International Journal of Innovative Research in Engineering & Management (IJIREM)
ISSN: 2350-0557, Volume-9, Issue-1, February 2022
https://doi.org/10.55524/ijirem.2022.9.1.69
Article ID IRP229167, Pages 341-344
www.ijirem.org

A Review Paper on Application of PCR-ELISA in the Molecular Diagnosis

Namrata Arya[1], and Dr. Arminder Kaur[2]

[1]Assistant Professor, Department of Biotechnology, Sanskriti University, Mathura, Uttar Pradesh, India
[2]Associate Professor, Department of Biotechnology, Sanskriti University, Mathura, Uttar Pradesh, India

Correspondence should be addressed to Namrata Arya; namrata.sobas@sanskriti.edu.in

ABSTRACT- PCR-ELISA (Enzyme-linkedlinked immunosorbent assay - polymerase chain reaction), immunodetection method used to count the PCR amplified creation just after the halt of biotinylated DNA on the microtiter bowl. Amplification, immobilization, and detection are the three phases of the synthesis chained response - enzymes coupled immunoassay assay procedure. PCR-ELISA is more sensitive than the conventional PCR method, it detects nucleic acids rather than protein with the lesser detection time and tinier analytical time. Due to its high sensitivity and specificity, it has potential application as a detection tool in the numerous industries like; agricultural, food industry, veterinary and medical. PCR-ELISA in industries used for the detection, diagnosis and quantitative monitoring. Current advancements in the Polymerase chain reaction - enzymes linked immunoassay test show that the assay is best known for its sensitive detecting limits, which reduces total diagnostic time and improves quality.

KEYWORDS- Applications, Diagnosis, Molecular Diagnosis, PCR-ELISA.

I. INTRODUCTION

The triple helix shape of deoxyribonucleic was initially discovered by James D. Macpherson and Francesco Mccartney in 1953. After the discovery of the dual helical construction of the DNA, numerous other molecular techniques were discovered like, Electrospinning, replication, and the development of the Polymerase are all commonly utilized biotechnological procedures. PCR was discovered by Kary Mullis in the 1983. Rather Polymerase chain reaction is the powerful technique but application of PCR can't be expressed without the detection tool. For PCR result identification, gel electrophoresis is mainly used but it confirms only the presence or absence of the particular gene. Real time PCR commonly used as a detection method. The research of DNA performs sparked a surge of interest in the 1980s. Various immunodetection approaches have been found and reported, one of which being Coutlee et alwork .'s on DNA usually works using invitrogen RNA tags. After that, various studies on DNA immunodetection were published using the enzyme linked immunosorbent assay (ELISA) techniques that leads to the introduction of combined PCR-ELISA technique. PCR-ELISA is the similar applications except the detection of nucleic acid instead of proteins. After the encapsulation of the biotinylated DNA, the synthesis chain response - enzymes attached immunoassay assay is utilized to measure the PCR expanded result.

Steps: PCR-ELISA completed

- Amplification
- Immobilization
- Detection

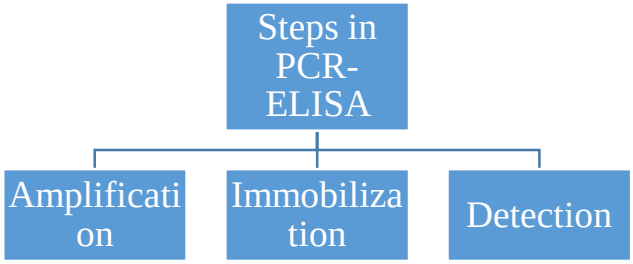

Figure 1: Steps involves in the process of PCR-ELISA

Firstly, Gene of interest will amplify with the help of polymerase chain reaction in presence of digoxigenin (DIG). Then, labeled product of PCR bind to the oligonucleotide probes & labelled with the biotin. Then, immobilizing that gene to streptavidin coated microtiter plates. Avidin-biotin interaction strong affinity forms the complex of avidinbiotin, thus compulsory only the PCR amplified products with specific gene to microtiter plate & washed off all other nonspecific products. After the immobilization, the biotinylated DNA detection is essential as these can't be detected with the naked eyes. For this, With the help of anti-DIG peroxidase conjugate through Surface: 2,2'-azino-di-3-ethylbenzthiazoline sulfonic amplicons can be detected. Then, with the help of spectrophotometer reaction can be measured (Figure 2).

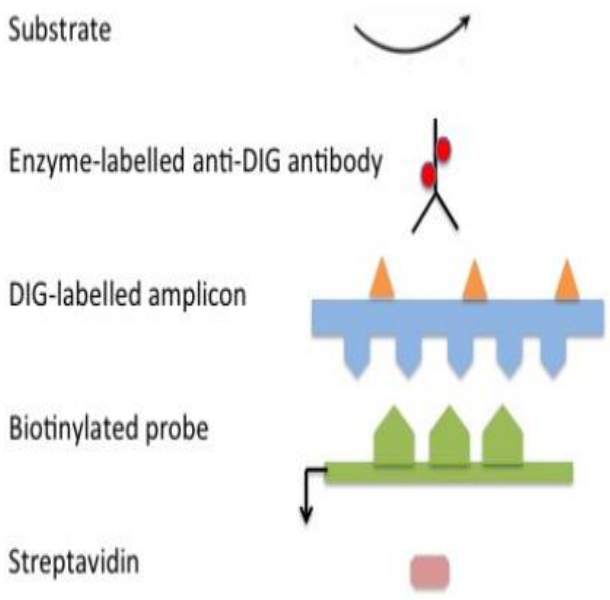

Figure 2: Schematic diagram of process of PCR-ELISA: (i) Intensification of DNA with the help of PCR product then bound to the specific probe, (ii) Halt of DNA to the microtiter plates, (iii) Detection of the biotinylated DNA with the help of anti-DIG peroxidase conjugate

A. PCR-ELISA has Many Benefits Over Conventional PCR-based Techniques

The viral chain reactions - enzymes tied immunosorbent test - has a number of benefits (PCR-ELISA) over other conventional ELISA and RT-PCR techniques. PCR-ELISA is an important Because of the precise bonding and enzymatic reaction, it is a better method than agar gel separation for increasing the signal of biotin-labeled Pcr primers [1]–[3].

B. Increased Specificity & Sensitivity

PCR-ELISA can detect the species specific sequence of DNA. With the help of PCR-ELISA, it is possible to differentiate between the different species of the bacterial, viral and fungal pathogens. In other hand, ELISA has disadvantages by the closely related species have similar antigens, leading to the cross reactivity of the antibodies and wrong positive results. With amplifying the DNA sequence, it increases sensitivity to detect the low plentiful sequence and this can be useful the detection and diagnosis of the viral pathogens. Thus PCR-ELISA showed increased sensitivity over conventional ELISA. Various articles comparing the difference of higher sensitivity ability between the qPCR and PCR-ELISA. Menotti et al. compare between the PCR-ELISA and qPCR for the detection of the Toxoplasma, parasite that cause life threatening infections. It was found that previous method yields negative results. These had been clinically confirmed to agonize from illness although the latter had accurate results through study. Various other authors (Table 1) supported this, whereby qPCR showed to be more sensitive. qPCR is a key method for detecting despite the existence of a competitor in terms of sensitivity. In compared to RT-PCR, PCR-ELISA was shown to be more cost effective. Thus, if the research not required high sensitivity, PCR-ELISA is the best option as it gives sufficient sensitivity at a minimum cost.

Other significant aspect of PCR-ELISA is that it enables large-scale testing utilizing ordinary laboratory instruments. That make it reliable for the use in the clinical labs. Despite its availability, respondents not switch to completely automated equipment for ELISA, likely because of the high buying and maintenance costs of the equipment and this assay's generally analytical time is also far shorter than the traditional PCR process thus, making it auspicious tool for the future uses particularly when dealing with the large amount of sample size. By new inventions, tools of the molecular biology need to be improved & developed for the efficient and faster results. Every new discovered invention has pros & cons after comparison to other technologies. Because PCR-ELISA necessitated the invention of a new test for an unfamiliar gene, traditional PCR with gel detecting will be an alternative of the primer which is very difficult to gain on the newly target gene. In short, pricing and level of sensitivity should be taken into account while selecting an innovative concept. Various studies compare the application of PCR-ELISA, qPCR and conventional PCR.

Table 1: Comparison between the three detection methods; PCR-ELISA, qPCR and traditional PCR with agar gel phoresis

Comparison	**PCR-ELISA**	**Conventional PCR**	**qPCR**
Equipment required	Standard lab requirement	Standard lab requirement	Fluorescence detection instrument
Detection limit	0.01 ng/μL	1-10 ng/μL	0.25 pg/μL
Reagent cost	Moderate	Low	Costly

C. Applications of PCR-ELISA

Many researchers suggested the implication of PCR-ELISA in a wide range of fields, detection, quantitative measurement/ Chronic illness surveillance, crop disease surveillance, and food allergy sensing (Figure 3).

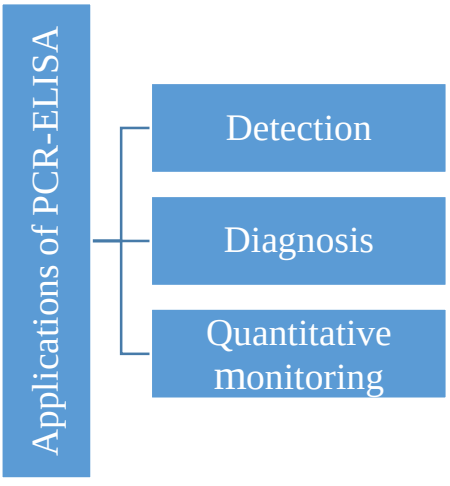

Figure 3: Applications of PCR-ELISA

D. Detection and Diagnosis

Because of the high specificity and sensitivity numerous studies proven the successful use of PCR-ELISA in the detection and the diagnosis. There are different publications that showed use of PCR-ELISA in the detection of food borne pathogens in the food industry like, B E Gillespie et al, showed the PCR-enzyme-linked western blot assay for Pseudomonas e. coli somatic subgroups C1 and E1 identification. *E. coli*, and *Vibrio parahaemolyticus.* PCR-ELISA can also use in the veterinary industries, Detection of the *Leishmania* parasite & avian virus in the chicken. It can detect the plant pathogens also include potato spindle tuber, and the plant virus in the woody plants. The PCR-ELISA method may also be utilized to determine the presence of dangerous waterborne microorganisms in liquid and industrial cooled towers effluent.

PCR-ELISA also used in the detection and identification of cancer cells. N Raji et al, showed PCR-ELISA was used to discover people papillomavirus 18 in cervical cancer specimens. (DIAPOPS), results of the research concluded that PCR-ELISA assay is more sensitive in comparison to other conventional PCR to detect the types of HPV, can be used for the diagnostics. Barbara Beifuss et al. demonstrated the direct identification of five prevalent pathogens taxa in medical specimens utilizing a quick and sensitive 24-hour PCR-ELISA technology open to protocol transfer, and the findings of the research revealed that PCR-ELISA may be used to identify pathogens showed reproducible & sensitive tool for detection and differentiate the dermatophytes at the species level. Presence of the type of hepatitis, Maryse St-Louis et al. demonstrated the PCR-ELISA for high plasma group testing for cardiac syndrome antigens.

PCR-ELISA is sensitive tool that can detect at small concentration. Usage of PCR-ELISA as an initial warning device can be expanded to identify latent disease symptoms. However, some research showed the detection and validation of the studies of expression of genes. Francesco Alessandro Palermo et al, showed the identification of oestrogen receptors mRNA activity and plasma vitellogenin production in young sole (Solea solea) subjected to waterborne 4-nonylphenol using PCR–ELISA and blood lipogenic induction using PCR–ELISA in juveniles sole exposed to aquatic 4-nonylphenol, results concluded that PCR-ELISA method showd good reproducibility, high sensitivity and Pulmonary ER mRNAs have the possibility for precise study. Pooria Gill et al, showed the Four -thalassemia points alterations were detected in Iranians utilizing a PCR-ELISA sequencing technique, with the findings indicating that PCR-ELISA had the same selectivity as other replication resistant mutant systems [4]–[6].

E. Quantitative Monitoring

Studies suggested that PCR-ELISA is useful for the rapid monitoring and especially in detecting the very small difference in the different pathogens. Mazyar Ziyaeyan et al, showed the quantification of the human cytomegalovirus DNA by a new capture hybrid polymerase chain reaction enzyme-linked immunosorbent. Results of the research concluded that PCR-ELISA had potential to monitor and diagnose cytomegalovirus infection who received transplantation of bone marro. Tetyana Kobets et al, showed the use of PCR-ELISA for the detection and monitoring the Leishmania parasites in the tissue. In this research, it was concluded that PCR-ELISA has potential to monitor and diagnose the others pathogens, particularly detecting the small changes in the different pathogens. However, PCR-ELISA is useful for the quantitative monitoring but more advancement is needed in the time to detect more preciously very tiny changes in the number of pathogens.

II. DISCUSSION

Enzyme linked immunoassay test - pcr method (PCR-ELISA), showed significant results in the diagnosis, detection and quantitative monitoring due to its high specificity and sensitivity but in the recent years, there are constants attempts for improving the wide applications of the Enzymes coupled immunoassay assay - viral chain reaction (PCR-ELISA). However, the Polymerase chain reactions (PCR) process is developed already then pain aspects of the improvement is the effect of streptavidin concentration on the microtiter plate. Hongmin Tahk, created an unique bidirectional reversed transcription polymerase chain response protease immunosorbent test (RT-PCR-ELISA) for the detection of several hepatitis viruses, including Hepatitis E virus (HEV) and Hepatitis A virus (HAV), In this study different viruses used for the specificity study and all were negative, hence this development in the PCR-ELISA can be used for the detection.

Tetyana, developed improved Enzyme coupled immunoassay test, synthesis chain reaction (PCR-ELISA) method for the quantification and detection of the Leishmania parasites in host tissues. This method removes the requirement for the separation step of hybridization of PCR with the probes. In this study, DIG & biotin labelled primers used for the production of PCR products. PCR products then attached with the streptavidin coated plate. This advance method also eliminates the all washing procedures in these steps. Overall this improvement in the PCR-ELISA reduces the time of washing and incubation and also the cost of reagents[7]–[9]. Nolasco, found that the asymmetric Enzymes coupled immunoassay analysis - polymerase chain reaction (PCR-ELISA) increases the concentration of DNA that makes it to the more sensitive than the TaqMan detection. This include less DIG labels that are costly, without reducing the sensitivity. However, no further studies evaluated the comparisons of these to identify the advantages and disadvantages but these advancements mainly focused on reducing the time and cost without reducing the specificity and sensitivity [10].

III. CONCLUSION

Assay for propeller chain response enzymes connected immunoassay polymerase chained response enzymes connected immunosorbent polymerase chained response enzymes connected immune (PCR-ELISA) method is more sensitive than available PCR with agarose gel method with less time and faster results. This method also allows for the multiple testing of samples. Polymerase chain reaction enzyme linked immunosorbent assay (PCR-ELISA) method reduces the chances of the contamination and it requires only standard lab equipment. Polymerase chain reaction enzyme linked immunosorbent method

showed less diagnostic time with high sensitivity makes it powerful tool for the detection in the field of veterinary, medical and food industry. Early detection with positive results allow to save more lives and prevent contaminated food to the consumers. Further, advancement is needed in the PCR-ELISA tool to be improved & developed for the efficient and faster results with reduces cost.

REFERENCE

[1] F. Eryuda, "HUBUNGAN SHIFT KERJA DAN KELELAHAN KERJA DENGAN STRES KERJA PERAWAT DI INSTALASI RAWAT INAP RSUD Dr. H. ABDUL MOELOEK BANDAR LAMPUNG," PLoS Negl. Trop. Dis., 2017.

[2] R. J. BARDA, "ANALISIS PENERAPAN KESELAMATAN PASIEN DI RUMAH SAKIT UMUM DAERAH INCHE ABDOEL MOEIS TAHUN 2017," PLoS Negl. Trop. Dis., 2017.

[3] Kemenkes, "Pedoman Nasional Pelayanan Kedokteran Tata Laksana Epilepsi Pada Anak," PLoS Negl. Trop. Dis., 2017.

[4] G. W. Utomo and B. Prabawani, "Pengaruh Brand Ambassador Dan Citra Merk Terhadap Keputusan Pembelian Sepeda Motor Suzuki Type Nex," Jur. Adm. Bisnis Fak. Ilmu Sos. Dan Ilmu Polit. Univ. Diponegoro, 2017.

[5] Zam-zam fauziah, "pengembangan media pembelajaran berbasis booklet pada mata pelajaran biologi untuk siswa kelas XI MIA madrasah aliyah alauddin pao-pao dan Man 1 makasar," PLoS Negl. Trop. Dis., 2017.

[6] gheraldin bella Aviolitasona, "pengaruh citra destinasi terhadap minat kunjung ulang wisatawan umbul sewu pengging, boyolali," PLoS Negl. Trop. Dis., 2017.

[7] R. Sriharyanti, "Pengembangan Desain Pembelajaran Menggunakan Model Pembelajaran Discovery Learning Berbasis Higher Order Thinking Skill Pada Siswa Kelas V Tema 6 Subtema 2 Di SD Negeri 2 Labuhan Ratu," Skripsi, 2017.

[8] J. R. Rizkinannisa, "Pengaruh Coping Stress Terhadap Burnout Anggota Polisi Resorg Malang," PLoS Negl. Trop. Dis., 2017.

[9] Athi Linda Yani, "Hubungan Perilaku Bullying dengan Tingkat Harga Diri Remaja Awal Yang Menjadi Korban Bullying," PLoS Negl. Trop. Dis., 2017.

[10] T. Arbós, "Actividad física y salud en estudiantes universitarios desde una perspectiva saludogénica.," 2017.