Third San Diego Conference on Nucleic Acid Probes  
October 26–28, 1988  
Practical Aspects of Molecular Probes

In a few short years the San Diego Conference has become a leading forum on the use of nucleic acid probes and recombinant molecules for diagnostic and therapeutic applications. The San Diego Conference, sponsored by the San Diego Chapter of AACC, is designed to bring together researchers from industry and academia with a common interest in applying recombinant DNA technology. Diverse topics such as areas of forensic testing, recombinant vaccines, gene therapy, and immunotherapy, as well as diagnosis of infectious diseases, have been symposium presentations. In some cases, the research has led to products already commercialized and utilized in clinical laboratories, as in the diagnosis of infectious disease and forensic testing. Other topics such as vaccines under development and use of recombinant lymphokines for adoptive immuno-therapy represent the near-term impact of technology in modern health care.

The following group of papers deal with some of the topics covered at the 1988 San Diego Conference, which concentrated on "Practical Aspects of Molecular Probes." Building on the success of the first three conferences, the Fourth San Diego Conference—to be held October 25–27, 1989—will explore areas of genetic fingerprinting, oncogenes, viral and bacterial identification, automation and instrumentation, and anti-sense technology, thereby continuing the conference goal of providing a forum for new or innovative techniques in probe technology.

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Application of a Rapid Non-Radioisotopic Nucleic Acid Analysis System to the Detection of Sexually Transmitted Disease-Causing Organisms and Their Associated Antimicrobial Resistances

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We devised a versatile method for detecting nucleic acids in crude lysates of biological samples. A controlled network of nucleic acid hybrids composed of the target fragment, several oligonucleotide probes, branched DNA amplifiers, and labeled oligonucleotides is produced on a solid phase to ultimately incorporate 60 to 300 molecules of alkaline phosphatase, which are detected with a chemiluminescent substrate. The visible light output can be recorded on a luminometer or on instant black-and-white film. Assays have been developed for the detection of Chlamydia trachomatis, Neisseria gonorrhoeae, and for genes conferring penicillin and tetracycline resistance. Conducted much like ELISAs, the assays are performed in about 4 h (for 96 samples) in microtiter dishes. The molecular detection limit of approximately 50,000 molecules of double-stranded DNA has permitted us to detect 1 to 10×10^3 of C. trachomatis and N. gonorrhoeae with specific probe sequences. Both plasmid and genomic target sequences can be detected by the same procedure. All of the assay components, except for a set of unmodified oligonucleotide probes, are universally applicable for all targets.

Recently we have reported the development of a rapid method for the detection of nucleic acids in crude biological samples (1). First applied to the analysis of hepatitis B viral DNA in human serum and plasma, the method involves the solution-phase hybridization of several synthetic oligonucleotide probes to the target nucleic acid sequence in crude cellular lysates and the subsequent capture of the probe-target complex onto a solid phase. All steps in the analysis system (probe binding, solid-phase capture, amplified labeling) consist of specific nucleic acid hybridizations. An enzymatically generated chemiluminescent signal has been used for the final readout. Although polystyrene beads were used as the solid support for the hepatitis B studies, we have modified the system to utilize microtiter wells. With this adaptation, assays are conducted much like an ELISA (Figure 1). One plate (96 samples) can be fully processed in 4 h.

Except for the solution-phase hybridization probes, which are unmodified synthetic oligonucleotides, all reagents, probes, components (including the microtiter