Archives of Virology 83, 3—15 (1985)

Archives of Virology
© by Springer-Verlag 1985

Nucleic Acid Probes in Diagnosis of Viral Diseases of Man

Brief Review

By

J. K. Kulski[1] and Mary Norval[2]

[1] Department of Microbiology, The Queen Elizabeth II Medical Centre, Nedlands, Western Australia, Australia

[2] Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh, Scotland, United Kingdom

Accepted May 22, 1984

Summary

With the recent, rapid advances in recombinant DNA technology, it has become possible to consider the use of nucleic acid probes in diagnosis of human viral diseases. Several examples are discussed which employ techniques of dot blot hybridization, sandwich hybridization and *in situ* hybridization. Typing of viral strains using restriction endonuclease digestion as an epidemiological tool is considered. Finally, the present limitations of molecular hybridization are discussed, and future developments including the production of non-radioactively labeled probes, are assessed.

Introduction

Recent advances in the area of recombinant DNA technology have led to the possibility of using nucleic acid probes in the diagnosis of viral infections in man. Integrated viral sequences may be assayed, viral nucleic acids quantitated and epidemiology carried out on a molecular level. Hybridization methods may be applied to aetiopathogenic studies particularly to viral infections where viral genome is the only remaining evidence of virus infection acquired in the distant past. In addition, techniques of *in situ* hybridization permit viral nucleic acid to be detected in specific cells of a tissue section. The specificity and speed of hybridization should allow this method to be a valuable adjunct to the conventional methods used at present, such as infection of susceptible cells and growth of virus in tissue culture, electron microscopy of specimens, examination for viral antigens by using immunofluorescence or immunoenzyme conjugates, radioimmunoassay or ELISA, and serological assays (1, 16).

Three methods of recombinant DNA technology have important applications for viral diagnosis. These are molecular cloning, nucleic acid hybridization and the use of restriction enzymes to cut DNA at specific sites (20, 34). This paper outlines some of the methods currently used to prepare the viral nucleic acid probes; then consideration is given to several methods of rapid diagnosis, viral histopathology and epidemiological studies using such reagents. Finally, the future of such a molecular approach is discussed.

Preparation of Nucleic Acid Probes

Molecular cloning procedures have been especially useful for obtaining abundant supplies of those viral nucleic acid probes which cannot be propagated in tissue culture systems, such as hepatitis B virus (HBV) and human papilloma virus (HPV). These two viruses which have genomes of less than 10 kilobases have been cloned using plasmid vectors (5, 12, 17, 30). With larger M.W. viral DNAs, a collection of recombinant plasmids, bacteriophage or cosmid clones have been made containing viral DNA fragments which collectively represent the entire genome. Some of the human viral genomes that have been cloned in this way are vaccinia (3), cytomegalovirus (CMV) (21), *Herpes simplex* virus type 1 (HSV-1) (25) and type 2 (HSV-2) (24), Epstein-Barr virus (EBV) (57) and adenovirus (50). The cloned viral DNA fragments can be further digested with restriction endonucleases and then sub-cloned into plasmid vectors. The collection of different cloned fragments, called genomic libraries, are an important source of defined and undefined DNA sequences for mapping and use as diagnostic probes. Whereas plasmids, most bacteriophages and cosmids yield double-stranded (ds) recombinant probes, single-stranded (ss) recombinant probes can be obtained using M13 phage systems (41).

DNA probes also have been obtained from RNA viruses such as rotavirus (51) and influenza A (13) by synthesizing a complementary DNA (cDNA) from the viral RNA using the enzyme "reverse transcriptase". After synthesizing the homologous strand, the cDNA can be inserted into a bacterial plasmid or phage and molecularly cloned to produce recombinant DNA probes.

Viral diagnostic probes are also prepared directly from viruses which have been propagated in eukaryotic cell-culture systems (43). However, the disadvantages of using eukaryotic cells are that the yield of virus from productive infections may be very low and viral nucleic acids may be contaminated with cellular nucleic acids during the isolation procedure. Extensive genomic drift also may occur on prolonged maintenance of virus in culture especially for RNA viruses (27).

Applications to Rapid Diagnosis

Several methods have been published recently which illustrate the potential of using nucleic acid probes in viral diagnosis. In all of these,

Table 1. *Comparison of conventional detection methods and hybridization*

Virus group	Conventional detection	Detection by hybridization and RE fragment analysis	Refs.
CMV	Growth in tissue culture (several weeks) or EM. No method of distinguishing types.	Dot blot hybridization (24 hours), 5 pg vDNA detectable, quantitative. Analysis of type by RE digestion.	1, 5, 28, 48
HSV	Growth in tissue culture (several days), EM, IF, RIA or ELISA. Types distinguished serologically or by biological properties.	Dot blot hybridization (20 hours), vDNA from less than 10^3 cells detectable.	49
		Analysis of type by RE digestion or by membrane hybridization under different conditions.	10, 26, 32
EBV	Heterophil antibody test, abnormal large lymphocytes, IF and CF.	Dot blot hybridization, 6 pg vDNA detectable (20 hours).	6
Adenovirus	Growth in tissue culture (several days), RIA or IF. Types distinguished by neutralization or CF.	Sandwich hybridization (several hours), 0.2 ng vDNA detectable. Analysis of type by RE digestion or by hybridization under different conditions.	1, 14, 39, 52, 53
HBV	Antigens and antibodies detected by RIA and ELISA.	Dot blot hybridization (24 hours).	4
		1—10 pg vDNA detectable, quantitative.	8, 44
	No cell culture system available to propogate virus.	Southern blotting and hybridization after RE digestion (48 hours). Integrated and free vDNA detectable.	
HPV	Free virus detected by EM or IF but difficult to find.	Analysis of type by RE digestion.	17, 18
	No cell culture system available to propogate virus.	Southern blotting and hybridization under different conditions.	30, 33
Rotavirus	Detection by EM, PAGE, RIA or ELISA.	Dot blot hybridization (several hours), 8 pg vRNA	22
		detectable, 10—100 × more sensitive than ELISA. Analysis of type by Northern blotting under different conditions of hybridization.	51

v viral; EM electron microscopy; ELISA enzyme linked immunosorbent assay; IF immunofluorescence; RIA radioimmunoassay; CF complement fixation; RE restriction endonuclease; PAGE polyacrylamide gel electrophoresis

radioactively labeled probes have been prepared and used for DNA-DNA hybridization in solution or on a solid support. A comparison of conventional and hybridization procedures for the detection of several different viral groups is summarized in Table 1.

One of the first reports on the use of hybridization to type HSV isolates was by Brautigam *et al.* (7); DNA-DNA hybridization was performed in solution with the products being analyzed by hydroxyapatite chromatography. A simpler hybridization method which can detect and type HSV DNA from less than a 1000 infected cells has since been described (49). DNA extracted from cells grown in one roller tube was alkali-denatured and immobilized on nitrocellulose membranes by dot blotting. This enabled a large number of samples to be processed simultaneously. A probe was made from ^{32}P-labeled DNA fragment of HSV-1. Low stringent washing of membranes after hybridization permitted the detection of both HSV-1 and HSV-2. Rewashing of membranes under conditions of high stringency, where only a few base mis-matches are tolerated (1), allowed discrimination between types 1 and 2 as no hybridization occurred with the latter. In this study, a *Hind*III-*Eco*RI DNA fragment was used as probe although its precise location in the HSV genome was not known (49). HSV probes for hybridization need to be selected with care as HSV-1 fragments cross-hybridize to HSV-2 with varying degrees (37) and homologies may occur between viral DNA of the herpes group and cellular DNA (and mRNAs) (38). Traditionally, HSV isolates are typed by determining differences in biological properties which are not always clear cut. Serological assays are also used and recently, monoclonal antibodies have been produced that can distinguish between HSV types 1 and 2 (35).

Other dot blot hybridizations have been carried out successfully. In 1980, Brandsma and Miller reported the screening of lymphoid cells for the presence of EBV (6). The cells were spotted on nitrocellulose filters, the DNA denatured and hybridization carried out with EBV-DNA, radiolabeled by nick translation. The sensitivity was 0.06 to 0.6 genome equivalents per cell and the method quantitative. Hybridizations are of particular importance in the study of viral infections such as EBV in which the infection is either nonproductive or virions are released in low quantity.

Flores *et al.* (22) described a dot blot technique for rotaviruses which are normally detected by electron microscopy, analysis of RNA on polyacrylamide gel electrophoresis, RIA or ELISA. The stool specimens were pre-treated with fluorocarbon and extracted with phenol before denaturation by boiling and dotting on nitrocellulose membranes. However, it was also shown that the pre-treatment procedures, apart from boiling, were not essential. Hybridization was carried out using ss rotavirus RNA labeled with ^{32}P-GTP by *in vitro* transcription or with ^{125}I by chemical iodination. Good correlation was obtained with diagnosis by other methods. There was

sufficient cross-homology between different strains of rotavirus that detection of unknown strains in the specimens was possible. Asymptomatic carriers were found. The limit of detection of 8 pg of viral RNA was 10 to 100 times more sensitive than the ELISA test. Although the dot blot was very sensitive and enabled rapid screening of a large number of samples, it lacked specificity within the rotavirus genus (22). Information on the diversity of strains can be further obtained by polyacrylamide gel electrophoresis and Northern blot hybridization as human strains differ in their RNA migration patterns (51).

Increasingly, programs are being undertaken to screen for the presence of CMV in urine or other specimens from immunosuppressed patients who are receiving bone marrow, heart and kidney transplants. By conventional methods of *in vitro* culture, clinical CMV isolates often produce recognizable cytopathic effects only after several weeks and quantitation by plaque assay is too slow to be of any benefit to a patient receiving therapy. Recently, a dot blot technique has been used to detect human CMV in urine samples that were clarified, then ultracentrifuged to deposit any virus (1, 15). The pellet was alkali-denatured, neutralized and immobilized on nitrocellulose filters before hybridization was carried out with a ^{32}P-labeled CMV DNA probe containing an *Eco*RI fragment representing 1/10 of the viral genome. This assay was rapid (being completed in one day), sensitive (detecting 5 pg viral DNA) and quantitative so that the impact of antiviral therapy on the patient could be easily measured. A similar dot blot procedure has been used to detect adenovirus types (1, 14), HSV types (49), HBV (4) and enteroviruses (29) in biological specimens.

A different hybridization system has been described to detect HBV DNA in liver and serum and to determine the presence of integrated and free viral DNA in liver cells (8). DNA was extracted from needle biopsies of liver; some was digested with the restriction endonuclease *Hind*III, and the fragments separated by electrophoresis on agarose before being transferred to a nitrocellulose membrane by the Southern blot technique (47). The hybridization was carried out using cloned HBV DNA labeled with ^{32}P by nick translation and denatured. One band (3.2 kilobase position) was found in the *Hind*III digested and undigested samples from all HBe antigen-positive patients which demonstrated unintegrated viral DNA. In addition, bands corresponding to high M.W. DNA were found in some digested samples indicating integration of HBV sequences. Serum samples were phenol extracted and assayed in a similar manner for free viral DNA. This was a more sensitive assay for serum infectivity than radioimmunoassay for HBe antigen. Thus, viral DNA replication, serum infectivity, integration, and the early stages of infection and its progression may be studied using hybridization. Also, the selection and monitoring of patients for antiviral therapy can be better assessed as it seems unlikely that drugs such as adenine arabinoside or interferon could affect integrated viral genomes. Recently,

the state of HBV DNA in various childhood liver diseases has been examined in a similar way (44).

A new test called sandwich hybridization has been described which is an adaptation of the dot blot hybridization (1, 39, 52). This has been applied to the detection of adenovirus in nasopharyngeal mucus aspirates. To test for subgroup C viruses, a fragment (29—42 per cent map position) of cloned adeno-2 was denatured and immobilized on nitrocellulose filters. It was hybridized with denatured DNA from the aspirate in the presence of the probe, ^{125}I-labeled ss adeno-2 fragment (42—45.3 per cent map position) which had been cloned in the phage M13 mp7 (39). Where the aspirate DNA was homologous to adeno-2, it annealed to both filter and probe, thus binding the probe to the filter. The sensitivity of the technique (5×10^6 molecules or 0.2 ng of adenovirus DNA) was similar to that of a radioimmunoassay for viral hexon protein (1). It is possible to manipulate the probes so that reagents may be prepared with either group or strain specificity and the technique is quantitative if suitable standards are available (1, 39, 52).

Human leukemias and lymphomas are currently being surveyed for sequences homologous to human T-cell leukemia virus DNA using probes prepared from the viral genome and provirus (56).

Applications to Viral Histopathology

Although *in situ* hybridization is not applied at the moment to rapid viral diagnosis, several examples illustrate its potential and its present use in histopathology. It is of particular value where a small proportion of cells in a population is infected or where the histological and pathological picture can be correlated with the distribution of viral genomes. In addition to localization of viral genomes, expression of viral mRNA may be monitored.

Burrell *et al.* (11) used *in situ* hybridization to examine the replication of HBV in liver sections from patients with chronic hepatitis. ^{3}H-labeled cloned HBV DNA was used as a probe to detect HBV DNA in single cells. They were able to show focal infection suggesting cell-to-cell spread of virus, and an association between HBV replication and hepatocyte injury at the cellular level. From these results they suggested that patients showing spread of virus would be the ones most likely to benefit from treatment with antiviral agents.

Some work has been done to detect viral genomes in various neurological conditions of unknown etiology using *in situ* hybridization. Sequiera *et al.* (45) showed that HSV type 1 DNA was present in cells from brain smears of several patients who had died with chronic psychiatric illness and neuropathological changes. In a similar way HSV mRNA was detected in ganglion cells in sections of paravertebral sympathetic ganglia at autopsy (23). Only a small percentage of neurons within the ganglia were positive. *In situ*

hybridization has also been used to detect viral DNA or mRNA in various neoplasms such as epidermodyplasia verruciformis (36), Burkitt's lymphoma (58), nasopharyngeal carcinoma (55) and cervical carcinoma (19).

The biggest drawback at the moment to using *in situ* hybridization as a tool for rapid viral diagnosis is the time taken for the autoradiograph to develop to visualize viruses in tissue sections. This may be in the order of several days for ^{125}I and ^{32}P probes, and up to several months for ^{3}H probes. If labeling with non-radioactive reagents such as antisera to nucleic acid hybrids (40), biotin (31) or fluorochrome (2) can be further developed, then more applications for *in situ* hybridization would become apparent. A start has been made in this direction by BRIGATI *et al.* (9) who reported the use of biotin-labeled DNA or RNA probes to detect viral genomes both in tissue culture (parvovirus, polyomavirus, HSV, adenovirus, retrovirus) and in paraffin sections of tissue (HSV and adenovirus). The whole procedure was completed in less than 24 hours, although the sensitivity was not equal, as yet, to autoradiographic methods.

Applications to Viral Epidemiology

It is often important to be able to trace the source of a virus which causes an infection, to test the relatedness of different strains or to follow the genomic changes over a period of time. Restriction endonuclease digestion followed by electrophoretic separation of fragments has allowed such studies to be made in recent years. This may be allied to Southern and Northern blotting, and subsequent hybridization with nucleic acid probes.

As serological reagents which are able to distinguish between types of CMV are not available, molecular epidemiological studies have provided valuable information in this area. Restriction endonuclease digestion of CMV from random clinical isolates has indicated that no 2 viruses generate the same pattern of DNA fragments, if more than one enzyme is used. Thus, HUANG *et al.* (28) showed that endogenous virus carried by mothers during pregnancy was the most frequent source of recurrent infection, and of transmission to their babies. Re-infection with another strain occurred rarely. In much the same way SPECTOR (48) demonstrated that CMV could be spread from one baby who had viruria to another in a hospital environment. This result has obvious implications for management of patients in renal and bone marrow transplant units and in pediatric wards.

HSV isolates have also been typed (32) and classified using restriction endonuclease digestion (1) as have some adenoviruses which are not clearly identified by serological methods (53). Genome mapping has provided valuable information about the source of herpetic infection in nosocomial outbreaks (10) in temporal clusters of encephalitis cases (26) and in monitoring transmission of virus from one patient to another (46).

The analysis of diversity in strains of viruses has been illustrated by human rotaviruses isolated over a period of several years (51). The segmented viral RNAs were separated by polyacrylamide gel electrophoresis, transferred by Northern blotting to diazobenzyloxymethyl (DBM) paper and hybridized with cDNA probes under conditions of low stringency (34 per cent base mis-match tolerated) and high stringency (no more than 8 per cent base mis-match tolerated). A distinct advantage of using DBM paper instead of nitrocellulose paper is that it may be used with various probes. This method permits strains to be compared in retrospect or as new clones become available. In addition, RNA electrophoretic patterns of viral isolates can be visualized directly on gels by using ethidium bromide or a sensitive silver staining procedure (54).

A further example of the use of hybridization and restriction endonuclease analysis has been to distinguish human papilloma virus (HPV) types of which 25 are presently recognized and to associate each with a particular type of lesion (18, 33). The recent discovery of HPV-16 in cervical tumors of women using molecularly cloned viral DNA probes, HPV-6 or HPV-11, demonstrates the value of employing hybridization conditions of varying stringencies to detect and isolate new types of virus (18). Free HPV has not been found in cervical tumors using traditional techniques of viral diagnosis, such as EM, immunofluorescence or cell culture systems to propogate virus. On the other hand, many cases of non-productive viral infection and new types of virus (HPV-6, 10, 11, 16 and 18) have been identified in cervical disease using recombinant DNA procedures (42).

Present Limitations and Future Developments

This review has illustrated the significant contribution which molecular hybridization and restriction enzyme fragment analysis can make to diagnostic virology and the examples given indicate the wide ranging applications in this area, especially when allied with other techniques. Cloning and characterization of the recombinant DNAs for use as diagnostic probes involve methods which are complex and time-consuming. It is clear that their preparation will be done in research centers and molecular biology companies. The availability of commercial diagnostic kits should enable hybridization techniques to become valuable adjuncts to the more traditional diagnostic methods.

At the moment, probes are normally radio-labeled to high specific activity with γ-emitters. This procedure is expensive, produces probes of short half-life and creates difficulties in handling in a routine diagnostic laboratory. The replacement of the radioactive label by other tracer substances of at least equal sensitivity is an obvious area for development. This should enable preparations of safer and more convenient reagents which are better suited to a diagnostic laboratory. Three alternatives are currently

being tested. The first is to use antibodies against the nucleic acid hybrids which would then be detected by immunocytochemistry or ELISA (40). Secondly, biotin can be covalently attached to the C-5 of pyrimidine and incorporated into the probe *in vitro*. This may then be detected using immunofluorescence, immunoperoxidase, avidin conjugated to an enzyme or affinity chromatography, based on the specific interaction between biotin and antibiotin IgG or avidin (31). Thirdly, fluorescein and rhodamine have been coupled to the 3′-terminus of the probe giving advantages in speed and resolving power for *in situ* hybridizations (2).

It would be useful if nucleic acid probes could be generated with various specificities, such as group, type or "virulence", and these quantitated under hybridization conditions of varying stringencies. Such methods are of special interest when viruses cannot be cultured *in vitro*, when they grow very slowly, when there are no antigenic markers to type them or when numbers of samples are very large. Dots of specimen DNAs or RNAs may be prepared in countries not equipped to do hybridizations and then posted to some central laboratory where the probes are available. Pre-treatment of samples before application to nitrocellulose paper leads to some extra work but has not proved essential in some systems. A distinct advantage of dot blotting is that it allows viral sequences to be assayed either singly or as numerous samples, simultaneously. *In situ* hybridization techniques may have important applications in the field of histopathology, especially if paraffin embedded sections of tissue can be used. Integrated viral genomes can be detected which may not be expressed and therefore not demonstrable by other methods. Such studies, including the ability to characterize single cells in a tissue section, may be particularly useful in the area of tumor etiology and in disorders of unknown etiology.

Epidemiology using restriction endonuclease digestion and molecular hybridization has revealed important information for virus groups which cannot readily be typed in other ways or for which detailed genetic analysis is required. The way is now clear for antigenic relatedness, genetic mapping and pathogenesis to be compared and correlated.

Acknowledgements

J. K. Kulski is supported by the National Health and Medical Research Council (Australia). Mary Norval wishes to acknowledge the financial assistance of the Cancer Research Campaign (U.K.) and a visiting Research Fellowship of the University of Western Australia.

References

1. Bachmann, P. A. (ed.): New Developments in Diagnostic Virology. (Curr. Top. Microbiol. Immun., Vol. 104.) 1983.
2. Bauman, J. G., Wiegant, J., Van Duijn, P.: Cytochemical hybridization with

fluorochrome-labeled RNA. II. Applications. J. Histochem. Cytochem. **29**, 238—246 (1981).

3. Belle-Isle, H., Venkatesan, S., Moss, B.: Cell-free translation of early and late mRNAs selected by hybridization to cloned DNA fragments derived from the left 14 million to 72 million daltons of the vaccinia virus genome. Virology **112**, 306—317 (1981).
4. Berninger, M., Hammer, M., Hoyer, B., Gerin, J. L.: An assay for the detection of the DNA genome of hepatitis B virus in serum. J. Med. Virol. **9**, 57—68 (1982).
5. Bichko, V. V., Kozlovskaya, T. M., Dishler, A., Pumpen, P., Janulaitis, A., Gren, E. J.: Restriction map of the hepatitis B virus DNA cloned in *Escherichia coli*. Gene **20**, 481—484 (1982).
6. Brandsma, J., Miller, G.: Nucleic acid spot hybridization: rapid quantitative screening of lymphoid cell lines for Epstein-Barr viral DNA. Proc. Natl. Acad. of Sci., U.S.A. **77**, 6851—6855 (1980).
7. Brautigam, A. R., Richman, D. D., Oxman, M. N.: Rapid typing of Herpes simplex virus isolates by deoxyribonucleic acid: deoxyribonucleic acid hybridization. J. Clin. Microbiol. **12**, 226—234 (1980).
8. Brechot, C., Hadchouel, M., Scotto, J., Degos, F., Charnay, P., Trepo, C., Tiollais, P.: Detection of hepatitis B DNA in liver and serum: a direct appraisal of the chronic carrier state. Lancet **ii**, 765—768 (1981).
9. Brigati, D. J., Myerson, D., Leary, J. J., Spalholz, B., Travis, S. Z., Fong, C. K., Hsiung, G. D., Ward, D. C.: Detection of viral genomes in cultured cells and paraffin-embedded tissue sections using biotin-labeled hybridization probes. Virology **126**, 32—50 (1983).
10. Buchman, T. G., Roizman, B., Adams, G., Stover, B. H.: Restriction endonuclease fingerprinting of herpes simplex DNA. A novel epidemiological tool applied to nosocomial outbreak. J. Infect. Dis. **138**, 488—498 (1978).
11. Burrell, C. J., Gowans, E. J., Jilbert, A. R., Lake, J. R., Marmion, B. P.: Hepatitis B virus DNA detection by *in situ* cytohybridization: implications for viral replication strategy and pathogenesis of chronic hepatitis. Hepatol. **2**, 85S—91S (1982).
12. Burrell, C. J., Mackay, P., Greenaway, P. J., Hofschneider, P. H., Murray, K.: Expression in *Escherichia coli* of hepatitis B virus DNA sequences cloned in plasmid pBR322. Nature **279**, 43—47 (1979).
13. Caton, A. J., Robertson, J. S.: New procedures for production of influenza virus-specific double-stranded DNA's. Nucleic Acids Res. **7**, 1445—1456 (1979).
14. Chiba, S., Nakata, S., Nakamura, I., Taniguchi, K., Urasawa, S., Fujinaga, K., Nakao, T.: Outbreak of infantile gastroenteritis due to type 40 adenovirus. Lancet **2**, 954—957 (1983).
15. Chou, S., Merigan, T. C.: Rapid detection and quantitation of human cytomegalovirus in urine through DNA hybridization. New Eng. J. Med. **308**, 921—925 (1983).
16. Davis, B. D., Dulbecco, R., Eisen, H. N., Ginsberg, H. S.: Microbiology, 3rd ed. Hagerstown, Md.: Harper and Row 1980.
17. De Villiers, E.-M., Gissmann, L., Zur Hausen, H.: Molecular cloning of viral DNA from human genital warts. J. Virol. **40**, 932—935 (1981).
18. Durst, M., Gissmann, L., Ikenberg, H., Zur Hausen, H.: A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. Proc. Natl. Acad. Sci., U.S.A. **80**, 3812—3815 (1983).
19. Eglin, R. P., Sharp, F., Maclean, A. B., Macnab, J. C., Clements, J. B.,

WILKIE, N. M.: Detection of RNA complementary to Herpes simplex virus DNA in human cervical squamous cell neoplasms. Cancer Res. **41**, 3597—3603 (1981).

20. EMERY, A. E.: Recombinant DNA technology. Lancet **ii**, 1406—1409 (1981).
21. FLECKENSTEIN, B., MULLER, I., COLLINS, J.: Cloning of the complete human cytomegalovirus genome in cosmids. Gene **18**, 39—46 (1982).
22. FLORES, J., BOEGGEMAN, E., PURCELL, R. H., SERENO, M., PEREZ, I., WHITE, L., WYATT, R. G., CHANOCK, R. M., KAPIKIAN, A. Z.: A dot hybridization assay for detection of Rotavirus. Lancet **i**, 555—559 (1983).
23. GALLOWAY, D. A., FENOGLIO, C., SHEVCHUK, M., McDOUGALL, J. K.: Detection of Herpes simplex RNA in human sensory ganglia. Virology **95**, 265—268 (1979).
24. GALLOWAY, D. A., SWAIN, M.: Cloning of *Herpes simplex* virus type 2 DNA fragments in a plasmid vector. Gene **11**, 253—257 (1980).
25. GOLDIN, A. L., SANDRI-GOLDIN, R. M., LEVINE, M., GLORIOSO, J. C.: Cloning of *Herpes simplex* type 1 sequences representing the whole genome. J. Virol. **38**, 50—58 (1981).
26. HAMMER, S. M., BUCHMAN, T. G., D'ANGELO, L. J., KARCHNER, A. W., ROIZMAN, B., HIRSCH, M. S.: Temporal clusters of herpes simplex encephalitis investigation by restriction endonuclease cleavage of viral DNA. J. Infect. Dis. **141**, 436—440 (1980).
27. HOLLAND, J., SPINDLER, K., HORODYSKI, F., GRABAU, E., NICHOL, S., VANDEPOL, S.: Rapid evolution of RNA genomes. Science **215**, 1577—1585 (1982).
28. HUANG, E.-S., ALFORD, C. A., REYNOLDS, D. W., STANGO, S., PASS, R. F.: Molecular epidemiology of cytomegalovirus infections in women and their infants. New Eng. J. Med. **303**, 958—962 (1980).
29. HYYPIA, T., STALHANDSKE, P., VAINIONPAA, R., PETTERSSON, U.: Detection of enteroviruses by spot hybridization. J. Clin. Micro. **19**, 436—438 (1984).
30. KREMSDORF, D., JABLONSKA, S., FAVRE, M., ORTH, G.: Biochemical characterization of two types of human papillomavirus associated with epidermodysplasia verruciformis. J. Virol. **43**, 436—447 (1982).
31. LANGER, P. R., WWALDROP, A. A., WARD, D. C.: Enzymatic synthesis of biotin-labeled polynucleotides: novel nucleic acid affinity probes. Proc. Natl. Acad. of Sci., U.S.A. **78**, 6633—6637 (1981).
32. LONSDALE, D. M.: A rapid technique for distinguishing herpes simplex virus type 1 from type 2 by restriction-enzyme technology. Lancet **1**, 849—852 (1979).
33. LUTZNER, M. A.: The human papillomaviruses. Arch. Dermatol. **119**, 631—635 (1983).
34. MANIATIS, T., FRITSCH, E. F., SAMBROOK, J.: Molecular cloning, a laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory 1982.
35. NOWINSKI, R. C., TAM, M. R., GOLDSTEIN, L. C., STONG, L., KUO, C. C., COREY, L., STAMM, W. E., HANDSFIELD, H. H., KNAPP, J. S., HOLMES, K. K.: Monoclonal antibodies for diagnosis of infectious diseases in humans. Science **219**, 637—644 (1983).
36. ORTH, G., FAVRE, M., BREIBURD, F., CROISSANT, O., JABLONSKA, S., OBALEK, S., JARZABEK-CHORZELSKA, M., RZESA, G.: Epidermodysplasia verruciformis: a model for the role of papilloma viruses in human cancer, pp. 259—282. In: ESSEX, M., TODARO, G., ZUR HAUSEN, H. (eds.), Viruses in Naturally Occurring Cancers. Cold Spring Harbor: Cold Spring Harbor Laboratory 1980.
37. PEDEN, K., MOUNTS, P., HAYWARD, G. S.: Homology between mammalian cell DNA sequences and human herpesvirus genomes detected by a hybridization procedure with high-complexity probe. Cell **31**, 71—80 (1982).

38. Puga, A., Cantin, E. M., Notkins, A. L.: Homology between murine and human cellular DNA sequences and the terminal repetition of the S component of Herpes Simplex Virus type 1 DNA. Cell **31**, 81—87 (1982).
39. Ranki, M., Palva, A., Virtanen, M., Laaksonen, M., Soderlund, H.: Sandwich hybridization as a convenient method for the detection of nucleic acids in crude samples. Gene **21**, 77—85 (1983).
40. Reddy, A. R., Sofer, W.: A rapid procedure to detect *in situ* DNA/RNA hybrids. Biochem. Biophys. Res. Commun. **103**, 959—967 (1981).
41. Ricca, G. A., Taylor, J. M., Kalinyak, J. E.: Simple rapid method for the synthesis of radioactively labeled cDNA hybridization probes utilizing bacteriophage M13mp7. Proc. Natl. Acad. Sci., U.S.A. **79**, 724—728 (1982).
42. Richart, R. M., Ferenczy, A., Meisels, A., Syrjanen, K., Zur Hausen, H.: Condyloma virus and cervical cancer—how strong a link? Contemp. Obstet. Gynec. **23**, 210—224 (1984).
43. Rigby, P. W.: Cloning vectors derived from animal viruses. J. gen. Virol. **64**, 255—266 (1983).
44. Scotto, J., Hadchouel, M., Hery, C., Alvarez, F., Yvart, J., Tiollais, P., Bernard, O., Brechot, C.: Hepatitis B virus DNA in children's liver diseases: detection by blot hybridization in liver and serum. Gut **24**, 618—624 (1983).
45. Sequiera, L. W., Jennings, L. C., Carrasco, L. H., Lord, M. A., Curry, A., Sutton, R. N.: Detection of Herpes-simplex viral genome in brain tissue. Lancet **ii**, 609—612 (1979).
46. Smith, I. W., Maitland, N. J., Peutherer, J. F., Robertson, D. H.: Restriction enzyme analysis of herpes-virus-2 DNA. Lancet **ii**, 1424 (1981).
47. Southern, E.: Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**, 503—533 (1975).
48. Spector, S. A.: Transmission of cytomegalovirus among infants in hospital documented by restriction-endonuclease-digestion analyses. Lancet **i**, 378—381 (1983).
49. Stalhandske, P., Pettersson, U.: Identification of DNA viruses by membrane filter hybridization. J. Clin. Microbiol. **15**, 744—747 (1982).
50. Stenlund, A., Perricaudet, M., Tiollais, P., Pettersson, U.: Construction of restriction enzyme fragment libraries containing DNA from human Adenovirus types 2 and 5. Gene **10**, 47—52 (1980).
51. Street, J. E., Croxson, M. C., Chadderton, W. F., Bellamy, A. R.: Sequence diversity of human Rotavirus strains investigated by Northern blot hybridization analysis. J. Virol. **43**, 369—378 (1982).
52. Virtanen, M., Palva, A., Laaksonen, M., Halonen, P., Soderlund, H., Ranki, M.: Novel test for rapid viral diagnosis: detection of Adenovirus in nasopharyngeal mucus aspirates by means of nucleic-acid sandwich hybridization. Lancet **i**, 381—383 (1983).
53. Wadell, G., De Jong, J. C., Wolontis, S.: Molecular epidemiology of adenoviruses: alternating appearance of two different genome types of adenovirus 7 during epidemic outbreaks in Europe from 1958 to 1980. Infect. Immun. **34**, 368—372 (1981).
54. Whitton, J. L., Hundley, F., O'Donnell, B., Desselberger, U.: Silver staining of nucleic acids. Applications in virus research and in diagnostic virology. J. Virol. Methods **7**, 185—198 (1983).
55. Wolf, H., Zur Hausen, H., Becker, V.: EB viral genomes in epithelial nasopharyngeal carcinoma cells. Nature New Biology **244**, 245—247 (1973).

56. Wong-Staal, F., Hahn, B., Manzari, V., Colombini, S., Franchini, G., Gelmann, E. P., Gallo, R. C.: A survey of human leukaemias for sequences of human retrovirus. Nature **302**, 626—628 (1983).
57. Yano, S., Faber, H. E., Lee, Y.-S., Nonoyama, M.: Cloning of Epstein-Barr virus (EBV) DNA fragments in pBR 322 and Charon 3A. Gene **13**, 203—208 (1981).
58. Zur Hausen, H., Schulte-Holthausen, H., Wolf, H., Dorries, K., Egger, H.: Attemps to detect virus-specific DNA in human tumors. II. Nucleic acid hybridization with complementary RNA of human herpes group viruses. Intl. J. Cancer **13**, 657—664 (1974).

Authors' address: Dr. J. K. Kulski, Department of Microbiology, The Queen Elizabeth II Medical Centre, Nedlands, Western Australia 6009.

Received December 22, 1983