.* **39:** 1157–1175.

YARCHOAN, R. and BRODER, S. (1987) Development of antiretroviral therapy for the acquired immunodeficiency syndrome and related disorders. *New Engl. J. Med.* **316:** 557–564.

YARCHOAN, R., BERG, G., BROUWERS, P., FISCHL, M. A., SPITZER, A. R., WICHMAN, A., GRAFMAN, J., THOMAS, R. V., SAFAI, B., BRUNETTI, A., PERNO, C. F., SCHMIDT, P. J., LARSON, S. M., MYERS, C. E. and BRODER, S. (1987) Response of human-immunodeficiency-virus-associated neurological disease to 3′-azido-3′-deoxythymidine. *Lancet* **i:** 132–135.

YOLKEN, R. H. (1980) Enzyme-linked immunosorbent assay (ELISA) a practical tool for rapid diagnosis of viruses and other infectious agents. *Yale J. Biol. Med.* **53:** 85–92.

YOLKEN, R. H. and TORSCH, V. M. (1980) Enzyme-linked immunosorbent assay for detection and identification of coxsackie A viruses. Abstract of the 80th Annual Meeting of the American Society for Microbiology, May 11–16, p. 222.

YOLKEN, R. H., KIM, H. W., CLEM, T., WYATT, R. G., CHANOCK, R. M., KALICA, A. R. and KAPIKIAN, A. Z. (1977) Enzyme-linked immunosorbent assay (ELISA) for detection of human reovirus-like agent of infantile gastroenteritis. *Lancet* **ii:** 263–266.

YOUNG, J. F. and PALESE, P. (1979) Evolution of human influenza A viruses in nature: Recombination contributes to genetic variation of HlNl strains. *Proc. Natn. Acad. Sci. U.S.A.* **76:** 6547–6551.

YOUNKIN, S. W., BETTS, R. F., ROTH, R. K. and DOUGLAS, R. G. (1983) Reduction in fever and symptoms in young adults with influenza A/Brazil/78 HlNl infection after treatment with aspirin or amantadine. *Antimicrob. Agents Chemother.* **23:** 577–582.

YOW, M. D., LAKEMAN, A. D., STAGNO, S., REYNOLDS, R. B. and PLAVIDAL, F. J. (1982) Use of restriction enzymes to investigate the source of a primary cytomegalovirus infection in a pediatric nurse. *Pediatrics* **70:** 713–716.

ZURHEIN, G. and CHOU, S. M. (1965) Particles resembling papovaviruses in human cerebral demyelinating disease. *Science* **148:** 1447–1479.

Books for supplementary reading

GALASSO, G. J., MERIGAN, T. C. and BUCHANAN, R. A. (1985) *Antiviral Agents and Viral Diseases of Man.* Raven Press, New York.

HSIUNG, G. D. (1982) *Diagnostic Virology*, 3rd Edn. Yale University Press. New Haven, Conn.

HSIUNG, G. D. and GREEN, R. H. (eds) (1978) *CRC Handbook Series in Clinical Laboratory Science. Section H: Virology and Rickettsiology.* CRC Press. West Palm Beach, Fl.

LENNETTE, D. A. and THOMPSON, K. D. (eds) (1979) *Diagnosis of Viral Infections: The Role of the Clinical Laboratory.* University Park Press, Baltimore, MD.

LENNETTE, E. H. and SCHMIDT, N. J. (eds) (1979) *Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections*, 5th Edn. American Public Health Association. Washington, DC.