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Review Article

Nucleic Acid Amplification Techniques (NAAT) in Post Genomics Era

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Abstract

Nucleic Acid Amplification Techniques (NAAT) have evolved quickly with new innovations which can provide unprecedented opportunities in the field of Health and food security. Over time different method of Nucleic Acid Extraction (NAE) has been evolved like from a chemical based or solution based method to solid based to magnetic beads bases method and now we are in the period of full automation, we got fully automated Nucleic Acid (NA) extractors, a chip based system for amplification which take much less time in comparison to other amplification systems. More evolution in amplification techniques with introduction of Helicase Dependent DNA Amplification (HDA) which is superior to all other methods in terms of ease of performance, cost, and its ability to perform a completely isothermal reaction. These techniques have ultimate impact on field of health care particularly in point of care diagnostics and it is continuously evolving to provide a better quality of life.

Keywords: Nucleic Acid; Nucleic Acid Extraction; Nucleic Acid Amplification; PCR; DNA; RNA

Introduction

In modern era the field of molecular biology known to be the next big thing in field of health care and in the field of food security. In the era of technology the combination of biology and the technology associated with it make a new field of biotechnology which made so many achievements in the field of medicine and food productivity and security. This all possible with the development biotechnology and most importantly with the development of NAAT, by which we are able to find out the mutant sequences, or the sequences which are responsible for a particular character by amplifying the particular sequence of nucleic acid or genome. It is helpful in finding the desired sequence in the genome whether it is for diagnosis of a disease or a sequence in the crop plant to modify it for a better species or for a better productivity result or it may be a sequence that can be used in the production of a better biofertilizer like in algae or a nitrogen fixing bacteria or to introduce nitrogen fixing genes in crop plant and transform it. By introduction of NAAT, the field of biotechnology or say molecular biology is completely revolutionized and more overly in matter of time saving, but to reach up to this point there is long course of evolution of these techniques which took over more than a century to reach to this point and it is still evolving. Over time different method of Nucleic Acid Extraction (NAE) has been evolved like from a chemical based or solution based method to solid based to magnetic beads bases method and now we are in the period of full automation, we got fully automated NA extractors, a chip based system for PCR which take much less time in comparison to other PCR systems. With fully automated system of NAE there is introduction of Cartridge Based NAE, which is a very cost effective system and with approximately no chance of contamination. In India Molbio Diagnostics Pvt. Ltd. is making automated systems under the Make in India project of Indian Government. Different amplification techniques developed after invention of PCR like ligase chain reaction, rolling circle reaction and most superior of them all is helicase dependent amplification. Now the most important influence of NAAT is on point of care

diagnostics, it evolved with time from a basic protocol of NA extraction to a fully automated system in which we saved labour but most importantly we are saving much more time and giving result of disease diagnosis in short period of time so that the treatment of affected patient can be started on a proper time and increasing quality of life.

History of NAE and NAAT

In year of 1869 almost by accident a Swiss physician and biologist by the name of Johannes Friedrich Miescher discovered a phosphorus rich acid in the nucleus of white blood cell or leucocyte and called it 'Nuclein' which is later renamed as DNA [1]. Albrecht Kossel in 1881 identified nuclein as a nucleic acid and provided its present chemical name Deoxyribonucleic Acid (DNA) and from a period of 1885 to 1901 he was able to isolate constituent of nucleic acid and named those five organic compounds as adenine, cytosine, guanine, thymine and uracil. These compounds are now known collectively as nucleobases, and they provide the molecular structure necessary in the formation of stable DNA and RNA molecules. Walther flemming in 1888 distinguish that thread like structure in nucleus as chromatin with the help of limited microscopy present at that time. He stained them with aniline dye which is basopihillic in nature and strongly absorb by nucleic acid. These later named as chromosome given by Wilhelm Waldeyer and band across them named chromomere by Edouard Balbiani and Edouard Van Beneden. In 1914 a scientist by name of Joachim Wilhelm Robert Feulgen developed a method for staining the DNA and called it as feulgen stain and demonstrated the presence of DNA in chromosomes. Frederick Griffith discovered that there are two strains present in pneumococcus which were Rough-strain (R) and Smooth-strain (S) in 1922 and in 1928 he discovered that when heat killed S-strain mixed with live R-strain then his experimental mice that he used, died due to transformation. The transfer formula was DNA was later discovered by Oswald T. Avery, Colin M. Mcleod and Maclyn McCarty in 1944, they showed that the bacterial DNA from pneumococcus was able to induce the transformation of other genetically different pneumococci. In 1944 Erwin Charguff who inspired by work of Oswald T. Avery analyzed the nitrogenous components and sugars of DNA from different species and discovered that in any double-stranded DNA, the number of guanine units equals the number of cytosine units and the number of adenine units equals the number of thymine units and no. of purine and pyramidine varies from one species to another, now these are known as Chargaff's Rules. Simultaneously John Griffith independently discovered that adenine attaract thymine and guanine attract cytosine [2]. Rollin Douglous Hotchkiss, Avery and Ephruss-Taylor's work on bacteria pnemococcus led to the establishment of role of DNA as genetic material [3]. French scientist Andre Boivin, Roger Vendrely, Colette Vendrely showed that the DNA content of cells was directly related to no. of chromosome contained in cell. In 1950's Seymour Benzer told that the unit of mutation and recombination both are nucleic base. Alexander Todd arranged chemical component of DNA showing that skeleton of DNA is made up of alternating phosphoryl and pentoxyl residue, concluding that phosphate, a group of oxygen and phosphorus atom linked to the next one by a deoxyribose sugar in DNA [4]. In 1952 Alfred Hershey and Martha Chase used T2 bacteriophage and proved that the genetic material of phage was DNA, not protein. This experiment finally proved Oswald T. Avery's experiment [5]. Raymond Gosling who was a student of Rosalind Franklin, they worked together and able to get two sets of high-resolution photos of crystallized DNA fibers in 1952 [6]. In 1953 James Dewey Watson and Francis Harry Compton Crick gave double helix structure of DNA by analyzing the X- ray crystallography by Rosalind Franklin and Maurice H.F. Wilkins and experiments of Erwin Chargaff and John Griffith [7]. After the revealing the structure of DNA scientists started working on the sequencing of DNA. In 1965 Robert Holley and colleagues were able to produce the first whole nucleic acid sequence of alanine tRNA from *Saccharomyces cerevisiae*. The major breakthrough that forever altered the progress of DNA sequencing technology came in 1977, with the development of Sanger's 'chain-termination' or dideoxy technique and Maxam and Gilbert method. A next generation sequencing method called pyrosequencing technique, pioneered by Pal Nyren and colleagues. A number of parallel sequencing techniques sprung up following the success of pyrosequencing. In third-generation DNA sequencing, nucleotide detection in a Zero-Mode Waveguide (ZMW) as featured in PacBio sequencers [8].

History of techniques used in NAAT

In the early 1970s one could not identify one single gene among thousands of fragments of DNA until Edward Southern introduced his eponymous powerful DNA transfer and probing technique in 1975. In 1977 George Stark and colleagues replicated the configuration of Southern's transfer apparatus in an effort to transfer cellular RNA to chemically activated cellulose paper and named it Northern blotting. Two years later, Stark developed an early protein blotting technique, relying on overnight capillary transfer from gel to activated cellulose paper. Harry Towbin's faster and

simpler approach for electroblotting proteins to nitrocellulose membranes is clearly preferred today. The name Western blot was first given to the technique by W. Neal Burnette in 1981 [9]. Restriction fragment length polymorphism (RFLP) technique invented in 1984 by the English scientist Alec Jeffreys during research into hereditary diseases. The breakthrough of DNA Fingerprinting was due to a different project that Professor Jefferys was working on. On September 10th, 1984, at Jefferys's lab in the University of Leicester (UK), the x-ray blot was developed [10]. Another technique Random amplified polymorphic DNA (RAPD) markers were developed and applied in plants by Welsh and McClelland in 1990 and was developed by Williams., et al. in 1990. Amplified fragment length polymorphism (AFLP) or AFLP PCR is a PCR-based tool used in genetics research, DNA fingerprinting, and in the practice of genetic engineering. Developed in the early 1990s by Keygene, AFLP uses restriction enzymes to digest genomic DNA, followed by ligation of adaptors to the sticky ends of the restriction fragments [11]. In 1971, Kjell Kleppe and the Nobel laureate, HarGobind Khorana, published a description of a technique that represented the basic principles of a method for nucleic acid replication which was a clear description of the process that is now recognized as the PCR [12]. The theoretical process was outlined by Kleppe and coworkers in 1971, and experimentally applied by Kary Mullis in 1985. After that in 1993 first real-time (RT) PCR detection experiments to show utility for DNA quantization reaction took place using EtBr detection. In post amplification techniques, Tiselius published his thesis in 1930, 'The Moving Boundary Method of Studying the Electrophoresis of Proteins', which was important as it represented a new technique for studies of the physico-chemical properties of proteins. In 1937 Tiselius introduced an electrophoresis cell with a rectangular cross-section and efficient cooling at 4°C where water has its maximum density. Shortly afterwards improved procedures were presented by Longsworth and Svensson. The works of Svensson, Longsworth and Dole were of fundamental importance for the development also of zone and displacement electrophoresis [13].

Method of nucleic acid extraction

Nucleic acid extraction (NAE) is one of the most pivotal steps in molecular biology, being routinely used in many areas of the biological and medical sciences, as this procedure marks a starting point in any molecular diagnostic kit. NAE can be roughly divided into four steps, which can be modulated depending on the sample and downstream applications: (i) cell lysis, (ii) removal of organic components, (iii) purification and binding of NA, (iv) NA elution [14].

Chemically Driven Methods or Solution Based Method

Cesium Chloride (CsCl) Gradient Centrifugation with Ethidium Bromide (EtBr)

Matthew Meselson, Franklin Stahl, and Jerome Vinograd developed cesium chloride, or CsCl, density gradient centrifugation in the 1950s. This technique is mainly based on the phenomenon of buoyant and specific density. Ethidium bromide (EtBr) is an intercalating agent, thus reporting the location of the double-stranded DNA under UV-light and allowing the easy visual separation of DNA molecules. After the ultracentrifugation, CsCl has to be dialyzed of the collected DNA [15]. The method can be used to extract DNA from bacteria, chloroplast DNA, or mitochondrial DNA. Being sensitive and provider of good yields of pure DNA, the method is laborious, time-consuming, and costly as compared to other purification protocols. There is concern about using EtBr, which is known to cause genotoxicity and frame shift mutations.

Guanidinium thiocyanate-phenol-chloroform extraction

Volkin and Carter reported the first use of guanidinium chloride in the isolation of RNA in 1951. In 1987, Chomczynski and Sacchi combined guanidinium thiocyanate with phenol-chloroform extraction under acidic conditions. A guanidinium thiocyanate (GuSCN) phenol-chloroform mixture allows for RNA extraction in a single-step procedure. Despite being less soluble in water than guanidine hydrochloride, another common salt of guanidine, GuSCN has stronger denaturing properties because both its ions are chaotropic. The basic principle of the method is the separation of RNA from DNA and proteins after extraction with an acidic solution, which consists mainly of GuSCN, sodium acetate, phenol, and chloroform, followed by centrifugation. Total RNA is then recovered through precipitation by isopropanol and can be used for subsequent process. Optimum pH plays a critical role in the separation process as DNA partitions to the organic phase under acidic condition [16]. The main drawback of this method is that phenol and chloroform are both hazardous chemicals.

Alkaline extraction

Alkaline extraction method is dedicated to plasmid DNA isolation, described by Birnboim and Doly in 1979. The basic principle of this method is selective alkaline denaturation of high molecular weight chromosomal DNA, while covalently bond circular plasmid DNA remains intact. After neutralization, chromosomal DNA renatures and makes an insoluble precipitate, while plasmid DNA remains in the supernatant. The method involves exposing of

bacteria to alkaline solution consisting basically of SDS and NaOH. SDS acts as detergent to lyse the cells and denature proteins, while alkaline condition denatures genomic DNA, plasmid DNA, and proteins. Potassium acetate addition neutralizes the mixture and results in renaturation of plasmid as well as genomic DNA. Further addition of ethanol or isopropanol precipitates genomic DNA, while plasmid DNA can be collected from the supernatant after a short two minute centrifugation [17]. The main drawback is vigorous mixing during lysis and neutralization phases can cause fragmentation of genomic DNA, resulting in contamination with plasmid supernatant.

Cetyltrimethylammonium bromide (CTAB) extraction alkaline extraction

In plants, a breakthrough in DNA extraction came in 1980 with the development of the CTAB protocol by Murray and Thompson. CTAB is a cationic detergent that is compatible with the high salt concentrations often used to dissociate DNA from chromosomal proteins and can also be used to selectively precipitate nucleic acid. The basic composition of CTAB extraction buffer includes 2% CTAB at alkaline pH, but, like many other extraction protocols, CTAB has been modified according to the need of each sample. CTAB works by precipitating nucleic acids and acidic polysaccharides in low ionic strength solutions, while proteins and neutral polysaccharides remain in solution. During the precipitation and washing steps, CTAB method uses various organic solvents and alcohols such as phenol, chloroform, isoamyl alcohol, and mercaptoethanol [18]. The main drawback of this procedure is that it is time-consuming and makes use of toxic chemicals like phenol and chloroform.

Purification of Poly(A)+ RNA by oligo(dT)-cellulose chromatography

The basic mechanism of this method is that poly(A) RNA hybridizes with an oligo(dT)-cellulose matrix, under high-salt conditions. Polyadenylated RNA with minimum 20 residues have the ability to attach to the oligo(dT)-cellulose matrix, which usually consists of 10 - 20 nucleotides. After washing out all the nonpolyadenylated RNAs, a low-salt buffer is used to disrupt the oligo (dT) - poly (A) bond, resulting in the elution of poly(A) RNAs. There are two methods commonly used in the selection of Poly (A) + RNA column chromatography on oligo (dT) columns and batch chromatography [19]. Its main drawback is that the method selects only mRNAs and naturally excludes important biological information present in other RNAs, such as miRNAs, rRNAs, and tRNAs.

Solid-Phase nucleic acid extraction

Solid-phase extraction (SPE) is one of the most efficient NAE techniques available in the market. It is based on liquid and stationary phases, which selectively separate the target analyte from the solution based on specific hydrophobic, polar, and/or ionic properties of both solute and sorbent.

Solid-phase micro extraction (SPME)

It is a relatively new development in solid-phase extraction technique, introduced in 1990s and being useful for various analytes including liquid, gaseous, and solid matrices. Two important steps are involved in SPME: (i) partitioning of analytes on fiber-coated extraction phase and (ii) handing over extract to separating instrument like gas chromatography where takes place desorption. SPME is a rapid and easy to use technique and have good detection limit (parts per trillion) for specific compounds [20]. The main drawbacks of SPME include difficulty in analyzing high molecular weight compounds, sample carryover, and the eventual shortage of commercially available stationary phases.

Silica matrices

In 1979, it was found that silicates have high binding affinity for DNA under alkaline conditions and increased salt concentration. DNA binds to the inorganic matrix and is released in heated water. The mechanism involved in this technique is the affinity between negatively charged NA and positively charged silica material, resulting in selective binding of nucleic acids to the silica matrices, while the rest of the cell components and other chemicals are washed out. Silica surface is covered by positive ions like Na+, Li+ and NH4+ which enhances the binding of negatively charged DNA. As a final step, NA can be eluted from silica matrix by nuclease-free water. Drawback of this technique is being unable to recover small fragments DNA efficiently, as small fragments binds tightly with the silica matrix.

Glass particles

Glass particles, powder and beads are useful for nucleic acid purification. The basic principle of binding of NA to glass particles is based on the adsorption affinity of the components present in the mixture of cellular components like DNA, proteins, etc. to the stationary phase of chromatography column (glass particles).

Diatomaceous earth

First described by Boom., *et al.* this procedure is mainly based on the binding of NA to a solid phase in the presence of chaotropic agents, followed by elution in water or low-salt buffer. Diatomaceous Earth (DE), alternatively known as kieselguhr, is mainly composed of silica, alumina, and hematite, NA binds to the silica present in DE, following the same principles of binding to silica matrices. This procedure has the advantage of reduced pipetting error, shorter protocol time, and a smaller number of steps for sample preparation. The drawback is high cost of this technique compare to other [21].

Magnetic beads-based nucleic acid purification

The technique involves the separation of nucleic acids from complex mixtures via complementary hybridization. It is enough to apply a magnet to the side of a vessel or tube containing the sample mixed with the functionalized magnetic beads which exclusively aggregate the target particles near the vessel wall. The extraction technique can be used in a diverse kind of samples and is relatively easy to execute, being one of the best choices for automation, high-throughput applications, and high sample processivity. The poly (A) RNA can be extracted by introducing magnetic beads coated with oligo (dT). RNA with a poly-A tail attach to the oligo (dT) [22]. The beads will then be drawn to the bottom of a tube removing mRNA directly from total RNA. The magnetic beads which are specially treated, minimize the nonspecific binding of other nucleic acids and ensure the purity of mRNA.

Anion exchange material

Anion exchange resin is one of the popular examples that utilized the anion-exchange principle. It is based on the interaction between positively charged diethyl amino ethyl cellulose (DEAE) groups on the resin's surface and negatively charged phosphates of the DNA backbone. The anion-exchange resin consists of defined silica beads with a large pore size, a hydrophilic surface coating and has a high charge density. The pH and salt concentration are the important aspects determining the binding or elution of NA to the anion exchange resin. Anion exchange has the advantage of extracting very pure DNA as compared to silica and the ability to reuse the resin upon renaturation [23]. This method used high-salt concentration in the elution step, thus requiring desalting for downstream applications.

Cellulose matrix

Absorbent cellulose-based paper is an interesting matrix for nucleic acids purification and storage. Cellulose-based paper are simply a thick layer of paper, embedded with a proprietary mix of buffers, detergents, and chelating agents, such as the ubiquitous Tris pH 8, SDS, and EDTA. Once cells are spotted onto the paper, the detergent lyses the membranes and EDTA chelates metal ions that are cofactors to nucleases, also preventing the growth of contaminating organisms [24]. Although cellulose matrix have many advantages regarding the easiness of use and storage, processing them to extract good yields of nucleic acids might be more complicated than expected, especially in diluted samples.

Cartridge based NAE

Detection of the nucleic acid using cartridge based nucleic acid extractor with the help of a cartridge based sample preparation device which is a light weight, portable and operates on portable mains or on a rechargeable battery. Cartridge based system uses a proprietary matrix enclosed in a cartridge to purify nucleic acids from clinical sample. The pretreated sample is added to the sample chamber of cartridge then the cartridge is placed in cartridge based sample preparation device for processing. Nucleic acid from the sample bound by the matrix and inhibitor in sample are washed out. At the end of the processing the bound nucleic acid is eluted and collected in elution chamber. The elute is transferred to Elute collection tube (ECT). The elute is then transferred to chips for further analysis on Real Time PCR analyzer [25].

Methods for amplification of nucleic acid

Polymerase Chain Reaction (PCR) was the first DNA amplification method developed by Kary B. Mullis in 1985. Today, numerous different methods are now wide use, including Rolling Circle Amplification, Ligase Chain Reaction, and Helicase Dependent DNA amplification. Many of these newer methods for DNA amplification attempt to fix some of the fundamental problems associated with PCR, such as its dependence on thermo cycling.

Polymerase chain reaction (PCR)

PCR is the classical technique used to amplify DNA. There are three major components involved with PCR, first DNA polymerase which is stable at high temperatures, second a mixture of four different deoxyribonucleotide triphosphates (dNTP) and third are

two small synthesized oligonucleotide primer designed to attach to the DNA sequence of interest. The first step of PCR begins with the denaturation of the double stranded DNA. In order to do this, the mixture must be raised to a high temperature, usually around 94°-95°celcius. After the DNA has been denatured, there is Annealing step takes place between 50°-65°celsius, in it the oligonucleotide primers hybridize to the denatured DNA at the specific targets that they were synthesized to bind with. In the final step, the DNA polymerase sequentially adds the dNTPs to the template DNA strands. This effectively synthesizes two new identical double stranded DNA molecules. This step occurs around 70°-75°celsius. These three steps make up one cycle of the PCR reaction [26]. There are many advantages associated with PCR reaction as it occurs rapidly and only takes a few hours to perform. This method is also extremely sensitive. The main drawback is that primers need to be synthesized earlier so the researcher needs to know at least some of the DNA sequence prior to amplification and this is not always the case when studying a new DNA sequence. Many variants of PCR are used in modern time with all having the same basic principle but with small modification in the standard PCR protocol we can achieve a desired goal. In Multiplex PCR we add different pair of primer to target multiple genes at a single time. Variable Number of Tandem Repeats (VNTR) PCR targets areas of the genome that exhibit length variation. Asymmetric PCR preferentially amplifies one strand of the target DNA. Nested PCR is used to increase the specificity of DNA amplification; in this two sets of primers are used in two successive reactions. Hot-start PCR is started by performing manual heating of reaction components to the DNA melting temperature before adding the polymerase. In Touchdown PCR the annealing temperature is gradually decreased in later cycles for efficient amplification at the end of the reaction. Assembly PCR is the synthesis of long DNA structures by performing PCR on a pool of long oligonucleotides with short overlapping segments, to assemble two or more pieces of DNA into one piece. Reverse Transcription PCR is used to reverse-transcribe and amplify RNA to cDNA also called RT-PCR. Ligation-mediated PCR uses small DNA oligonucleotide linkers that are first ligated to fragments of the target DNA. Methylation-specific PCR is used to identify patterns of DNA methylation at cytosine-guanine (CpG) islands in genomic DNA. Quantitative PCR (q-PCR) is used to measure the specific amount of target DNA or RNA in a sample in real time so also called Quantitative Real-Time PCR. And many more variants are invented over time [27].

Ligase chain reaction (LCR)

The LCR reaction is similar to PCR as both use synthesized primers to amplify the DNA. LCR oligonucleotide primers are long as they are designed to cover the entire sequence to be amplified and LCR based on temperature cycling in order to break the double stranded DNA. The first step in LCR is the denaturation of the double stranded DNA by heating the reaction mixture. After denaturation, the two complimentary pairs of primers bind to their respective DNA strands. One pair is complimentary to one strand of the DNA while the other is complimentary to the other template DNA strand. These primers cover the entire length of the sequence that will be amplified by the reaction. There is no space between these primers as the space between the two is then sealed by a thermo stable DNA ligase. This generates a fragment that is as long as the total length of each pair of primers. These products of one cycle then serve as the templates for subsequent cycles. The main advantage of this type of amplification is that a single point mutation in the original template DNA can prevent the reaction. Therefore, an absence of product from this type of amplification can be an indicator of mutations. There are many drawbacks to the LCR reaction as this reaction is very specific and only the sequences encoded by the primers are replicated, any mutation outside of this amplified sequence is not detected [28].

Rolling circle amplification (RCA)

With RCA, first two ends of the DNA of interest are joined together using a DNA ligase to form a circular single stranded DNA template. Next, the primer is attached to this template during an annealed step similar to PCR. The amplification is then carried out by a suitable DNA polymerase, which extends the primer until the circle is complete at which point the synthesized strand is displaced due to the intrinsic property of the polymerase. The main advantage that this method has is that the synthesis can occur at room temperature and does not require heating and this reaction occurs relatively quickly, only taking a few hours. The main drawback of RCA is that the protocol is extremely complicated, and it is not capable of amplifying a satisfactory length of nucleic acids and also the initial step in which the single stranded cyclic DNA is formed does require an initial heat denaturation step [29].

Helicase-Dependent DNA amplification (HDA)

HDA occurs like the DNA replication found in cells in that it relies on a DNA helicase to separate the double stranded DNA to

generate the single stranded DNA templates. After the helicase has unwound the DNA, single-stranded binding proteins bind to the unwound DNA, which prevent these unwound pieces from re-annealing to one another or degrading. Next two sequence specific primers are annealed to the single stranded DNA, and a DNA polymerase extends these primers until a new double stranded DNA molecule is formed. These newly synthesized double stranded DNA molecules are then used as templates for the next round of amplification. The major advantage to HDA is that the DNA helicase can operate at room temperature and therefore the thermal cycling as well as thermal cycler is not required. The only disadvantage of HDA is that, it is not much efficient as PCR as it takes a little longer to amplify the same amount of DNA to the target concentration using HDA than it would by using PCR [30].

Significance of NAAT in medical science and agriculture

In recent times the importance and use of NAAT is increasing day by day in the field of health care. Commercial use of different nucleic acid amplification techniques for clinical tests which helps in quantitative as well as qualitative analysis of genome of pathogens includes bacteria, viruses, fungi, yeast and parasites and also have play a significant role in diagnosis of autoimmune disorders [31,32]. It helps in detection of cancer and helps in guiding cancer treatment and nucleic acid testing is being increasingly used to provide personalized and precision medicine and avoid adverse reactions from drugs in individuals. It also helps in screening for genetic disorders and mitochondrial diseases [33]. Detection of new disease at early stage by using NAAT helps in saving millions of lives by detection of pathogenic sequence in the pathogen causing the disease before becoming epidemic.

In Agriculture, field NAAT used widely in genetic engineering of crop plants for maintaining qualitative aspects like nutrition quality or composition of nutrients in the staple food around the world as well as quantitative aspects [34]. It helps in study of different kind of genes responsible for survival as well as maintaining nutrition quality of crop plant in harsh condition and their manipulation for another crop plant. It is a significant part of green revolution around the world [5]. It is also playing a significant role in development of bio fertilizers helps crop plant in proper nitrogen fixation and other functions.

Conclusion

After almost 150 years after the first successful isolation of DNA by Friedrich Miescher, nucleic acids are now central to obtaining biological information in areas as distinct as specimens' identification for conservational purposes to the realms of personalized medicine. Protocols and devices used for NAE have evolved from thiocyanate-phenol-chloroform manual techniques to user-friendly column-technology and automated platforms, but no general gold-standard method has yet been established. This review analyzed the history of NAE from the time of Friedrich Miescher to the sequencing of NA and history of bio techniques that are the part of NAAT and their evolution with time and the methods of NAE and their working principle of each available method, as well as their advantages and disadvantages. In Amplification techniques PCR and LCR require thermocycling to occur throughout the DNA amplification reaction and RCA requires an initial heating step before the reaction can take place and is a lengthy. HDA is superior to all other methods in terms of ease of performance, cost, and its ability to perform a completely isothermal reaction. PCR seems the most efficient of all the amplification reactions and PCR has been in practice for a long time because of which many automated machineries are available which perform the temperature cycling and only require the researcher to add all the reagents at the beginning of the reaction. Although molecular biology techniques are sensitive and accurate methods, they require a rather well established laboratory setting and expensive instruments, as well as skilled personnel to run the tests and analyze the results particularly in the field of point of care diagnostics.

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Volume 2 Issue 9 December 2018