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Review Article

POLYMERASE CHAIN REACTION AND ITS CLINICAL APPLICATION: A REVIEW

Sonia Tomar *
Ram Gopal College of Pharmacy, Gurgaon, Haryana, India
*Corresponding Author Email: sonitomar26@gmail.com

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ABSTRACT

Polymerase chain reaction is a popular method in molecular diagnostic in which small amount of DNA undergo five steps of denaturation, primer attachment, again repeating to form number of copies, in analogous way continued round of amplification many identical copies can be produced. There are numerous factors which play significant role in this technique like denaturation temperature (94°C), Annealing temperature (40 to 60°C), Extension temperature (72°C) also with type of DNA polymerase enzyme use like natural (Taq) or recombinant (Amplitaq). All polymerase varies in term of their properties (Their thermostability, exonuclease activity, extension rate, reverse transcriptase activity, molecular weight, resulting DNA end and so on). Its applicability is very wide ranging from genetic fingerprinting, forensic investigation, cloning, mutation detection, microarray. It is still in its development phase new advances are made every day, still need to overcome some of its drawback like variation in parameters, starting material quantification.

Key words: Polymerase chain reaction, qRT-PCR, Fluorescence signal, Cycle number, Multiplex PCR

INTRODUCTION

These days research in biochemistry and genetic play a key role in the field of health sciences. It gives a better interpretation of processes and as horizon in the development of new diagnostic and therapeutic strategies. From the molecular techniques, Polymerase Chain Reaction PCR is used widely in identification and characterization of viral, bacterial, parasitic and fungal agents, helping in the investigation and diagnosis of a growing number of diseases microbial infections and their epidemiological studies. It is used to amplify a single or a few copies of a piece of DNA across several orders of magnitude. It uses widen in investigation and diagnosis of a growing number of diseases, quantification of changes in gene expression. PCR is used in research laboratories in DNA cloning procedures, Southern blotting, DNA sequencing, recombinant DNA technology and DNA fingerprinting. In clinical microbiology, forensics laboratories PCR is especially useful because only a tiny amount of original DNA is required[1]. DNA of microorganism in body fluids, foodstuffs or drinking water is being detected by using PCR. Both qualitative and quantitative PCR play a crucial role in the fight against. A newly derived variant i.e. Digital PCR has its advantage and applicability in quantification of low-level small pathogens, genetic sequences, copy variety variations, and relative organic phenomenon in single cells, as it is being amplification enabled by single-step digital PCR may be a key think about reducing the time and value of the "next generation sequencing" methods and hence enabling personal genomics. Inverse PCR is especially useful for the determination of insert locations[2].

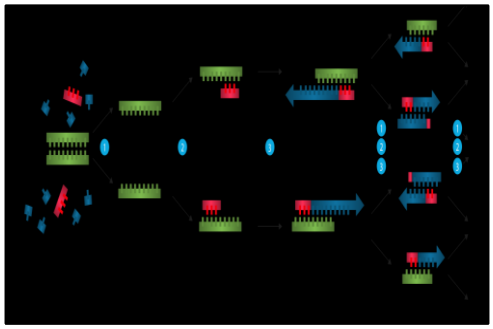
HISTORY AND DEVELOPMENT

A 1971 paper in the Journal of Molecular Biology by Kleppe and co-workers first described a method using an enzymatic assay to replicate a short DNA template with primers in vitro . In 1983, Kary Mullis, PhD, conceived PCR, its impact on molecular biology was given by Cetus scientists. In1985, first presented at the American Society for Human Genetics annual meeting[3]. In 1986, Cetus scientists isolated the Taq polymerase from Thermus aquaticus. In 1987, PerkinElmer, another US-based biotech company, launched a thermal cycler. In 1991, Roche bought the rights to PCR from Cetus and invested in refining the science for use in molecular diagnostics to detect diseases[4].

BASIC PRINCIPLE OF PCR

Time duration of PCR reaction is around two hours which depends on the specific conditions of the reaction. DNA Polymerase enzyme assist the synthesis of deoxyribonucleic acid from deoxynucleotide substrates on a fiber deoxyribonucleic acid templet. deoxyribonucleic acid enzyme adds nucleotides to the 3` finishes of oligonucleotide once it's toughened to an extended templet deoxyribonucleic acid[5]. It contains an area complementary to the oligonucleotide. deoxyribonucleic acid enzyme will use the oligonucleotide as a primer associated in elongation of its 3`-end finish to come up with an extended region of double stranded deoxyribonucleic acid. second in denaturation, deoxyribonucleic acid templet is heated up to 94° C that breaks the weak atomic number 1 bonds that hold deoxyribonucleic acid strands along in a very helix, forming single stranded deoxyribonucleic acid by separating, followed by tempering during which the mixture is cooled by lowering temperature to 50-70° C, permitting primers to bind to their complementary

sequence within the templet deoxyribonucleic acid. throughout extension, the reaction is heated to 72° C, to act. deoxyribonucleic acid enzyme extends the primers, adding nucleotides onto the primer in a very serial manner, oppress the target deoxyribonucleic acid as a template[6]. In one single cycle, one section of double-stranded deoxyribonucleic acid templet is amplified into 2 separate items of double-stranded deoxyribonucleic acid. These two are then out there for amplification within the next cycle. As variety of cycle unceasingly replicates, and increased exponentially[7].



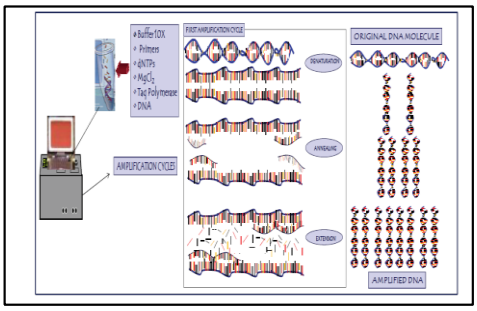


Fig 1: Steps involved in PCR

TYPES OF PCR AND FACTORS AFFECTING

Various variants have been developed from the basic PCR method to improve performance and specificity, and to achieve the amplification of other molecules of interest in research. In Real-time PCR, the amount of DNA is measured after each cycle using fluorescent dyes that yield increasing fluorescent signal in direct proportion to the number of PCR product molecules or amplicons generated. The change in fluorescence is measured by combines thermal cycling with fluorescent dye scanning capability which further determined by plotting fluorescence against the cycle[8]. Models available are QuantStudio™ 3, QuantStudio™ 5, or StepOnePlus™ system or user interchangeable, as within the ViiA seven system, the Applied Biosystems™ QuantStudio vi and seven Flex systems, also the Applied Biosystems™ QuantStudio 12K Flex system with sort of reaction vessels: 48-well plates, 96-well plates, 384-well plates, 384-microwell cards, 3,072–through-hole plates. Its instrument conjointly includes software package for information assortment and analysis. Aerosol barrier tips and screwcap tubes will facilitate decrease cross-contamination issues. 10 to 1,000 copies of templet macromolecule use during this for every time PCR reaction. varied Primer style software package programs. Its instrument also includes software for data collection and analysis. The amplification plot represents the accumulation of product in the entire duration of the entire PCR. s, real-time. It is dynamic, accurate and precise. In qRT-PCR, the first-strand deoxyribonucleic acid synthesis reaction and time PCR reaction takes place within the same tube, simplifying & easing down reaction setup that any reduces contamination. This method there is a need of Gene-specific primers. Primers should be 18–24 nucleotides in length. It should provide optimal annealing temperature & compatible melting temperatures (within 1°C) and contain approximately 50% GC content., DNA polymerase, dNTP, reverse transcriptase, DNA polymerase., Magnesium concentration. Aerosol barrier tips and screwcap tubes can help decrease cross-contamination problems. 10 to 1,000 copies of template nucleic acid use in this for each real-time PCR reaction[9]. Various Primer design software programs available are Invitrogen™ OligoPerfect™ designer and Applied Biosystems™ Primer Express™ software. Ct is defined as cycle number where fluorescence signal produced by reaction reaches above ZaAZVA designated threshold level, it is used to measure DNA copy number used initially, as Ct value is inversely proportional to starting DNA level. RT PCR is needed to be quantified in the early part of the exponential phase[10,11].

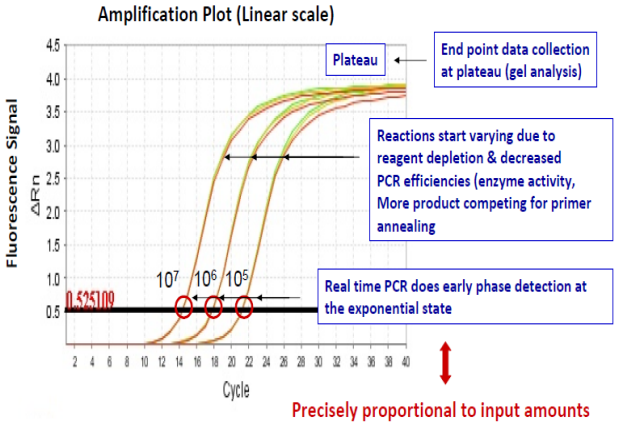


Fig 2: Fluorescence signal v/s Cycle

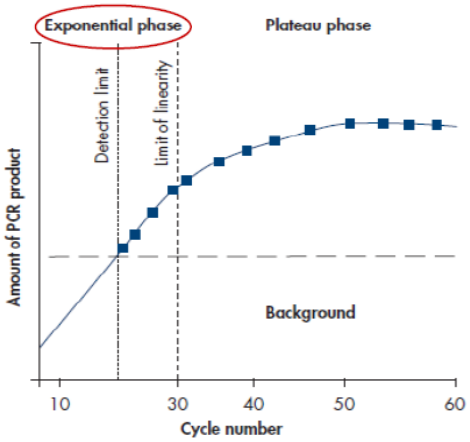


Fig 3: Amount of PCR product v/s Cycle number

The amplification cycle shows that there is increase in fluorescence intensity with increase in amplicon concentration. Reverse transcription- Polymerase chain reaction (RT-PCR) is a technique commonly used in molecular biology to detect RNA expression, gene expression through creation of complementary DNA (cDNA) transcripts from RNA, and quantify the expression of mRNA, the starting template for a PCR reaction can be DNA or RNA[12,13]. DNA is usually the appropriate template for studying the genome of the cell or tissue and for the detection of DNA viruses. RNA is better than genomic DNA for detecting structural changes in long genes, as genomic sequence greatly reduces the length of DNA. A small quantity of target RNA can be detected. The template for RT-PCR can be total RNA or poly (A)+ selected RNA. Parameter and component affecting amplification efficiency in the RT-PCR process include the efficiency of reverse transcription, Mg2+/ dNTPs/ primer concentrations, enzyme activity, pH, annealing temperature, cycle number, temperature variation, tube to tube variation etc[14]. RT-PCR cannot be used for quantitative analysis so PCR amplification variant can be done in two-step process by using either a single tube or multiple tube with multiple reaction steps by using external template as the internal control, fresh-frozen tissue, FFPE tissue. In reverse transcription method mRNA is converted to a cDNA molecule by RNA-dependent DNA polymerase & cDNA is required for PCR amplification[14]. The primers used can be either unique or common. Poly-A tail of mRNA molecules use Oligo-dT primers and allow synthesis of cDNA only from mRNA molecules. However, In Multiplex PCR is a widespread molecular biology time efficient technique for amplification of multiple targets in a single PCR experiment by using multiple primer pairs in a reaction mixture[15]. There was an introduction of Nested PCR (nPCR) which provides good sensitivity and specificity of DNA (or RNA = nRT-PCR) amplification and may be used to confirm the integrity of a specific amplicon. In Nested PCR, two pairs of PCR primers commonly used for a single center. The first pair amplifies and second pair of primers (nested primers) binds within the first PCR product and produce a second PCR product that will be shorter than the first one. It uses two consecutive PCR's, involving 30 cycles of amplification in each. The first PCR is having an external pair of primers, while in second there are two nested primers are used which are internal to the first primer pair, also called hemi-nested primer. The larger fragment produced by the first reaction is used as template for the second PCR where the specificity of the first amplicon is being confirmed by its ability to serve as template for the nPCR Its variant is 'hot' nPCR in which one of the nested primers is labelled using radio or non-radioactive markers[16].

Long Range PCR was introduced to amplify up to 30 kb and beyond. For complex, genomic templates, 20 kb is a typical target. Single cell PCR was introduced in which first step toward single cell PCR is isolation of single cells[16,17]. In Fast Cycling PCR, buffer facilitates amplification of specific PCR products with significantly reduced cycling time. which increases the affinity of Taq DNA polymerases for short single stranded DNA fragments, reducing the time for annealing to just 5 seconds. Methylation specific PCR comes in focus of molecular biology which is sensitive and specific for methylation of virtually any block of CpG sites in a CpG island. Methylation-specific PCR (MSP) is a method for analysis of DNA methylation patterns in CpG islands. PCR is performed with two primer pairs, which are detectable methylated and unmethylated DNA. Hot start PCR is a modified form of Polymerase chain reaction (PCR) which avoids a non-specific amplification of DNA by inactivating the taq polymerase at lower temperatures. High-fidelity PCR, use a DNA polymerase having minimal error possibility and providing high degree of accuracy in the replication of the DNA of interest. Variable Number of Tandem Repeats (VNTR) PCR targets areas of the genome that exhibit length variation. In Asymmetric PCR, the conditions are the same as those of symmetric PCR except for the ratios of primers used. The ratios of primers were varied from 20/2, 20/ 1, 20/0.2, 20/0.04 to 20/0. Repetitive element sequence-based PCR is a typing technique which enables the generation of DNA fingerprinting that discriminates bacterial strains. The overlap extension polymerase chain reaction also called Splicing by overlap extension or Splicing by overhang extension (SOE) PCR[18]. It is used for mutations insertion / addition at specific points in a sequence it can splice smaller DNA fragments into a larger polynucleotide. Intersequence-Specific PCR (or ISSR-PCR) is method for analyzing DNA fingerprinting, uses primers from segments which are repeated throughout a genome producing unique fingerprint of amplified product lengths. In Ligation-mediated PCR, the exponentially amplify segments of DNA located between two specified primer hybridization sites. A single-sided PCR method needs specification of only one primer hybridization site; the second is recognized by the ligation-based addition of a unique DNA linker. This linker, together with the flanking gene-specific primer, allows exponential amplification of any fragment of DNA. Touchdown PCR, which differentiated from other in which the annealing temperature is gradually reduced in later cycles[19]. The annealing temperature in the early cycles is usually 3–5 °C above the standard T_m of the primers used, while in the later cycles it is a similar amount below the T_m[20]. COLD-PCR also called co-amplification at lower denaturation temperature-PCR is a PCR variant that enriches variant alleles from a mixture of wildtype and mutation-containing DNA. Solid phase amplification (SPA) use surface-bound PCR instead of freely-diffusing primers to amplify DNA. This limits the amplification to two-dimensional surfaces and therefore allows the easy parallelization of DNA amplification in a single system[21]

APPLICATION OF PCR & ITS VARIANT IN CLINICAL DIAGNOSIS

PCR is used in the investigation and diagnosis of a growing number of diseases, allowing the determination and quantification of changes in gene expression, used in in forensics laboratories, Nested PCR is one of these protocols for detection of only a small number of bacteria. It plays a key role in many genetics research laboratories, along with uses in DNA fingerprinting for forensics and other human genetic cases Qualitative PCR used to detect not only human genes but also genes of bacteria and viruses, it is used to detect the samples of body fluid, foodstuff, drinking water, also in the field of infectious diseases is AIDS. PCR is used for herpes simplex virus, varicella-zoster virus, and human papillomavirus infections analysis because of its high sensitivity and robustness. Both qualitative and quantitative PCR play a crucial role in the fight against cancer. Other diagnostic uses, includes tests for genetic diseases, cancers, and other infectious diseases, are evolving. Digital PCR has several potential applications, as well as the detection and quantification of low-level pathogens, rare genetic sequences, copy variety variations, and relative organic phenomenon in single cells. RT-PCR is usually used in studying the virus genome. Radiolabeled PCR-based assay for identifying point mutations in the ras protooncogene[22]. Sestini et al. demonstrate the utility of competitive PCR in determining gene amplification in tissue (breast cancer) from both fresh and paraffin-embedded material. Real-time PCR is used in detection of M. tuberculosis in clinical samples as a method to aid diagnosis. Guiver et al. (2000) described the first real-time PCR assay for the detection of N. meningitides in whole blood, CSF, plasma and serum samples which further improved the recognition of the prevalence of meningococci in meningitis and is therefore an important model for other infectious diseases. A real-time QPCR assay for VTEC was described by Sharma and Dean-Nystrom in 2003 which targeted the stx1, stx2, and eae genes. The real-time assay was used for analysis & diagnostic purpose of gastrointestinal parasite as described by Oberst et al. (1998) in which a 5' nuclease assay is done for detection of E. coli O157:H7. Real-time PCR methods have several applications in clinical parasitology like detection of fecal parasites, quantitation of minute parasitic burden during infection develop new treatments during drug trials and for diagnosing low level infections[23]. Hermsen et al. (2001) described a real-time 5' nuclease QPCR assay targeting the 18S rRNA gene of Plasmodium falciparum used for measuring parasitaemia for the detection and quantification of malaria parasite. (Schulz et al., 2003) apply real-time LC PCR Cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL) identification by targeting the 18S rRNA gene, more over it applicability was also found in detection of influenza virus by van Elden et al. (2001). A real-time QPCR assay for the detection of herpes simplex viruses (HSV was described by Ryncarz et al. (1999). A real-time QPCR is used for the detection of pathogen herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), varicella zoster virus (VZV), and the human herpes viruses 6, 7, and 8 (HHV-6, HHV-7, HHV-8). DENV the causative agent of dengue. Real-time PCR assays targeting detection differentiation and quantification of all four different DENV serotypes in acute phase serum. nPCR using primers for MPB 64 gene appears to be a useful technique for detection of M. tuberculosis in paucibacillary extrapulmonary specimens studied by (K. Lily Therese, 2004). Nested PCR was used by Scarpelline et al in-AIDS patient CSF to detect M. Tuberculosis DNA[24]. Identification of microorganisms by PCR has led to the selection and quality assurance of blood that blood banks are using for patients with different pathologies. Identification of mutations associated with ocular diseases has been widely used for the study of families at risk, demonstrate genetic heterogeneity in congenital fibrosis of extraocular muscles (Woloschak et al. 1994). Input copy number is determined by absolute quantification, usually by relating the PCR signal to a standard curve. The $2^{-\Delta\Delta C}{}_T$ method is used to analyze the relative changes in gene expression from real-time quantitative PCR by

Kenneth J. Livak et al in 2000. Real-time RT-PCR used high-density filter arrays which confirmed the change in expression of 17 of 24 (in 71%) genes. Genes with strong hybridization signals and at least twofold difference were likely to be validated by real-time RT-PCR studied by Mangalathu et al in 2001. Use of Q-PCR in biotechnology studied by Archie Lovatt in 2002 applied in determination of bio distribution of gene therapy vectors in animals; quantification of the residual DNA in final product therapeutics; detection of viral and bacterial nucleic acid in contaminated cell banks and final products; quantification of the level of virus removal in process validation viral clearance studies; specific detection of retroviral RT activity in vaccines with high sensitivity; and transgene copy number determination for monitoring genetic stability during production. A quantitative assay for viral RNA in plasma or sera that differs in several aspects from those reported previously was developed for quantitation of human immunodeficiency virus type 1 RNA in plasma and determining acute retroviral infection by Mulder et al in 1994. Blakely in 2001 introduced PCR to identify radiation-responsive molecular biomarkers, including gene expression targets and DNA mutations. Type II synthase ($PhaCl_{ps}$) for polyhydroxyalkanoate (PHA) was subjected PCR-mediated mutagen-esis using invitro studies to analyze and enhance the function of $PhaCl_{ps}$ to significantly produce poly(3-hydroxybutyrate) [P(3HB)] in recombinant bacteria, in the method to site were used (Ser325 and G1n481) for mutation & there was increases in P(3HB) synthesis after the method. NADH-cytochrome c reductase activity decreased mildly with age, whereas succinate cytochrome c reductase activity did not show significant age-dependent changes. Deletions in the muscle mitochondrial DNA (mtDNA) by use of PCR techniques were investigated. Multiplex quantitative fluorescent polymerase chain reaction (PCR) is a new molecular biological technique capable of quantifying in-situ DNA without the need for cell culture. Our objective was to test the reliability of PCR using fetal DNA from amniotic fluid (Amino-PCR) for the rapid prenatal diagnosis of the common trisomy's. Amnio-PCR is a reliable technique that aids the clinical management of pregnancy (Levett et al in 2001). PCR permits early diagnosis of malignant diseases such as leukemia and lymphomas, which is currently the highest-developed in cancer research and is already being used routinely[23]. PCR assays can be done on genomic DNA samples to detect translocation-specific malignant cells having sensitivity that is at least 10,000 fold higher Model-based strategy to identify genes suited to normalize quantitative RT-PCR data from colon cancer and bladder cancer in this study mathematical model of gene expression were used by Aanderson et al in 2004 the study involve not only calculation and analyzation of the overall variation of the candidate normalization genes but also of the variation between sample subgroups of the sample set which is further applied to Bladder and Colon Cancer Data Sets. PCR can identify genes that have been implicated in the development of cancer. Multiplex PCR is being used in detection of STD[26], stomach flu and other viral diseases in which sample extraction made from swab or protein. Duchenne and Becket muscular dystrophies (DMD/BMD) are diseases resulting from disruptions within the dystrophin gene Rapid Detection of Polymorphic Muscular Dystrophies cab be done with the help of Multiplex PCR. qPCR is used to evaluate biodegradation potential or activity in contaminated groundwater. qPCR is used to detect and quantify bacteria such as dehalococcoides McCarty as well as vinyl chloride reductase functional genes which cause biodegradation of chlorinated solvents, e.g trichloroethene and tetrachloroethylene . It also quantify functional genes involved in the biodegradation of solvents like benzene, toluene, ethylbenzene, and xylenes.

LIMITATION AND FUTURE DEVELOPMENT

PCR is rapid technique which can be used for large numbers of samples can be simultaneously. It can identify organisms that are difficult to culture. Differential PCR assays. It can amplify very small amounts of genetic material; thus, it can detect very low numbers of organisms in a sample. However, lack of antimicrobial sensitivity data, complexity of the assay, and the price of PCR. Interpretation of positive and negative PCR results can be challenging. There is always a need of DNA sequence information, DNA short size and limiting product are the limiting factors. Along that difficulty in designing primer pairs with appropriate specificity and in the need to use purified nucleic acids can limit the usefulness of PCR in a clinical setting because it increases the chance of contamination. So, overall PCR is limited with three significant limitations that are sensitivity, multiplexing and quantitation. In rare mutation analysis of samples like cancer cells, getting the right sensitivity, is a challenge. Costs need to be lower for fields like diagnostics and better assay design on which work need to be done. One positive target may inhibit other reactions. There are various inhibitors of PCR which exert their effects through direct interaction with DNA or interference with thermostable DNA polymerases. Magnesium cofactor is requisite for PCR and agents that reduce Mg2+ availability & interfere with binding of Mg2+ to the DNA polymerase can inhibit PCR. KCl, NaCl and salts, ionic detergents like; sodium deoxycholate, sarkosyl and SDS are ethanol, isopropanol and phenol are the materials and reagents that met samples during processing or DNA purification and act as inhibitors in the process. For overcoming limitation and future developments scientist trying to use PCR on microRNAs, looking for faster reactions and better sensitivity. Illumina ECO RT-PCR system improve the conductivity in PCR technology by use of metal blocks of varying size & also reduce heating applying higher thermal mass. These also make possible to enhance the speed of thermal, but caution need to be taken for temperature variation between individual wells and samples for DNA. BJS Biotechnologies has creator an ultra-high speed thermal cycler, known as xxpress. This can run 40 cycles of PCR in 15 minutes or less, potentially making it the fastest PCR thermal cycler in the world. It is having innovative heating and cooling techniques. In Cepheid, a Smart Cycler has disposable reaction tubes is there that is having a good fit in I-CORE ceramic heater plates, with respect to shape and size & its designing is done to increase heat uniformity and heat transfer. Real-time PCR (qPCR) is becoming a mature technique, a third-generation droplet digital PCR (ddPCR) has arisen. For developing multiplex PCR Luminex® is developed in which 100 tests can be performed altogether. Incorporation of internal controls is fundamental way to ensure a negative result in an assay is correct and not due to the sample containing the target organism rather than through inhibition of the assay.

CONCLUSION

Advances in molecular biology have revolutionized daily clinical practice and practitioners to adapt PCR approach, as it is coming as an essential tool for improving human health and human life due to its high sensitivity and specificity. It is more superior than other conventional diagnostic methods, which risen interest in

scientist to see advance improvement and future development in PCR technology. PCR is true point care of molecular diagnostic whose shortcoming overcome by its effectiveness. PCR along with diagnostic, can quantitatively demonstrate how much of a sequence type is present. The future of PCR is promising, combining various assays and approaches to produce greater insight into various gene combinations. In the future new developments in PCR technologies will lead to further benefits to the patient consolidating the role of PCR as an essential tool.

REFERENCES

1. Mullis KB. The unusual origin of the polymerase chain reaction. Scientific American. 1990 Apr 1 ;262(4):56–61.
2. Weier HU, Gray JW. A programmable system to perform the polymerase chain reaction. DNA. 1988 Jul 01, 7(6):441-447.
3. Ochman, H., Gerber, A. S., Hartl, D. L. "Genetic applications of an inverse polymerase chain reaction". Genetics. 1988 Nov 3.; 120 (4): 621–623
4. Erlich, H. A; Gelfand, D; Sninsky, J. J. Recent Advances in the Polymerase Chain Reaction. Science, 1991, Jan 14; 252 (50) 1643-1651.
5. Chagovetz, A and Blair, S. Real-time DNA microarrays: reality check. Biochem. Soc. Trans. 2009 , Nov. 25; 37 (5):471-5.
6. Smith, CJ and Osborn, AM. Advantages and limitations of quantitative PCR (QPCR)-based approaches in microbial ecology. FEMS Microbiol. Ecol.2009, Jun 4; 67(1):6-20.
7. Giasuddin, AS; Jhuma, KA; Haq AM. Applications of free circulating nucleic acids in clinical medicine: recent advances. Bangladesh Med. Res. Counc. Bull. 2008 Feb16; 34(1):26-32.
8. Higuchi R, Fockler C, Dollinger G, Watson R. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Biotechnology 1993 Oct 9; 11(12), 1026–1030.
9. Kariyazono H, Ohno T, Ihara K et al. Rapid detection of the 22q11.2 deletion with quantitative real-time PCR. Mol. Cell. Probes, 2001 Sep 14; 15 (1), 71–73.
10. Gibson, U.E.; Heid, C.A.; Williams, P.M. A novel method for real time quantitative RT-PCR. Genome Res. 1996 Mar 5, 6 (9), 995–1001.
11. Jou, NT; Yoshimori, RB; Mason, GR; Louei, JS; Liebling, MR. Single tube, nested, reverse transcriptase PCR for detection of viable Micobacterium tuberculosis. J. Clin. Microbiol. Jan 18, 2003; 35 (12), 1161-1165.
12. Salomon, RN. Introduction to quantitative reverse transcription polymerase chain reaction. Diag. Mol. Pathol. 1995 May 6; 4 (1):82-84.
13. Jackson, CR; Fedorka-Cray, PJ; Barret, JB. Use of a Genus and Species Specific Multiplex PCR for amplification of Enterococci. J. Clin Microbiol. 2004 Mar 9; 42 (11): 3558-3565.
14. Jann-Yuan, W; Li-Na, N; Chin-Sheng, C; Chung-Yi, H; Shu-Kuan, W; Hsin-Chih, L; PoRen, H; Kwen-Tay, L. Performance assessment of a Nested-PCR assay and the BD ProbeTec ET system for detection of Micobacterium tuberculosis in clinical specimens. J. Clin. Microbiol. 2004 Sep 5; 42 (9): 4599-4603
15. Tan, Y. et al. A novel long-range PCR sequencing method for genetic analysis of the entire PKD1 gene. J. Mol. Diagn. 2012 Nov 3;14 (3), 305–313
16. Stemmer WP, Crameri A, Ha KD, Brennan TM, Heyneker HL. "Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides". Gene. 1995 Jan17; 164 (1); 49–53.
17. Hernández, H; Tse, MY; Pang, SC; Arboleda, H; Forero, DA. "Optimizing methodologies for PCR-based DNA methylation analysis.". Biotechniques. 2013 Jun 3; 55(4) 181–197
18. Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS . "'Touchdown' PCR to circumvent spurious priming during gene amplification". Nucleic Acids Res. 1991 Mar 3; 19 (14): 4008-17
19. M. J. Espy,* J. R. Uhl, L. M. Sloan, S. P. Buckwalter, M. F. Jones, E. A. Vetter, J. D. C. Yao, N. L. Wengenack, J. E. Rosenblatt, F. R. Cockerill, III, and T. F. Smith. Real-Time PCR in Clinical Microbiology: Applications for Routine Laboratory Testing. Clin Microbiol Rev. May 12 2006; 19(1): 165–256.
20. O. I. Skotnikova, A. Yu. Sobolev, V. V. Demkin, N. P. Nikolaeva, E. Yu. Nosova, E. L. Isaeva, A. M. Moroz, V. I. Litvinov. Application of nested-PCR technique for the diagnosis of tuberculosis. Bulletin of Experimental Biology and Medicine. 2000, Feb 11; 129 (5):612-18
21. V H J van der Velden, A Hochhaus, G Cazzaniga, T Szczepanski, J Gabert and J J M van Dongen. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. Leukemia. 2003, Mar 21; 17 (5), 1013–1034.
22. Sunzeri, F.J., T.-H. Lee, R.G. Brownlee, and M.P. Busch. Rapid simultaneous detection of multiple retroviral DNA sequences using the polymerase chain reaction and capillary DNA chromatography. Blood. 1991, Jul 8; 77 (5): 879-886.
23. Panet A, Khorana HG. Studies on Polynucleotides. J. Biol. Chem. 1974 oct 24. 249(16): 5213–21.

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