0273–2289/86/1203–0301$02.00

PATENTS AND LITERATURE

ROBERT J. LINHARDT

Division of Medicinal Chemistry and Natural Products, University of Iowa, College of Pharmacy, Iowa City, IA 52242

The objective of this section is to keep readers aware of significant inventions and trends in industrial research, as well as to highlight those areas of research that may lead to new biotechnological opportunities. In addition to applications of polysaccharides, covered in the last issue, three other subject areas will be examined in later issues: protein engineering, mammalian cell culture, and microbial transformations. The subject of the third Patents and Literature Section of 1986 is DNA probes for clinical applications.

DNA Probes for Clinical Applications

PATENTS

This section identifies and gives a brief description of patents from the US patent literature from January 1975 to January 1986. The major search headings were deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), with the cross-terms: assay, clinical, probe, reagent, hybridization, and complementary. Both patent abstracts and titles were searched. Copies of US Patents can be obtained for $1.50 each from the Commissioner of Patents and Trademarks, Washington DC 20231.

Falkow, S. and Moseley, S. L.
SPECIFIC DNA PROBES IN DIAGNOSTIC MICROBIOLOGY
US 4,358,535, Nov. 9, 1982
Assignee: Board of Regents of the University of Washington

A method and composition for infectious disease diagnosis and epidemiology is described, involving labeled nucleotide probes complementary to nucleic acid coding for a characteristic pathogen product. Clinical

isolates are cultivated, increasing the number of microorganisms, the resulting colonies lysed and the genome denatured and fixed. Alternatively, clinical samples (stool, sputum, pus, and the like) are spotted onto an inert support. The sample is treated to liberate DNA from microbes in the sample and complexed onto the support. The DNA is denatured and fixed in this process. Subsequently, a labeled polynucleotide probe specific for a DNA sequence characteristic of a pathogenic product suspected of being present in the clinical sample is contacted with the fixed genomic single-stranded nucleic acid under hybridizing conditions. Hybridization of probes to the single stranded nucleic acid is diagnostic of the presence of the pathogen.

Gillespie, D., Brodsky, I., and Bresser, J.
IMMOBILIZATION OF MESSAGE RNA DIRECTLY FROM CELLS ONTO FILTER MATERIAL
US 4,483,920, Nov. 20, 1984
Assignee: Hahnemann University

Message RNA is immobilized directly from cells to filter material. Immobilization is carried out by solubilizing cellular components with a chaotropic salt, passing the solubilized cellular components through a filter that selectively binds messenger RNA, and baking the filter-containing bound messenger RNA. The preferred chaotropic salts are sodium iodide, potassium iodide, or sodium perchlorate. Prior to solubilizing, the cells may be washed and lysed. The bound messenger RNA can be hybridized to a labeled probe and the amount of messenger RNA measured. Prior to baking, the filter-containing bound RNA may be incubated in a solution that acelylates basic protein and other molecules that might interfere with molecular hybridization.

Kiovsky, J. R. and Hendrick, C. L.
APPARATUS FOR NUCLEIC ACID QUANTIFICATION
US 4,526,690, Jul. 2, 1985
Assignee: Millipore Corp.

A multiwell apparatus for the assay of microliter quantities of body fluids is described, which prevents fluid loss by lateral migration or gravity flow through a microporous membrane or ultrafilter. A sample is passed through a membrane to which an antibody has been bonded in order to harvest complementary viral antigens in the sample. The harvested viral antigen is converted to single-stranded DNA or RNA, which is then deposited on a second membrane. The deposited single-stranded DNA or RNA is capable of reacting with labeled DNA or other suitable detection probe and correlated with antigen concentration in the sample.

Lavi, S.
ASSAYS FOR THE DETERMINATION OF CARCINOGENICITY
US 4,532,220, Jul. 30, 1985
Assignee: Yeda Research and Development Co. Ltd.

An assay is described for the determination of the carcinogenicity of chemical and physical agents. A selected line of tumor virus transformed cells is exposed to the agent to be tested. It is then determined whether such exposure induces endogenous viral DNA replication in such cells. This induction is indicative of the carcinogenicity of the tested agent and is determined by *in situ* hybridization. A kit for carrying out such assay is described.

Leder, P.
DETECTING REARRANGED HUMAN c-*myc* DNA FRAGMENTS ASSOCIATED WITH ONCOGENES
US 4,542,096, Sep. 17, 1985
Assignee: President and Fellows of Harvard College

Rearranged DNA fragments associated with oncogenes can be detected by DNA hybridization to a labeled probe comprising DNA fragment subject to rearrangement.

Maas, R.
COLONY HYBRIDIZATION METHOD
US 4,533,628, Aug. 6, 1985
Assignee: New York University

A colony hybridization technique is described in which bacterial colonies are replica-plated onto filter paper, and their DNA is hybridized with labeled DNA containing a specific sequence. A 100-fold increase in sensitivity is obtained by subjecting the colonies to a stream of steam in the presence of alkali for about three minutes prior to hybridization.

Owens, R. A. and Diener, T. O.
SENSITIVE AND RAPID DIAGNOSIS OF VIROID DISEASES AND VIRUSES
US 4,480,040, Oct. 30, 1984
Assignee: The United States of America, represented by the Secretary of Agriculture

A rapid and sensitive method for diagnosing plant viroid diseases and viruses is described. Plant sap is bound to a solid support and the bound sample probed with a radioactively labeled DNA that is complementary to the viroid or nucleic acid of the virus being diagnosed. The ra-

dioactively labeled cDNA hybridizes with that viroid or virus RNA or DNA for which it is specific. The DNA–RNA and DNA–DNA hybrids are detected by autoradiography.

Rippe, D. F.
INSOLUBILIZED DEOXYRIBONUCLEIC ACID (RNA)
US 4,234,563, Nov. 18, 1980
and
US 4,251,514, Feb. 17, 1981
and

METHOD OF DETECTING ANTIBODIES TO HUMAN THYROGLOBULIN
US 4,254,097, Mar. 3, 1981
Assignee: American Hospital Supply Corp.

A method of detecting antibodies to native deoxyribonucleic acid (n-DNA), anti-nuclear antibodies, antibodies to nonhistone-containing protein, rheumatoid factors, and antibodies to thyroglobulin is described. An insoluble conjugate of methylated bovine serum albumin (mBSA)–n-DNA with serum from patients with systemic lupus erythematosus (SLE) or other autoimmune diseases is incubated for a sufficient length of time, the precipitate of flocculate is washed and separated and the supernatant fluid discarded. A fluorescein-labeled anti-immunoglobulin antibody is then added to the flocculate and incubated, washed, and separated. The pellet is suspended in a liquid medium to determine its fluorescence, which is proportional to the concentration of antibodies to deoxyribonucleic acid in serum specimen. The insoluble conjugate of mBSA–n-DNA retains the double-stranded form of n-DNA.

Rubin, H.
DETECTION AND ISOLATION OF ENDORPHIN mRNA USING A SYNTHETIC OLIGODEOXYNUCLEOTIDE
US 4,358,586, Nov. 9, 1982
and
US 4,416,988, Nov. 22, 1983

A synthetic oligodeoxynucleotide complementary to endorphin mRNA and a method of using it to detect and isolate endorphin mRNA and cDNA from human and rabbit pancreas is described. A unique 15-base oligodeoxynucleotide, dCATGAACCCGCCGTA, has been found to be complementary to endorphin mRNA. To isolate endorphin mRNA, total RNA is first extracted from human brain, and mRNA–poly A is isolated. This RNA is then treated with the oligodeoxynucleotide, and the resulting hybridizing RNA is enzymatically converted to endorphin mRNA:cDNA, which can then be purified and used in a conventional manner to produce endorphin by cloning techniques.

Rubin, H.
DETECTION AND ISOLATION OF ENDORPHIN mRNA USING A SYNTHETIC OLIGODEOXYNUCLEOTIDE
US 4,358,586, Nov. 9, 1982
and
US 4,416,988, Nov. 22, 1983

A synthetic oligodeoxynucleotide complementary to endorphin mRNA and a method of using it to detect and isolate endorphin mRNA and cDNA from human and rabbit pancreas is described. A unique 15-base oligodeoxynucleotide, dCATGAACCCGCCGTA, has been found to be complementary to endorphin mRNA. To isolate endorphin mRNA, total RNA is first extracted from human brain, and mRNA–poly A is isolated. This RNA is then treated with the oligodeoxynucleotide, and the resulting hybridizing RNA is enzymatically converted to endorphin mRNA:cDNA, which can then be purified and used in a conventional manner to produce endorphin by cloning techniques.

Shafritz, D. A.
DIAGNOSTIC TEST FOR HEPATITIS B VIRUS
US 4,562,159, Dec. 31, 1985
Assignee: Albert Einstein College of Medicine, A Division of Yeshiva University

A method and test kit are described for detecting the presence of hepatitis B virus in a test specimen containing at least a portion of the DNA of the virus. A test reagent consisting of cloned hepatitis B virus (HBV) DNA that has been repurified by treatment with a restriction enzyme and labeled to high specific activity with a radioactive label. The sample to be tested is fixed to a solid matrix, incubated in the presence of the test reagent under hybridization conditions, and detected by hybridization to the labeled DNA probe. The uncombined HBV DNA (labeled) is removed from the substrate, and the hybridized HBV DNA determined by scintillation counting or by autoradiography of the substrate.

Wahl, G. M. and Stark G. R.
TRANSFER AND DETECTION OF NUCLEIC ACIDS
US 4,302,204, Nov. 24, 1981
Assignee: The Board of Trustees of Leland Stanford Junior University

Improvements in the transfer and detection of separated nucleic acids, both RNA and DNA, are described. For analysis of large DNA, the molecular-weight-segregated fractions of DNA are depurinated and fragmented to provide fractions having less than about two kilobases (kb) as single strands. After resolution, the nucleic acid fractions containing both RNA and DNA are transferred and covalently fixed to a chemically treated support. The immobilized nucleotides are hybridized with labeled nucleotide probes and a volume exclusion agent.

Weinberg, R. A., Tabin, C. J., and Bradley, S. M.
CHARACTERIZATION OF ONCOGENES AND ASSAYS BASED THEREON
US 4,535,058, Aug. 13, 1985
Assignee: Massachusetts Institute of Technology

Experiments designed to define the differences between an oncogene isolated from human bladder cancer cells and its corresponding proto-oncogene are described. By a series of in vitro recombinations, the difference was initially isolated to a 350-kb segment of DNA; sequencing defined the difference as a charge in the Gly(12)codon, causing the p21 protein of the oncogene to contain valine at a location where the p21 protein of the proto-oncogene contained glycine. Assays for detecting carcinogenesis based on such differences are also described. In one type of assay, a restriction enzyme specific for either the altered or nonaltered DNA segment of the genes are employed to detect carcinogenesis. In another type of assay, seralogical reagents, such as antibody specific for either p21 protein expressed from the proto-oncogene or oncogene, or a common site, are described.

Wilson, L. B., Wilson J. T., and Geever, R. F.
METHOD FOR THE DIRECT ANALYSIS OF SICKLE CELL ANEMIA
US 4,395,486, Jul. 26, 1983
Assignee: Medical College of GA Research Institute, Inc.

A direct diagnosis of sickle cell anemia by a restriction endonuclease assay with an enzyme, such as Dde I, which recognizes the nucleotide base sequence, CTNAG (where *N* is any base), for the sickle cell allele (beta(s)gene) through molecular hybridization, is described. Following enzyme cleavage, the resulting DNA restriction fragments are separated by molecular weight and transferred to filter paper. A probe is utilized for hybridization that is complementary to the 5′ end of the beta-globin gene. The banding pattern of individuals with normal hemoglobin shows two bands (approximately 175 and 201 bp), sickle cell trait individuals exhibit an additional band (approximately 376 bp), and individuals with sickle cell anemia show the band at approximately 376 bp, with a concommitant loss of the band at approximately 175 bp. Prenatal and postnatal diagnosis of sickle cell anemia is possible with the present method.

LITERATURE

This section surveys the literature in the area of DNA Probes for clinical applications published from December 1984–January 1986. This section is not intended to be all encompassing and lists only some of the major articles and reviews that appeared during this time period.

REFERENCES

1. Ambinder, R. F., Wingard, J. R., Burns, W. H., Hayard, S. D., Saral, R., Perry, H. R., Santos, and Hayward, G. S. (1985), Detection of Epstein-Barr Virus DNA in Mouthwashes by Hybridization. *J. Clin. Microbiol.* **21,** 353–356.
2. 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.
3. Arthur, R. R., Beckmann, A. M., Li, C. C., Saral, R., and Shah, K. V. (1985), Direct Detection of the Human Papovavirus BK in Urine and Bone Marrow Transplant Recipients: Comparison of DNA Hybridization with ELISA. *J. Med. Virol.* **16,** 29–36.
4. Bar-Joseph, M., Segev, D., Twizer, S., and Rosner, A. (1985), Detection of Avocado Sunblotch Viroid by Hybridization with Synthetic Oligonucleotide Probes. *J. Virol. Methods* **10,** 69–73.
5. Barker, J. M., McInnes, J. L., Murphy, P. J., and Symons, R. H. (1985), Dot-Blot Procedure with [^{32}P]DNA Probes for the Sensitive Detection of Avocado Sunblotch and Other Viroids in Plants. *J. Virol. Methods* **10,** 87–98.
6. 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.
7. Bhasin, V. K., Clayton, C., Trager, W., and Cross, G. A. (1985), Variations in the Organization of Repetitive DNA Sequences in the Genomes of *Plasmodium falciparum* Clones. *Mol. Biochem. Parasitol.* **15,** 149–158.
8. Blum, H. E., Figus, A., Haase, A. T., and Vyas, G. N. (1985), Laboratory Diagnosis of Hepatitis B Virus Infection by Nucleic Acid Hybridization Analyses and Immunohistologic Detection of Gene Products. *Dev. Biol. Stand.* **59,** 125–139.
9. Bodkin, D. K., and Knudson, D. L. (1985), Assessment of Sequence Relatedness of Double-Stranded RNA Genes by RNA–RNA Blot Hybridization. *J. Virol. Methods* **10,** 45–52.
10. Boileau, C. R., d'Hauteville, H. M., and Sansonetti, P. J. (1984), DNA Hybridization Technique to Detect Shigella Species and Enteroinvasive *Escherichia coli*. *J. Clin. Microbiol.* **20,** 959–961.
11. Brahic, M., Smith, R. A. Gibbs, C. J., Garruto, R. M., Tourtellotte, W. W., and Cash, E. (1985), Detection of Picornavirus Sequences in Nervous Tissue of Amyotrophic Lateral Sclerosis and Control Patients. *Ann. Neurol.* **18,** 377–343.
12. Chou, S. W., Roark, L., and Merigan, T. C. (1984), DNA of 21 Clinical Cytomegalovirus Strains Detected by Hybridization to Cloned DNA Fragments of Laboratory Strain AD-169. *J. Med. Virol.* **14,** 263–268.
13. Churchward, G., Belin, D., and Nagamine, Y. (1984), A pSC101-Derived Plasmid Which Shows no Sequence Homology to Other Commonly Used Cloning Vectors. Gene **31,** 165–171.
14. Clewley, J. P. (1985), Detection of Human Parvovirus Using a Molecularly Cloned Probe. *J. Med. Virol.* **15,** 173–181.
15. Cremer, C., Rappold, G., Gray, J. W., Muller, C. R., and Ropers, H. H. (1984), Preparative Dual-Beam Sorting of the Human Y Chromosome and *In Situ* Hybridization of Cloned DNA Probes. *Cytometry* **5,** 572–579.
16. Delozier-Blanchet, C. D., Old, J. M., and Engel, E. (1985), Prenatal Preven-

tion of Drepanocytosis: Analysis of Cellular DNA in 2 Cases. *J. Genet. Hum.* **33,** 171–178.
17. Echeverria, P., Seriwatana, J., Sethabutr, O., and Taylor, D. N. (1985), DNA Hybridization in the Diagnosis of Bacterial Diarrhea. *Clin. Lab. Med.* **5,** 447–462.
18. Fackler, J. C., Nagel, J. E., Adler, W. H., Mildvan, P. T., and Ambinder, R. F. (1985), Epstein-Barr Virus Infection in a Child with Acquired Immunodeficiency Syndrome. *Am. J. Dis. Child.* **139,** 1000–1004.
19. Fournier, J. G., Tardieu, M., Lebon, P., Robain, O., Ponsot, G., Rozenblatt, S., and Bouteille, M. (1985), Detection of Measles Virus RNA in Lymphocytes from Peripheral-Blood and Brain Perivascular Infiltrates of Patients with Subacute Sclerosing Panencephalitis. *N. Engl. J. Med.* **313,** 910–915.
20. Fukushima, M., Okagaki, T., Twiggs, L. B., Clark, B. A., Zachow, K. R., Ostrow, R. S., and Faras, A. J. (1985), Histological Types of Carcinoma of the Uterine Cervix and the Detectability of Human Papillomavirus DNA. *Cancer Res.* **45,** 3252–3255.
21. Garbutt, G. J., Wilson, J. T., Schuster, G. S., Leary, J. J., and Ward, D. C. (1985), Use of Biotinylated Probes for Detecting Sickle Cell Anemia. *Clin. Chem.* **31,** 1203–1206.
22. Geffrotin, C., Popescu, C. P., Cribiu, E. P., Boscher, J., Renard, C., Chardon, P., and Vaiman, M. (1984), Assignment of MHC in Swine to Chromosome 7 by *In Situ* Hybridization and Serological Typing. *Ann. Genet.* (Paris) **27,** 213–219.
23. Gendelman, H. E., Moench, T. R., Narayan, O., Griffin, D. E., and Clements, J. E. (1985), A double Labeling Technique for Performing Immunocytochemistry and *In Situ* Hybridization in Virus Infected Cell Cultures and Tissues. *J. Virol. Methods* **11,** 93–103.
24. Grimont, P. A., Grimont, F., Desplaces, N., and Tchen, P. (1985), DNA Probe Specific for *Legionella pneumophila*. *J. Clin. Microbiol.* **21,** 431–437.
25. Haase, A. T., Gantz, D., Blum, H., Stowring, L., Ventura, P., Geballe, A., Moyer, B., and Brahic, M., (1985), Combined Macroscopic and Microscopic Detection of Viral Genes in Tissues. *Virology* **140,** 201–206.
26. Haase, A. T., Walker, D., Stowring, L., Ventura, P., Geballe, A., Blum, H., Brahic, M., Goldberg, R., and O'Brien, K. (1985), Detection of Two Viral Genomes in Single Cells by Double-Label Hybridization *In Situ* and Color Microradioautography. *Science* **227,** 189–192.
27. Ho, M., Miller, G., Atchison, R. W., Breinig, M. K., Dummer, J. S., Andiman, W., Starzl, T. E., Eastman, R., Griffith, B. P., and Hardesty, R. L., (1985), Epstein-Barr Virus Infections and DNA Hybridization Studies in Posttransplantation Lymphoma and Lymphoproliferative Lesions: The Role of Primary Infection. *J. Infect. Dis.* **152,** 876–886.
28. Hyypia, T., Larsen, S. H., Stahlberg, T., and Terho, P. (1984), Analysis and Detection of Chlamydial DNA. *J. Gen. Microbiol.* **130,** 3159–3164.
29. Hyypia, T. (1985), Detection of Adenovirus in Nasopharyngeal Specimens by Radioactive and Nonradioactive DNA Probes. *J. Clin. Microbiol.* **21,** 730–733.
30. Hyypia, T., Jalava, A., Larsen, S. H., Terho, P., and Hukkanen, V. (1985), Detection of *Chlamydia trachomatis* in Clinical Specimens by Nucleic Acid Spot Hybridization. *J. Gen. Microbiol.* **131,** 975–978.
31. Jeffreys, A. J., Wilson, V., and Thein, S. L. (1985), Individual-Specific "Fingerprints" of Human DNA. *Nature* **316,** 76–79.
32. Jeffreys, A. J., Brookfield, J. F., and Semeonoff, R. (1985), Positive

Identification of an Immigration Test-Case Using Human DNA Fingerprints. *Nature* **317,** 818–819.

33. Kulski, J. K., and Norval, M. (1985), Nucleic Acid Probes in Diagnosis of Viral Diseases of Man. Brief Review. *Arch. Virol.* **83,** 3–15.
34. Landry, M. L., and Fong, C. K. (1985), Nucleic Acid Hybridization in the Diagnosis of Viral Infections. *Clin. Lab. Med.* **5,** 513–529.
35. Loning, T., Ikenberg, H., Becker, J., Gissmann, L., Hoepfer, I., and zur Hausen, H. (1985), Analysis of Oral Papillomas, Leukoplakias, and Invasive Carcinomas for Human Papillomavirus Type Related DNA. *J. Invest. Dermatol.* **84,** 417–420.
36. Love, J., George, J., and Elliot, M. (1985), DNA Probes to Characterize Leukemia and Lymphoma Cells. *Am. Clin. Prod.* **4,** 12–16.
37. McFarlane, R. G., and Thawley, D. G. (1985), DNA Hybridization Procedure to Detect Pseudorabies Virus DNA in Swine Tissue. *Am. J. Vet. Res.* **46,** 1133–1136.
38. McLaughlin, G. L., Edlind, T. D., Campbell, G. H., Eller, R. F., and Ihler, G. M. (1985), Detection of *Plasmodium falciparum* Using a Synthetic DNA Probe. *Am. J. Trop. Med. Hyg.* **34,** 837–840.
39. Moench, T. R., Gendelman, H. E., Clements, J. E., Narayan, O., and Griffin, D. E. (1985), Efficienty of *In Situ* Hybridization as a Function of Probe Size and Fixation Technique. *J. Virol. Methods* **11,** 119–130.
40. Nagata, Y., Yokota, H., Kosuda, O., Yokoo, K., Takemura, K., Kikuchi, T., (1985), Quantification of Picogram Levels of Specific DNA Immobilized in Microtiter Wells. *FEBS. Lett.* **183,** 379–382.
41. Negro, F., Berninger, M., Chiaberge, E., Gugliotta, P., Bussolati, G., Actis, G. C., Rizzetto, M., and Bonino, F. (1985), Detection of HBV–DNA by *In Situ* Hybridization Using a Biotin-Labeled Probe. *J. Med. Virol.* **15,** 373–382.
42. Nishibuchi, M., Ishibashi, M., Takeda, Y., and Kaper, J. B. (1985), Detection of the Thermostable Direct Hemolysin Gene and Related DNA Sequences in *Vibrio parahaemolyticus* and Other *Vibrio* Species by the DNA Colony Hybridization Test. *Infect. Immun.* **49,** 481–486.
43. Palva, A., and Ranki, M. (1985), Microbial Diagnosis by Nucleic Acid Sandwich Hybridization. *Clin. Lab. Med.* **5,** 475–490.
44. Pirtle, E. C., Wathen, M. W., Paul, P. S., Mengeling, W. L., and Sacks, J. M. (1984), Evaluation of Field Isolates of Pseudorabies (Aujeszky's Disease) Virus as Determined by Restriction Endonuclease Analysis and Hybridization. *Am. J. Vet. Res.* **45,** 1906–1912.
45. Pollice, M., and Yang, H. L. (1985), Use of Nonradioactive DNA Probes for the Detection of Infectious Bacteria. *Clin. Lab. Med.* **5,** 463–473.
46. Polsky-Cynkin, R., Parsons, G. H., Allerdt, L., Landes, G., Davis, G., and Rashtchian, A. (1985), Use of DNA Immobilized on Plastic and Agarose Supports to Detect DNA by Sandwich Hybridization. *Clin. Chem.* **31,** 1438–1443.
47. Ramsay, M., Levin, S. L., Rosendorff, J., Pinto, M., and Berstein, R. (1985), *Chorionic villus* Sampling for First Trimester Diagnosis of beta-Thalassaemia. Report of the First South African Case. *S. Af. Med. J.* **68,** 672–675.
48. Rijntjes, P. J., Van Ditzhuijsen, T. J., Van Loon, A. M., Van Haelst, U. J., Bronkhorst, F. B., and Yap, S. H. (1985), Hepatitis B Virus DNA Detected in Formalin-Fixed Liver Specimens and its Relation to Serologic Markers and Histopathologic Features in Chronic Liver Disease. *Am. J. Pathol.* **120,** 411–418.
49. Rotbart, H. A., Levin, M. J., and Villarreal, L. P. (1984). Use of Subgenomic

Poliovirus DNA Hybridization Probes to Detect the Major Subgroups of Enteroviruses. *J. Clin. Microbiol.* **20,** 1105–1108.

50. Schrier, R. D., Nelson, J. A., and Oldstone, M. B. (1985), Detection of Human Cytomegalovirus in Peripheral Blood Lymphocytes in a Natural Infection. *Science* **230,** 1048–1051.
51. Shimizu, Y., Ida, S., Matsukura, T., and Yuasa, T. (1984), Determination of Hepatitis B Virus DNA in Serum by Molecular Hybridization. *Microbiol. Immunol.* **28,** 1117–1123.
52. Sixbey, J. W., and Pagano, J. S. (1985), Biotin-Labeled DNA Probes for Detection of Epstein-Barr Virus by *In-Situ* Cytohybridization. *Clin. Lab. Med.* **5,** 503–512.
53. Southern, P. J., Blount, P., and Oldstone, M. B. (1984), Analysis of Persistent Virus Infections by *In Situ* Hybridization to Whole-Mouse Sections. *Nature* **312,** 555–558.
54. Spector, S. A., Neuman, T. R., and Hirata, K. K. (1985), Rapid Determination of Molecular Relatedness of Isolates of Human Cytomegalovirus. *J. Infect. Dis.* **152,** 755–759.
55. Squire, K. R., Chuang, R. Y., Chuang, L. F., Doi, R. H., and Osburn, B. I. (1985), Detecting Bluetongue Virus RNA in Cell Culture by Dot Hybridization with a Cloned Genetic Probe. *J. Virol. Methods* **10,** 59–68.
56. 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.
57. Takiff, H. E., Seidlin, M., Krause, P., Rooney, J., Brandt, C., Rodriguez, W., Yolken, R., and Straus, S. E. (1985), Detection of Enteric Adenoviruses by Dot-Blot Hybridization Using a Molecularly Cloned Viral DNA Probe. *J. Med. Virol.* **16,** 107–118.
58. Tani, K., Takizawa, T., and Yoshida, A. (1985), Normal mRNA Content in a Phosphoglycerate Kinase Variant with Severe Enzyme Deficiency. *Am. J. Hum. Genet.* **37,** 931–937.
59. Taylor, M. A., Wise, K. S., and McIntosh, M. A. (1985). Selective Detection of *Mycoplasma hyorhinis* Using Cloned Genomic DNA Fragments. *Infect. Immun.* **47,** 827–830.
60. Tompkins, L. S. (1985), Recombinant DNA and Other Direct Specimen Identification Techniques. *Clin. Lab. Med.* **5,** 99–107.
61. Tracy, S. (1985), Comparison of Genomic Homologies in the Coxsackievirus B Group by Use of cDNA:RNA Dot-Blot Hybridization. *J. Clin. Microbiol.* **21,** 371–374.
62. 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.
63. Wagner, D., Ikenberg, H., Boehm, N., and Gissmann, L. (1984), Identification of Human Papillomavirus in Cervical Swabs by Deoxyribonucleic Acid *In Situ* Hybridization. Obstet. Gynecol. **64,** 767–772.