N92-22699

NUCLEIC ACID PROBES IN DIAGNOSTIC MEDICINE

Phillip A. O'Berry
National Technology Transfer Coordinator for Animal Science
National Soil Tilth Laboratory, USDA, ARS, OCI
2150 Pammel Drive, Ames, Iowa 50011

ABSTRACT

The need for improved diagnostic procedures will be outlined and variations in probe technology will briefly be reviewed. A discussion of the application of probe technology to the diagnosis of disease in animals and humans will be presented. A comparison of probe vs. nonprobe diagnostics and isotopic vs. nonisotopic probes will be made and the current state of sequence amplification will be described. The current market status of nucleic acid probes will be reviewed with respect to their diagnostic application in human and veterinary medicine. Representative product examples will be described and information on probes being developed that offer promise as future products will be presented.

INTRODUCTION

New technology will be required to meet the exponentially increasing world food needs. World population is projected to double in the four decade period from 1960-2000 to reach 6.1 billion [1]. World livestock and poultry numbers have also increased significantly. Healthy animals contribute immeasurably to the nutritional status, economic productivity, land resource utilization and overall well-being of our global society. Molecular approaches to agricultural production and animal disease diagnosis provide a ray of hope that world food needs can be met. Nucleic acid probes provide a powerful new technology that is just now being applied to improve our ability to more rapidly detect diseases and their causative agents.

Diagnostic Technology

Diagnosis of the hundreds of infectious and noninfectious diseases that affect man and animals is not simple. It often involves clinical examination, serologic tests, cultural or microscopic examination of a wide range of samples, fluids, or tissues, postmortem examination and the gathering and assessment of additional information. Adequate diagnostic tests do not exist for many important diseases. Even in instances where adequate tests do exist, there is a critical need for an improved test that is more rapid, more economical or more reliable. Thus, the field of diagnostics lends itself to the application of nucleic acid probe technology.

In simplest form, a nucleic acid probe is a specific piece of single stranded nucleic acid, usually composed of highly conserved nucleotide sequences, that contains an isotopic or nonisotopic label allowing it to be visualized when it finds and binds (hybridizes) to another piece of nucleic acid that has complementary nucleotide bases. It has been known since 1953 that the structure of DNA is a double helix [2]. It was later found that two separate but complementary strands of DNA reassociate (hybridize) under specific conditions to form a double helix [3]. These and other principles were used in the late 1960's to identify specific pieces of DNA in the nuclei of cells fixed to a microscope slide using labelled complementary pieces of nucleic acid. Thus probe technology was born.

Probes are made from such materials as target-specific sequences of cloned genes, complementary DNA, natural or synthetic single-stranded oligonucleotides or ribosomal RNA. Sample material usually is solubilized, and either its proteins are electrophoretically separated and bound to an appropriate support membrane (Western blotting) or they are applied to nitrocellulose filters (Dot blotting) for reaction with a probe. The probe, labeled by incorporation of a radioisotope (e.g. ^{32}P or ^{125}I) or enzyme, (e.g. alkaline phosphatase or horseradish peroxidase) then searches the sample on the support system for homologous nucleotide sequences, or "target" DNA. Probe-target DNA hybrids are then visualized or enumerated by autoradiographic or direct scintillation counting or by enzyme-substrate color change reactions.

Most diagnostic probes developed thusfar utilize radioisotopes as labels with resulting advantages and disadvantages. The advantages are high sensitivity, easy signal detection and permanent record of test results. The disadvantages are the risk of exposure to harmful levels of radiation, expensive safety protection equipment, strict waste disposal procedures and relatively limited probe storage life. The diagnostic community has been slow to develop, market and use these radioactive probes.

Nonisotopic probes offer some distinct advantages over their isotopic counterparts. They are more simple to run, obviously pose no radiation hazard and require no specialized safety equipment or disposal procedures. These probes tend to have longer storage life. The recent development and marketing of chemiluminescent substrates for use with nonisotopic probes allow for exposure of x-ray film in the probe reaction with the production of a hard-copy of the test results in only a few minutes. These and other recent improvements in probe development technology have made nonisotopic probes more sensitive and practical than ever before.

Powerful new procedures to amplify target DNA sequences in sample material offer considerable promise in enhancing the application of probe technology to diagnostic veterinary medicine. The best known of these is the polymerase chain reaction, (PCR) formally introduced in 1986 [4] and patented in 1987. A recent review article [5] describes PCR in detail and discusses its application to probe technology. Target DNA is heat denatured and specific paired primers are repeatedly annealed and extended with a specific polymerase until amplification is sufficient to allow signal detection even in a complex mixture. The relatively simple procedure uses a single thermostable enzyme, can be performed in a few hours and in a single tube, and lends itself to automation. Among the problems in the procedure are nonspecific hybridization, the number of steps involved in the thermal cycling of the reaction, the number, and sometimes efficiency, of product molecules per cycle is rather low thus necessitating an increased number of cycles to reach a sufficient amplification level. In addition, RNA targets and DNA targets are indistinguishable in some mixed nucleic acid samples. However, new developments in PCR techniques allow amplifications of over 100 billion-fold, meaning that a single molecule can be amplified to a point of visibility on a simple agarose gel.

Several other target amplification procedures are also available. They are amplification by RNA transcription (TAS, 3SR) and amplification by ligation (LAR/LAS). The comparative advantages and disadvantages of these procedures and PCR are well described. It is likely that newer and even more useful procedures will come in the years ahead.

Applications of Probe Technology

Nucleic acid probes are uniquely and specifically well suited to detect even minute amounts of their homologous counterparts. Applications of this powerful new technology are rapidly increasing and virtually limitless.

Detection of disease agent nucleic acids in diagnostic samples is an obvious application. Molecular approaches to infectious disease diagnosis has been recently reviewed [6]. Systems must be specifically developed for use on such varied diagnostic samples as tissues, cells, blood, urine, saliva, feces, mucosal swabs, skin scrapings, semen or other specimen. All must be compared against and be more advantageous than current conventional cultural, histopathologic, microscopic or immunologic diagnostic methods.

Nucleic acid probes can also aid in the differentiation between closely related microorganisms. Detection of the presence of a specific serovar or strain can influence the therapeutic or preventive measures that should be taken. Clinical samples are often complicated by the presence of nucleic acids of closely related nonpathogens or pathogens of such low virulence as to be unimportant.

The typing of DNA using probes has many practical applications. They include, but are not limited to, the detection of genetic defects, genome mapping, forensic uses, sex determination in sperm cells, paternity determination and microbial classification.

Some applications of nucleic acid probe methodology have been made to the detection and prognosis of certain malignancies. Some conditions have been associated with viral infections, such as cervical cancer and human papilloma. Others, such as certain breast and ovarian carcinomas, have been associated with oncogene amplification. Certain leukemias and lymphomas have been connected to specific clonal gene arrangements and chronic myelogenous leukemia has been shown by DNA probe use to be a specific gene translocation. Thus nucleic acid probes offer considerable promise as malignancy diagnostic, prognostic and perhaps even therapeutic applications.

An additional application of nucleic acid probe technology is in the area of quality control of biologics. Probes represent a powerful new tool to screen for and detect adventitious microorganisms in biologicals. Certain microorganisms are commonly found in cell cultures or production materials, like serum, and are quite difficult to detect by current and more conventional methods.

At this point in the development of nucleic acid probe technology, the most common application is in research. Probes are powerful research tools used for a wide variety of nondiagnostic purposes. They have contributed significantly to the gathering of new information on pathogenesis, epidemiology, latency, microbial genetics, taxonomy and microbial ecology. Probes are also used to develop new and improved immunogens, diagnostic tests and/or therapeutic agents.

Probe Products in Human Diagnostics

The market was surveyed to determine the number and types of nucleic acid probes available for diagnosis of human diseases. All companies in a recent product listing [7] were contacted, literature was surveyed and numerous contacts were made with Food and Drug Administration officials.

Over 200 DNA or RNA probes are currently commercially available. These products are marketed by 23 companies and the American Type Culture Collection with most specified for research purposes only. They cover such diverse areas as viruses (Human Immunodeficiency Virus, Human Papillomavirus, Herpes Simplex Virus, Epstein-Barr Virus and Hepatitis A and B viruses), bacteria (Legionella, Mycobacteria, Campylobacter, Mycoplasma and Haemophilus), mycotic agents (Histoplasma, Blastomyces and Coccidioides), drugs (digoxin and phenobarbital), neoplasias (Chronic Myelogenous Leukemia, Acute Lymphocytic Leukemia and certain Lymphomas), genetic diseases (Cystic Fibrosis and Duchene Muscular Dystrophy) as well as a host of oncogenes, proto-oncogenes, all 24 human chromosomes and over 100 probes for specific human Restriction Fragment Length Polymorphisms.

Nucleic acid diagnostic probes are considered to be devices by the FDA and as such are required to receive premarket approval if marketed for diagnostic use. None are licensed by FDA but 61 have received FDA approval in the past 6 years. One company, Gen-Probe Inc., San Diego, has received approval for 30 probes. Probes marketed for research purposes only receive limited FDA oversight.

It is difficult to compile information on probe product sales revenues. However, two companies active in this area (Enzo Biochem, Inc. and Oncogene Science) reported gross sales from all products in 1990 of $19 million and $5 million respectively [8]. It is not known how much of these gross sales were derived from probe sales. The market for probes in the second half of this decade has been estimated to be $500 million [9].

Probe development and use for human diagnostics has grown exponentially in the last few years. It is likely that many new and perhaps more powerful probes will be developed in the very near future. Promising new probes are being developed for Cryptosporidia [10], Borrelia burgdorferi [11], neoplastic tissue [12], E. coli in foods [13], Salmonella in foods [14], Listeria in foods [15], and Human Papillomavirus in Pap smears [16]. Ultimately, the value of these tests and any other probe tests that reach the market will be determined in the same way as for all new diagnostic products - by independent scrutiny of their clinical performance, convenience and relative cost [17].

Probe Products in Veterinary Diagnostics

A similar market survey was conducted to determine the number and types of nucleic acid probes available for diagnosis of animal diseases. Literature and scientific publications were surveyed and contacts were made with U.S. Department of Agriculture officials in the Animal and Plant Health Inspection Service.

Over 100 probes developed against a wide range of animal viral and bacterial pathogens have been reported [18]. Although premarket approval is not mandatory, one probe has received USDA approval to be marketed for veterinary diagnostic purposes. It is a DNA probe developed by IDEXX Corporation of Portland, ME for the detection of Mycobacterium paratuberculosis in fecal samples. A second probe, developed by scientists at Montana State University is used by Agricultural Bioengineering, Inc. of Bozeman, MT to test samples submitted to it for Tritrichomonas foetus, the flagellated protozoan that causes the reproductive disease bovine trichomoniasis. Little information is available on these probe product sales since both were introduced this year.

The future looks promising that additional probes will enter the veterinary diagnostics market. They will cover an expanding number of pathogens and diseases. Many will have patent protection and exclusive license provisions. Several of the more promising probes are specific for such diverse organisms as Treponema hyodysenteriae [19], Campylobacter fetus and Campylobacter hyointestinalis [20], Anaplasma marginale [21], Bovine Virus Diarrhea Virus [22], Eperythrozoon suis [23] and Bovine Leukocyte Adhesion Deficiency [24], a genetic disease of cattle.

SUMMARY

A powerful new technology utilizing labeled nucleic acid is being applied to human and veterinary diagnostics. Over 300 nucleic acid probes have been developed in the past few years. This new technology will be increasingly transferred into the U.S. and global marketplace with considerable promise of improvement in human and animal health.

REFERENCES

1. Pritchard, W. R. JAVMA 198, No. 1, Jan. 1, 1991: 46-51.
2. Watson, J. D. and Crick, H. C. Nature 171, 1953: 964-967.
3. Marmur, J. and Doty, P. J. Molec. Biol., 3, 1961: 595-617.
4. Mullis, K. Faloona, F. Scharf, S., Saiki, R., Horn, G. and Erlich, H. Cold Spring Harbor Symposium on Quantitative Biology 51, 1986: 263.
5. Arnhelm, N. and Levenson, C. H. Chem. & Eng. News, Oct. 1, 1990: 36-47.
6. Charache, P. The Fifth San Diego Conference. Nucleic Acids: New Frontiers. Nov. 14-16, 1990.
7. Scherago, E. J. Science 247, Part II, March 23, 1990: G83-92.
8. Thayer, A. M. Chem. & Eng. News, April 1, 1991: 21.
9. Parr, R. S. Biotech Patent News 5, No. 3, March 1991: 4.
10. Turner, M. H. The Scientist 4, No. 21, Oct. 29, 1990: 4-7.
11. Goodman, J. L., Jurkovich, P., Kramber, J. M. and Johnson, R. C. Infection and Immunity 59, No. 1, Jan. 1991: 269-278.
12. Parr, R. S. Biotech Patent News 5, No. 2, Feb. 1991: 8.
13. Hsu, H. Y., Chan, S. W. Sobell, D. I., Halbert, D. N. and Groody, E. P. J. Food Protection 54, No. 4, April 1991: 249-255.
14. Wilson, S. G., Chan, S. W., Deroo, M., Vera-Garcia, M., Johnson, A., Lane, D. and Halbert, D. N. J. Food Protection 55, 1990: 1394-1398.
15. King, W., Raposa, S., Warshaw, J., Johnson, A., Halbert, D. and Klinger, J. D. Int. J. Food Microbiol. 8, 1989: 225-232.
16. Parr, R. S. Biotech Patent News 5, No. 3, March 1991: 4.
17. Engleberg, N. C. ASM News 57, No 4, 1991: 183-186.

18. Knowles, D. P. and Gorham, J. R. Rev. Sci. Tech. Off. Int. Epiz. 9, No. 3, 1990: 733-757.
19. Jensen, N. S., Stanton, T. B. and Casey, T. A. U.S. Patent & Trademark Office Serial No. 07/496,579, March 21, 1990.
20. Wesley, I. U.S. Patent & Trademark Office Serial No. 07/603,503, Oct. 26, 1990.
21. Goff, W. L., Stiller, D., Roeder, R. A., Johnson, L. W., Falk, D., Gorham, J. R. and McGuire, T. C. Vet. Microbiol. 24, 1990: 381-390.
22. Kelling, C. L., Kennedy, J. E., Rump, K. K., Stine, L. C., Paul, P.S. and Partridge, J. E. Am. J. Vet. Res. 52, No. 8, Aug. 1991: 1237-1244.
23. Oberst, R. D., Hall, S. M., Jasso, R. A., Arndt, T. and Wen, L. Am. J. Vet. Res. 51, No. 11, Nov. 1990: 1760-1764.
24. Kehrli, M. E. and Shuster, D. U.S. Patent & Trademark Office, Sept. 20, 1991.