IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 20, Issue 9 Ser.2 (September. 2021), PP 54-57
www.iosrjournals.org

A review of nucleic acid tests in diagnostic microbiology

Ruvin Haidar[1] Muneera[2]
Post Graduate[1,2]
Dept of Microbiology, Singhania University[1], Dept of Oral Pathology and Microbiology, Kothiwal Dental College & Research Centre[2]

Abstract
Introduction. *A variety of nucleic acid methods are currently utilized for detection/identification of organisms and their virulence factors/resistance determinants. Since the advent of the PCR, numerous applications in infectious diseases diagnostics have been developed. Real-time PCR allowed this transition of the scientific technology from basic research and reference into the mainstream clinical laboratories having the efficiency of detecting group B Streptococcus (GBS) and influenza virus within no time.*
Methods*. Nucleic acid testing can be separated into amplified and non-amplified methods. Among the No amplified most common methods used are DNA-labeled or RNA-labeled probes that has target binding sites in the nucleic acid sequence and then accordingly form a signal in the attached reporter molecule. By the method of Target amplification different types of post amplification technologies can characterize the amplified targets nucleic acids.*
Discussion. *In recent decades' different modalities and techniques have been revolutionized in diagnostic microbiology. Nuicleic acid tests are one among them, these tests are wholly and solely dependent on the detection of DNA or RNA of pathogenic microorganisms.*
Conclusion. *By concluding the molecular diagnostics of infectious diseases will be focusing to extract the increased amount of information with markedly simple, rapid techniques were complex software analysis are required so as to resolve the data for use in clinical decision-making.*
Key words. *Hybridization, Molecular diagnostics, Molecular Typing, real-time PCR, sequencing*

Date of Submission: 25-08-2021 Date of Acceptance: 09-09-2021

I. Introduction

The introduction of nucleic acid based tests that lead to the multiplication of a few target molecules to a detectable level are gradually replacing or complementing culture based,biochemical, and immunological assays in routine microbiology laboratories. Moreover, testing of the drug susceptibility for proper treatment decision is also made by these advanced techniques. Not only at the individual level but these tests have an important role at the community level through epidemiological typing of the isolated and identified bacterial species. PCR is a Revolution in Science that relies on, cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Great mind behind this PCR was **Kary Banks Mullis** who Developed PCR in 1985 and was awarded nobel prize in 1993[1]. Modification of PCR the real-time PCR which is based on phase hybridization methods in which fluorescent-labeled probes are used for amplification. There are various probe technologies using the different real-time thermocycling instruments[2,3]. On the literature review various home-brew PCR assays are described based on species specific DNA fragments, genes encoding for toxins as well as virulence factors[4]. In the beginning main focus of these in vitro diagnostic companies was development methods for virological applications. But now these assays are designed mainly for identification of slow-growing or uncultivable organisms. Other than DNA or RNA amplification ligase chain reaction (LCR) and nucleic acid sequence-based amplification (NASBA) for bacterial diagnostics are used respectively. Because of the large scale research on the microbial genetics new methodologies based on nucleic acid have been developed that results in the extensive use of these molecular assays in the microbiology[5].

This review provides a brief overview of molecular diagnostic methods used for detection, identification, and bacterial typing as well as discuss the comparison of these new diagnostic methods with the conventional this microbiology.

II. Methods

Nucleic acid testing can be separated into amplified and unamplified methods. These methods mainly detect the DNA or RNA sequences specific for the specific organisms. These sequences can be amplified or not amplified, on that basis classification of nucleic acid tests are done. Because of the specificity and highly sensitivity of these tests all types of microorganisms can be detected.

Unamplified testing

The methods in which there is no needs of amplification for example where culture of the organisms has been done and sufficient concentration of specimen is present

Nucleic acid amplification

The amplification procedures are required where neither culture of the organism can be done nor they can be identified by other methods for example mycobacteria, viruses and fungi etc. By the amplification methods these nucleic acids DNA or RNA can be replicated several times so that the sufficient amount of specimen is available for the identification.

There are various tests in which amplification is done:

- One is Target amplification which includes PCR, reverse transcriptase–PCR (RT-PCR), strand displacement amplification, transcription amplification
- Second method is Signal amplification which includes branched DNA assays, hybrid capture
- Third method is Probe amplification which includes ligase chain reaction, cleavase-invader, cycling probes

In PCR many copies of specific DNA sequence is formed. By this method pictograms of nucleic acids can be amplified to micrograms in a very short period of time. The reason of the specificity and rapidity of PCR it has replaced every other NA procedure in clinical labs.various types of PCR are: Colony PCR ,Nested PCR, Multiplex PCR,Hot Start PCR ,Inverse PCR,Asymmetric PCR,Long PCR,Reverse Transcriptase PCR,Real time PCR,Touchdown PCR .

Steps in PCR are:

DENATURATION: At the temperature ≥95°C target DNA is denatured (melted). Denaturation sustains until the temperature remains ≥95°C and strands remain melted
, at this temperature base pairs can be recovered.

ANNEALLING: In this step addition of primers is done for binding to their respective complementary target regions. These primers are synthetic oligonucleotides made for annealing with the known target nucleic acid sequence. The temperature required for this step vary greatly from 40°C to 70°C.

EXTENSION: On addition of DNA Pol at the temperature of 72°C replication of DNA occurs just like the natural replication and formation of DNA copies occurs adjacent to the template DNA and extend across the areas between the primers. The temperature varies according to the DNA Pol used.

CYCLE: In programmed thermocycler most commonly 30-35 cycles are performed by alternate heating and cooling. Approximately 235 copies are required for one template of DNA. .

Gel electrophoresis with or without hybridization can be used for identification of Amplified DNA (amplicons)[1].

Reverse Transcriptase (RT) PCR

To detect the RNA virus an enzyme namely reverse transcriptase (RT), is used for reverse transcription of RNA into cDNA. Hence it can be used for detection of various infectious diseases caused due to RNA virus. Besides that various expression studies are conducted for determination of difference between normal and diseased tissues[6] .

Real-Time (rt) PCR and/or Quantitative PCR (qPCR)

One of the fastest growing PCR technology is real time PCR. Fluorescent dye, SYBR Green is used for the detection of DNA amplicons. Specific oligonucleotides are used for detection of target DNA. But the limitation of the syber Green is that it can bind to double stranded DNA and upon excitation emits light and can result an overestimation of the target concentration[2].

Signal amplification with branched DNA assays

This method is based on the signal amplification of branched DNA probe that binds with the specific nucleotide sequence. Between the target-specific oligonucleotides sequence of interest is captured hence this method is also called sandwich nucleic acid hybridization method. This method was initially used for viral loads (HIV-1 and HCV) detection. But now this method is used for microarray validations well as biomarker discovery to predictive toxicology[7].

Probe amplification by ligase chain reaction

The Ligation of adjacent oligonucleotides was first investigated by Besmer et al. in 1972. This method was initially used for identification of specific genetic disorders like sickle cell animia but now it is used for detection of various infectious diseases.It is now method of choice for urogenital infections caused because of Chlamydia trachomatis .Four types of thermostable ligases used in LCR reactions are Taq ligase, Pfu, Tth, Amplicase[8].

Loop Mediated Isothermal Amplification (LAMP)

At the temperature of 63^0c both the Amplification and detection of gense is possible in a single step, by incubating all the constituents like primers DNA polymerase and substrates. *By* this method both DNA as well RNA of the pathogenic microorganisms can be detected earlier than PCR. There is no need of thermocycler for this method. In a modification that is real time accelerated reverse transcription loop mediated isothermal amplification (RT-LAMP) assays in a very short interval detection of the organisms like West Nile, Dengue, Japanese encephalitis H5N1- highly pathogenic avian influenza is possible[9].

Random Amplified Polymorphic DNA (RAPD) PCR

This methods is used for the study purpose at the individual level as well as to the species by genetic identity,moreover that it has an application in the gene mapping.But the popularity of this method is very poor because of its inappropriate inferences resulted due to week reproducibility, faint or fuzzy products as well as difficulty in scoring bands[10,11].

Restriction Fragment Length Polymorphism (RFLP)

This method is based on the enzyme Restriction Endonucleases. Differentiation of organisms is done by analyzing the patterns which is derived from cleavage of their DNA. RELP can be scored either by Southern hybridization or by PCR. Initially this method was used for genetic map construction, but now it has an application in the phylogenetic studies[12].

III. Discussion

In recent decades' different modalities and techniques have been revolutionized in diagnostic microbiology. Nuicleic acid tests are one among them,these tests are wholly and solely dependent on the detection of DNA or RNA of pathogenic microorganisms.A vast number of nucleic acid tests have been developed like Colony PCR ,Nested PCR, Multiplex PCR,Hot Start PCR ,Inverse PCR,Asymmetric PCR,Long PCR,Reverse Transcriptase PCR,Real time PCR,Touchdown PCR .These tests have been implemented into routine usage in many laboratories worldwide[13].Not only in laboratory setup but these test are developed in such a manner that they can be used in house also. Introduction of these tests replaced a number of traditional bacteriology tests. By the rapidity of these tests an effective and prompt treatment can be given to the patients

IV. Conclusion

By concluding the molecular diagnostics of infectious diseases will be focusing to extract the increased amount of information with markedly simple, rapid techniques were complex software analysis are required so as to resolve the data for use in clinical decision-making. By the introduction of these new technologies vast number of viral and other microbial diseases have been diagnosed in a very short period of time. Although Conventional microbiology will not be replaced by these methods but multiparameter identification of various pathogens using techniques like, rapid realtime PCR are incorporated in the routine laboratories. Furthermore, this is not an end more and more molecular research as well as development of more sophisticated technologies are required for investigation of the diversity and pathogenicity of microbes and the relationship with their host.

References

[1]. Shampo, M. A.; Kyle, R. A. (2002). "Kary B. Mullis – Nobel Laureate for procedure to replicate DNA". *Mayo Clinic Proceedings*. **77** (7): 606.

[2]. De Clerck, E., Van Mol, K., Jannes, G., Rossau, R., and De Vos, P. (2004) Design of a 5´ exonuclease-based real-time PCR assay for simultaneous detection of *Bacillu licheniformis*, members of the '*B. cereus* group' and *B. fumarioli* in gelatine.*Lett. Appl. Microbiol.* **39,** 109–115.

[3]. Cheung, P. Y., Chan, C. W., Wong, W., Cheung, T. L., and Kam, K. M. (2004) Evaluation of two real-time polymerase chain reaction pathogen detection kits for *Salmonella* spp. in food. *Lett. Appl. Microbiol.* **39,** 509–515.

[4]. Templeton, K. E., Scheltinga, S. A., van der Zee, A., et al. (2003) Evaluation of real-time PCR for detection of and discrimination between *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella holmesii* for clinical diagnosis. *J. Clin. Microbiol.* **41,** 4121–4126.

[5]. Rodriguez-Lazaro, D., Lloyd, J., Herrewegh, A., et al. (2004) A molecular beacon-based real-time NASBA assay for detection of *Mycobacterium avium subsp. paratuberculosis* in water and milk. *FEMS Microbiol. Lett.* **237,** 119–126.

[6]. Chernesky MA, Jang DE. APTIMA transcription-mediated amplification assays for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *Expert Rev Mol Diagn.* 2006;6:519–25. [PubMed] [Google Scholar]

[7]. Pachl C, Todd JA, Kern DG, et al. Rapid and precise quantification of HIV-1 RNA in plasma using a branched DNA signal amplification assay. *J Acquir Immune Defic Syndr Hum Retrovirol.* 1995;8:446–54. [PubMed] [Google Scholar]

[8]. Monis PT, Giglio S. Nucleic acid amplification-based techniques for pathogen detection and identification. *Infect Genet Evol.* 2006;6:2–12. [PMC free article] [PubMed] [Google Scholar]

[9]. Whiting S.H. and Champoux,J.J. (1998) *J. Mol. Biol.*, **278**, 559–577. [PubMed] [Google Scholar]
[10]. Mbwana J, et al. Molecular characterization of Haemophilus ducreyi isolates from different geographical locations. J Clin Microbiol. 2006 Jan;44(1):132-7. PMID: 16390960
[11]. Williams JG, et al. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 1990 v 25;18(22):6531-5. PMID: 1979162
[12]. Saiki, R.; Scharf, S; Faloona, F; Mullis, K.; Horn, G.; Erlich, H.; Arnheim, N (1985). "Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia". Science. **230** (4732): 1350–1354.
[13]. Lawn SD, Mwaba P, Bates M, et al. Advances in tuberculosis diagnostics: The Xpert MTB/RIF assay and future prospects for a point-of-care test. *Lancet Infect Dis.* 2013;13:349–61. [PMC free article] [PubMed] [Google Scholar]
[14]. Antonenka U, Hofmann-Thiel S, Turaev L, et al. Comparison of Xpert MTB/RIF with ProbeTec ET DTB and COBAS TaqMan MTB for direct detection of *M. tuberculosis* complex in respiratory specimens. *BMC Infect Dis.* 2013;13:280. [PMC free article] [PubMed] [Google Scholar]
[15]. Banada PP, Chakravorty S, Shah D, Burday M, Mazzella FM, Alland D. Highly sensitive detection of *Staphylococcus aureus* directly from patient blood. *PLoS One.* 2012;7:e31126. [PMC free article] [PubMed] [Google Scholar]
[16]. Compton J. Nucleic acid sequence-based amplification. *Nature.* 1991;350:91–2. [PubMed] [Google Scholar]
[17]. Chang CC, Chen CC, Wei SC, Lu HH, Liang YH, Lin CW. Diagnostic devices for isothermal nucleic acid amplification. *Sensors (Basel)* 2012;12:8319–37. [PMC free article] [PubMed] [Google Scholar]
[18]. Chernesky MA, Jang DE. APTIMA transcription-mediated amplification assays for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *Expert Rev Mol Diagn.* 2006;6:519–25. [PubMed] [Google Scholar]
[19]. Pachl C, Todd JA, Kern DG, et al. Rapid and precise quantification of HIV-1 RNA in plasma using a branched DNA signal amplification assay. *J Acquir Immune Defic Syndr Hum Retrovirol.* 1995;8:446–54. [PubMed] [Google Scholar]
[20]. Van Der Pol B, Warren T, Taylor SN, et al. Type-specific identification of anogenital herpes simplex virus infections by use of a commercially available nucleic acid amplification test. *J Clin Microbiol.* 2012;50:3466–71. [PMC free article] [PubMed] [Google Scholar]
[21]. Killick V, Kell P, Plumb H, Hurd N, Turner P. Screening for *Neisseria gonorrhoeae* using the BD ProbeTec nucleic acid amplification test in a low prevalence genitourinary medicine clinic. *Int J STD AIDS.* 2012;23:e1–3. [PubMed] [Google Scholar]
[22]. Walker GT, Fraiser MS, Schram JL, Little MC, Nadeau JG, Malinowski DP. Strand displacement amplification – an isothermal, in vitro DNA amplification technique. *Nucleic Acids Res.* 1992;20:1691–6. [PMC free article] [PubMed] [Google Scholar]
[23]. Gillio-Tos A, De Marco L, Carozzi FM, et al. Clinical impact of the analytical specificity of Hybrid Capture 2: Data from the NTCC study. *J Clin Microbiol.* 2013 [PMC free article] [PubMed] [Google Scholar]
[24]. Hazelton BJ, Thomas LC, Unver T, Iredell JR. Rapid identification of Gram-positive pathogens and their resistance genes from positive blood culture broth using a multiplex tandem RT-PCR assay. *J Med Microbiol.* 2013;62:223–31. [PubMed] [Google Scholar]
[25]. Elbir H, Henry M, Diatta G, et al. Multiplex real-time PCR diagnostic of relapsing fevers in Africa. *PLoS Negl Trop Dis.* 2013;7:e2042. [PMC free article] [PubMed] [Google Scholar]
[26]. Kim H, Jeong SH, Kim M, Lee Y, Lee K. Detection of *Clostridium difficile* toxin A/B genes by multiplex real-time PCR for the diagnosis of *C. difficile* infection. *J Med Microbiol.* 2012;61:274–7. [PubMed] [Google Scholar]
[27]. Njouom R, Yekwa EL, Cappy P, Vabret A, Boisier P, Rousset D. Viral etiology of influenza-like illnesses in Cameroon, January–December 2009. *J Infect Dis.* 2012;20(6 Suppl 1):S29–35. [PMC free article] [PubMed] [Google Scholar]
[28]. Park Y, Kim BS, Choi KH, et al. A novel multiplex real-time PCR assay for the concurrent detection of hepatitis A, B and C viruses in patients with acute hepatitis. *PLoS One.* 2012;7:e49106. [PMC free article] [PubMed] [Google Scholar]
[29]. Chang SS, Hsieh WH, Liu TS, et al. Multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis – a systemic review and meta-analysis. *PLoS One.* 2013;8:e62323. [PMC free article] [PubMed] [Google Scholar]
[30]. Fitting C, Parlato M, Adib-Conquy M, et al. DNAemia detection by multiplex PCR and biomarkers for infection in systemic inflammatory response syndrome patients. *PLoS One.* 2012;7:e38916. [PMC free article] [PubMed] [Google Scholar]

Ruvin Haidar, et. al. "A review of nucleic acid tests in diagnostic microbiology." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 20(09), 2021, pp. 54-57.