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PCR and Its Clinical Applications – Dr. Th. Dhabali Singh MD

The polymerase chain reaction (PCR) is a powerful core molecular biology technique. It is an efficient and rapid ***in vitro*** method for enzymatic amplification of specific DNA or RNA sequences from nucleic acids of various sources. It is an efficient way to copy or "amplify" small segments of DNA or RNA. Using PCR, millions of copies of a section of DNA are made in just a few hours, yielding enough DNA required for analysis. This innovative yet simple technique allows clinicians to diagnose and monitor diseases using a minimal amount of sample, such as blood or tissue. It allows for accurate diagnosis of underlying conditions which may not be currently clinically active but have a likelihood of developing in the future.

THE PCR TECHNIQUE

The genetic material of each living organism – plant or animal, bacterium or virus – possesses sequences of its nucleotide building blocks (usually DNA, sometimes RNA) that are uniquely and specifically present only in its own species. Complex organisms such as human beings possess DNA sequences that are uniquely and specifically present only in particular individuals. These unique variations make it possible to trace genetic material back to its origin, identifying with precision at least what species of organism it came from, and often which particular member of that species. Such an investigation requires that enough of the DNA under study is available for analysis – which is where PCR comes in.

PCR exploits the remarkable natural function of the enzymes known as polymerases. These enzymes are present in all living things, and their job is to copy genetic material (and also proof-read and correct the copies). Sometimes referred to as "molecular photocopying", PCR can characterise, analyse and synthesise any specific piece of DNA or RNA. It works even on extremely complicated mixtures, seeking out, identifying and duplicating a particular bit of genetic material from blood, hair or tissue specimens.

DIAGNOSTIC APPLICATIONS OF PCR

PCR is extensively used in analysing clinical specimens for the presence of infectious agents, including HIV, hepatitis, Mycobacterium tuberculosis (the causative agent for tuberculosis) and human papillomavirus (the causative agent for cervical cancer) among others.

PCR AND HIV: PCR is particularly invaluable in the early detection of HIV in infants as it can identify the DNA of the virus within human cells immediately following infection, as opposed to the antibodies that are produced weeks or months after infection. PCR can also be used to determine the viral load (i.e. how much virus is circulating around the body), which is a useful measure of prognosis. Thus, in short, for HIV, infections can be detected earlier, donated blood can be screened directly for the virus, newborns can be immediately tested for infection, and the effects of antiviral treatment can be quantified.

PCR AND HEPATITIS: PCR is useful for the detection of hepatitis B virus – DNA, and hepatitis C virus – RNA in serum and liver tissue. Detection of hepatitis c virus – RNA in serum may be the only means of confirming acute hepatitis C infection and also identifying viraemia in the chronic disease. It can also be used for direct evaluation of mother-to-child hepatitis C virus transmission. PCR can be used for monitoring reinfection with hepatitis C virus after liver transplant, and has proved invaluable in identification of different hepatitis C virus genotypes. The efficacy of antiviral treatment can also be monitored using PCR technique.

PCR AND TB: Some disease organisms, such as that for Tuberculosis, are difficult to sample from patients and slow to be grown in laboratories. PCR-based tests have allowed detection of small

numbers of disease organisms (both dead and alive), in convenient samples. Detailed genetic analysis of the *Mycobacteruim* can also be used to detect antibiotic resistance, allowing immediate and effective therapy. The effects of therapy can also be evaluated.

PCR IN CANCER DIAGNOSTICS: PCR provides invaluable information on patient's prognosis, and predicts response or resistance to therapy.

LIMITATIONS OF PCR

PCR is an extremely sensitive technique but is prone to contamination from irrelevant DNA, leading to false positive results. Another potential problem is the cross-contamination between samples. Moreover, reagents and equipment are costly and hence, cannot be afforded by small laboratories. However, the PCR equipment in recent years are designed as "closed systems" and hence, the possibility of cross-contamination as stated above are minimised significantly.

THE FUTURE OF PCR

The present technology for doing PCR, about the size of a microwave oven and costing several thousand rupees, is destined for further improvements. Researchers have already reported success at copying larger and larger pieces of DNA, including the entire genome of HIV.

Extraordinary miniaturisation of the hardware is also underway, as experimenters squeeze PCR onto chip-sized devices. Before long, it may become quite routine to diagnose an infectious disease, right in the doctor's chamber.

PCR is doing for genetic material what the invention of printing press did for written material - making copying easier, inexpensive and accessible.

Previously considered more as a research application, PCR today, is extensively adopted by diagnostic laboratories for diagnosis and monitoring of various infectious diseases. It is becoming inexpensive and more easily accessible than ever before.

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(The writer is Senior Consultant Pathologist and Managing Director, BABINA Diagnostics, Imphal)