Molecular diagnosis of infections in the new millennium

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Technology: Polymerase chain reaction (PCR)
Use: PCR is a highly sensitive technique that is used to amplify and detect small amounts of specific DNA sequences in biological material. It is made possible by the availability of specific DNA polymerases (e.g., Taq or Tth) that are produced by bacteria in hot springs. These thermostable enzymes allow for repeated cycles of denaturation and DNA synthesis in a test tube, theoretically generating $2^n$ or $1 \times 10^9$ copies of a given genetic sequence in a matter of hours. The PCR assay can be used in diagnosis to detect unculturable or fastidious microorganisms that cannot be identified by conventional methods. It provides the rapid identification of common strains that cause epidemics and of microorganisms that can take weeks to culture. It was used to identify Bartonella henselae as the cause of bacillary angiomatosis, and is commonly used to measure the amount of human immunodeficiency virus in the blood as a marker of disease progression, and used to measure the amount of human immunodeficiency virus (HIV) in plasma.

History: Dr. Kary Mullis, the genius behind this technology, conceived of the PCR process while working at Cetus Corporation (Emeryville, Calif.) in 1983 and was later awarded the Nobel prize in chemistry for it. The patent for the technology was later sold for $300 million, and by 1994 PCR had been referenced in over 7000 scientific publications.

Promise: The sheer speed of PCR-based assays (often 2–5 hours) means treatment can be implemented earlier. PCR is often the only test that can provide a diagnosis of HIV in neonates in the first week of life; the resulting early antiviral treatment may help prevent devastating neurologic complications. Although PCR-based assays are more expensive than conventional diagnostic tests, there is a significant potential for savings through improved health care outcomes.

Problems: The meaning of positive PCR results has not been validated for all infections. For instance, does the detection of cytomegalovirus indicate latent infection or current disease? PCR is also technologically more difficult to perform than conventional tests, and false-positive or negative results due to technical problems, sample contamination, lack of specificity, or amplification may hinder rather than help the clinician make decisions. The lack of standardization and reproducibility of many PCR-based assays have also limited many applications to "in-house" research use.

Prospects: PCR is a powerful tool with many legitimate applications; however, its premature acceptance as the universal standard may lead to diagnostic inaccuracies with significant clinical implications. In particular, its ability to detect nonviable genetic material presents problems of interpretation. Although PCR-based assays have been available for almost 20 years, they have not yet revolutionized the field of infectious diseases. There is a perception that the assays are complex and beyond the expertise of many laboratories. It is likely that the availability of automated workstations to perform sample processing, analysis and product detection will lead many to reconsider the use of PCR assays. The cost has also influenced the decision to retain more traditional and less costly procedures. It is important that the clinical benefit of a test outweigh its cost and complexity and that this is demonstrated in rigorous experimental conditions. In the study of HIV and AIDS, where such evidence exists, PCR-based assays have been embraced, and novel assays developed at Laval University may soon lead to widespread acceptance of this approach in bacteriology.

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References