Biologicals (1996) **24**, 187–188

Probes in DNA/RNA Hybridization

Mathew Longiaru
Gen-Probe Incorporated, 9880 Campus Point Drive, San Diego, CA 92121, U.S.A.

Gen-Probe has been developing nucleic acid probe-based diagnostics since it was incorporated over ten years ago. The core technologies that have been developed at Gen-Probe are the basis for the company's successful product development efforts and acceptance of those products in the diagnostic and clinical microbiology laboratory. During the presentation, three of these core technologies will be described, including the use of ribosomal RNA as targets for nucleic acid probe hybridizations, the Hybridization Protection Assay (HPA),[1] and the target amplification technology known as Transcription Mediated Amplification (TMA).[2]

Combining the abundant ribosomal RNA sequences that are present in up to 10 000 copies per bacterial cell with the homogeneous detection system known as HPA, Gen-Probe has been able to develop products that detect a wide variety of bacterial pathogens. These include sexually transmitted disease agents, such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (the PACE® 2 System),[3–5] the AccuProbe® line of culture identification assays for confirmation of Gram positive and negative bacteria, mycobacteria, and fungal pathogens, and an assay for the direct detection of *Group A Streptococci* in throat swab specimens (Group A Strep Direct[6–8]).

In order to develop tests that have the required sensitivity to detect pathogens that are present in extremely low levels in clinical specimens, a target amplification system known as Transcription Mediated Amplification (TMA) has been developed. TMA is a transcription-based target amplification system using only reverse transcriptase and RNA polymerase to increase the number of nucleic acid (RNA or DNA) target molecules by one billion-fold or more. The target sequence is isolated by means of specific primers, one of which contains a promoter sequence for RNA transcription. TMA is isothermal with no repetitive temperature cycling required and is a process that repeats autocatalytically. It makes use of the coordinated operation of the synthetic, nicking and unwinding activities of reverse transcriptase to produce a transcription complex for the synthesis of RNA amplicon by RNA polymerase. TMA has been combined with HPA into a single tube assay format that is referred to as the Gen-Probe Amplified System. The amplified assay format does not require any physical separation or wash steps and thus makes possible a format in which all reagents are added into the tube and nothing needs to be pipetted out of the tube. This format is very effective in minimizing carry-over contamination which is a major concern when utilizing such powerful target amplification technologies.

The first product that uses the Gen-Probe Amplified System is the Amplified *Mycobacterium tuberculosis* Direct (MTD) Test. The increasing awareness of tuberculosis as a major health threat and the rapid emergence of drug-resistant strains of TB has strengthened the demand for rapid culture independent methods for the detection of *M. tuberculosis* in clinical specimens. The Gen-Probe MTD test is able to detect the rRNA in a single TB cell and the time to result for testing 50 clinical specimens is 4–5 h. Gen-Probe's amplified MTD test was the first direct detection assay for TB to be sold in Europe and Japan and received FDA approval for sale in the United States in December 1995. Numerous articles have been published from laboratories around the world which have evaluated the Amplified MTD assay.[9–14] From these evaluations, it has been accepted and is routinely used in microbiology laboratories where TB is currently isolated and identified.

At this time, Gen-Probe is developing products using the amplified technology for the detection of other infectious disease pathogens including *Chalmydia trachomatis*, *Mycobacterium avium complex* (MAC), HIV and HBV.

References

1. Arnold LJ Jr, Hammond PW, Wiese WA, Nelson NC. Assay formats involving acridium ester labeled DNA probes. Clin Chem 1989; 35: 1588–1594.
2. Shinnick TM, Jonas V. Molecular approaches to diagnosis of tuberculosis. In: Bloom B, ed. Tuberculosis: Pathogenesis, Protection and Control. 1994; ASM Press, 517–530.

1045–1056/96/030187+02 $25.00/0

3. Kluytmans JA, Goessens WH, van Rijsoort-Vos JH *et al*. Improved performance of PACE 2 with modified collection system in eombination with probe competition assay for detection of *Chlamydia trachomatis* in urethral specimens from males. J Clin Microbiol 1994; 32: 568–570.
4. Warren R, Dwyer B, Plackett M *et al*. Comparative evaluation of detection assays for *Chlamydia trachomatis*. J Clin Microbiol 1993; 31: 1663–1666.
5. Hale YM, Melton ME, Lewis JS, Willis DE. Evaluation of PACE 2 *Neiseria gonorrhoeae* assay by three public health laboratories. J Clin Microbiol 1993; 31: 451–453.
6. Steed LL, Korgenski EK, Daly JA. Rapid detection of *Streptococcus pyogenes* in pediatric patient specimens by DNA probe. J Clin Microbiol 1993; 31: 2996–3000.
7. Heifer BJ, Bourbeau PP. Comparison of the Gen-Probe Group A Streptococcus direct test with culture and a rapid Streptococcal antigen detection assay for diagnosis of streptococcal pharyngitis. J Clin Microbiol 1993; 31: 2070–2073.
8. Pokorski SJ, Vetter EA, Wollan PC, Cockerill FR. Comparison of Gen-Probe Group A Streptococcus direct test with culture for diagnosing streptococcal pharyngitis. J Clin Microbiol 1994; 32: 1440–1443.
9. Abe C, Hirano K, Wada M *et al*. Detection of *Mycobacterium tuberculosis* in clinical specimens by polymerase chain reaction and Gen-Probe Amplified Mycobacterium Tuberculosis direct test. J Clin Microbiol 1993; 31: 3270–3274.
10. Jonas V, Alden MJ, Curry JI *et al*. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by amplification of rRNA. J Clin Microbiol 1993; 31: 2410–2416.
11. Pfyffer GE, Kissling P, Wirth R, Weber R. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by a target-amplified test system. J Clin Microbiol 1994; 32: 918–923.
12. Miller N, Hernandez SG, Cleary TZJ. Evaluation of Gen-Probe amplified *Mycobacterium tuberculosis* direct test and PCR for direct detection of *Mycobacterium tuberculosis* in clinical specimens. J Clin Microbiol 1994; 32: 393–397.
13. LaRocco MT, Wanger A, Ocera H, Macias E. Evaluation of the Gen-Probe amplified *Mycobacterium tuberculosis* direct test on processed sputum sediments. Eur J Clin Microbiol Infect 1994; 13: 726–731.
14. Ehlers S, Pirmann M, Zaki W, Hahn H. Evaluation of a commercial rRNA target amplification assay for detection of *Mycobacterium tuberculosis* complex in respiratory specimens. Eur J Clin Microbiol Infect Dis 1994; 13: 827–829.