The Polymerase Chain Reaction - Expectations and Realities

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A major aim of clinical microbiologists over the last century has been to demonstrate the presence of pathogenic microorganisms in clinical or pathological specimens associated with infectious diseases. With the development of molecular genetics over the last 20 years, new technologies have become available that allow morn sensitive and specific determinations to be made in a short period of time. The latest development in this area is a technique of gene amplification known as polymerase chain reaction (PCR) which enabled clinicians to diagnose difficult pathogens. PCR is an in vitro technique of producing copies of a given gene sequence so that within few hours 10^6 copies of a single gene can be produced. The pioneenng work in this field was done by the members of Cetus Corpomtion who described a rapid prenatal test for sickle-cell anaemia[1]. The test was based on the ability to identify a single base mutation within the entire foetal genome. Soon the PCR was used widely because of its simplicity and potential range of applications including the study of infectious diseases. To perform PCR, a target DNA sequence tobe amplified must first be chosen, typically several hundred to thousands base pairs (bp) in length. For example, to detect H. pylori in a clinical sample, a region of H. pylori urease A gene might be amplified[2]. A pair of synthetic oligc nucleotide primers (single stranded DNA oligonucleotide pri; :r of 10-20 bp in length) are required, one complementary to each of the targeted DNA strands and flanking the target sequence. At the start of the PCR all reagents (e.g., oligonucleotide primers, deoxyribonucleotides, PCR buffer and DNA polymerase) are added to the incubation mixture. A specimen to be amplified (human, bacterial, fi.ingal, plant and/or viral DNAs) was added in the last to incubation mixture. As expected PCR-based diagnostic test developments focussed initially towards the organism for which existing methods are eiher very time consuming, produce ambiguous results, or inadequate or simply not available. For example, Mycobacterium tuberculosis testing needs weeks of culture; serological testing of lyme disease produce ambiguous results and sensitive direct testing for the human retroviruses is not possible by other means. As with most diagnostic techniques, PCR is appropriate for detection of some infectious diseases, but may not be for others. In some cases, it will not be of practical diagnostic use, but will provide valuable epidemiological information. The criteria which might indicate that PCR would be useful are: i) that available methods are not ideal in terms of cost, time, specificity, sensitivity or have technical difficulties and ii) that it is possible to obtain genetic material (DNA or RNA) inaclinical sample. Although there are unlimited uses for the PCR, its application to study viral infections can be divided into following categories: 1) diagnosis of viral infections in the failure of conventional tests; 2)to understand viral pathogenesis; 3) to elucidate viral causes of nomniectious diseases and 4) diagnosis of simultaneous infections with closely related viral pathogens. So far the greatest impact has been made in diagnosing viral infections, e.g., hepatitis A[3], B[4] and C virus[5], cytomegalovirus[6], Epstein Barr virus[7], Herpes Simplex virus[8], human papilloma virus[8], Pavovirus[10], Rotavirus[11], HIV-l[12], HIV-2[13], HTLV-I[14], HTLV-ii[15], Enterovirus[16], Influenza virus[17], Mumps virus[18]. The extension of PCR to other infectious diseases has been slower. Many of the species for which PCR was developed were difficult or impossible to grow, or other available methods are inadequate. Amongbactenal pathogens diagnostic PCR was reported for M. tuberculosis[19], M. leprac[20], Botrelia burgdorgeri21, Treponema pallidum[22], Legionella pneumophila[23], Shigella[24], H. pylori[2] Bordetella pertusis[25], Clostridium difficile[26], Enterotoxigenic Eschenchia coil[27], Mycoplasma pneumoniae[28], Chiamydia trachomatis[29] and Rickettsia rickettsii[30]. PCR was also developed for some of the parasitic 2pathogens, e.g., Toxoplasma

gondii[31] and Trypnosoma cruzi[32]. Despite the development of diagnostic PCR for many microorganisms, PCR is still a research test and limited to large hospital and specialized laboratories. Although the promises and expectations of PCR-based diagnostic tests are great, they will not be totally useful without significant advances in instruments and automation. On the other hand, a cost effective and clinically useful system needs to be designed which can amplify and discriminate multiple organisms and gene system simultaneously. For example, a liver panel might consist of tests for hepatitis A, B, C, D and E viruses, cytomegalovirus and Epstein-Barr virus. This can be done by the development of panel of primer pairs that support discrete amplification of above mentioned organisms simultaneously. If molecular diagnosticians want to revolutionize diagnostic microbiology, they must offer a product which is not only specific, but must be clinically useful for primary care provider.

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