From Tradition to Innovation: Diverse Molecular Techniques in the Fight Against Infectious Diseases

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Abstract

Infectious diseases impose a significant burden on global health systems due to high morbidity and mortality rates. According to the World Health Organization, millions die from infectious diseases annually, often due to delays in accurate diagnosis. Traditional diagnostic methods in clinical microbiology, primarily culture-based techniques, are time-consuming and may fail with hard-to-culture pathogens. Molecular biology advancements, notably the Polymerase Chain Reaction (PCR), have revolutionized infectious disease diagnostics by allowing rapid and sensitive detection of pathogens' genetic material. PCR has become the gold standard for many infections, particularly highlighted during the COVID-19 pandemic. Following PCR, Next- Generation Sequencing (NGS) has emerged, enabling comprehensive genomic analysis of pathogens, thus facilitating the detection of new strains and antibiotic resistance tracking. Innovative approaches like CRISPR technology are also enhancing diagnostic precision by identifying specific DNA/RNA sequences. However, the implementation of these methods faces challenges, particularly in low- and middle-income countries due to infrastructural and financial constraints. This review will explore the role of molecular diagnostic methods in infectious disease diagnosis, comparing their advantages and limitations, with a focus on PCR and NGS technologies and their future potential.

Keywords: infectious diseases; molecular diagnostics; next-generation sequencing (NGS); polymerase chain reaction (PCR)

1.Introduction

Infectious diseases pose a significant burden on health systems worldwide due to high morbidity and mortality rates. According to the World Health Organization (WHO), millions of people die from infectious diseases each year, often because early and accurate diagnosis is not achieved [1]. This reality highlights the critical importance of rapid and accurate diagnosis of infectious diseases.

For many years, traditional diagnostic methods used in clinical microbiology laboratories were generally based on culture-based techniques. However, these techniques are not only time- consuming but also inadequate for pathogens that are difficult or impossible to cultivate under laboratory conditions [2]. This limitation is particularly evident in the case of slow-growing microorganisms or pathogens requiring highly specific culture conditions. Additionally, the sensitivity of immunological tests may significantly decrease during the early stages of infection, when specific antibody formation has not yet occurred [3].

In recent years, advancements in molecular biology have led to significant transformations in the diagnosis of infectious diseases, ushering in a new era in diagnostic processes. Among these advancements, the Polymerase Chain Reaction (PCR), developed by Kary Mullis in the late 1980s, stands out [4]. The primary advantage of PCR is its ability to rapidly and sensitively amplify the

genetic material of the infectious agent, enabling specific detection. This capability has quickly made PCR an indispensable tool in clinical microbiology laboratories and established it as the gold standard for diagnosing many infectious diseases. During the COVID-19 pandemic, PCR technology became the most widely used diagnostic method worldwide [5,6]. The rapid and reliable results provided by PCR-based tests during the pandemic have once again highlighted the critical importance of molecular diagnostic methods for public health and clinical practice.

One of the most remarkable innovations following PCR is Next-Generation Sequencing (NGS) technology. NGS allows for the sequencing of entire genomes of pathogens in a single run, enabling the detection of new strains and genetic variations [7]. This technology offers significant advantages, particularly in tracking antibiotic-resistant strains, identifying virulence factors, and understanding the molecular epidemiology of outbreaks. With the growing availability of bioinformatics tools and data analysis capabilities, NGS is increasingly being used in clinical diagnostics. The rapid identification of resistance genes and virulence factors has greatly accelerated the management of infections [8].

Molecular diagnostic methods have expanded beyond PCR and NGS to include innovative approaches such as CRISPR-based diagnostic systems. In addition to its gene-editing potential, CRISPR (short for "clustered regularly interspaced short palindromic repeats") technology stands out as a precise and rapid method for detecting specific DNA or RNA sequences in the diagnosis of infectious diseases. This technology marks the beginning of a new era in the diagnosis of infectious diseases [9,10].

However, the implementation of molecular diagnostic methods in clinical practice presents certain challenges, especially in low- and middle-income countries. The lack of advanced infrastructure, trained personnel, and sufficient funding poses significant barriers to the widespread adoption of these technologies [11]. Nonetheless, ongoing efforts to develop portable and more affordable PCR and NGS systems may help overcome these challenges [12,13].

With the rapid advancement of molecular diagnostic methods, the advantages and limitations of each technology have become highly significant for clinical applications. Techniques such as PCR, NGS, Loop-Mediated Isothermal Amplification (LAMP), and CRISPR offer speed, sensitivity, and specificity in the diagnosis of infectious diseases, yet each has its own distinct strengths and weaknesses. Table 1 provides a comparative overview of the advantages, disadvantages, and applications of these technologies, offering guidance on which methods may be preferred under different conditions in molecular diagnostic processes.

Technology	Advantages	Disadvantages	Applications
PCR	High sensitivity, early diagnosis, broad clinical use	Requires thermal cycler, may produce false positives/negatives in some cases	Diagnosis of viral, bacterial, fungal, and parasitic infections
qPCR (Real-Time PCR)	Allows quantitative analysis, simultaneous detection of multiple pathogens	Expensive equipment, requires thermal cycling	Viral load monitoring, tracking disease progression
Multiplex PCR	Detection of multiple pathogens at once	Complex primer design, requires optimization	Respiratory infections, co-infections
LAMP	Isothermal operation, rapid results, portable, and low cost	Difficult primer design, usually targets a single pathogen	Tropical infections, rapid field diagnosis
NGS	Genome scanning, detection of new variants and resistance genes	High cost, requires technical infrastructure and expertise	Antibiotic resistance, outbreak monitoring, discovery of new pathogens
CRISPR	Precise detection of specific DNA/RNA sequences, rapid results	Limited clinical applications so far, technically complex	Detection of viral infections, genetic variants

Table 1. Advantages, disadvantages, and clinical applications of molecular diagnostic methods.

This review will provide a detailed examination of the role of molecular diagnostic methods in the diagnosis of infectious diseases, how they are used in clinical applications, their advantages, and the challenges encountered. Particular emphasis will be placed on advanced diagnostic methods such as PCR and NGS, highlighting the advantages they offer over traditional methods and discussing potential future developments.

2. Molecular Diagnostic Methods

Molecular diagnostic methods are advanced biotechnological tools that enable the rapid, sensitive, and specific detection of infectious diseases. The slow nature and limited sensitivity of traditional diagnostic methods pose a significant disadvantage, especially for infections that require prompt intervention. In contrast, molecular methods target the genetic material (DNA and RNA) of pathogens, providing faster and more accurate results. The ability of these techniques to be used in the early stages of infection and to detect even low amounts of pathogenic material makes them indispensable in clinical applications [14,15].

Today, the most commonly used techni; ques in molecular diagnostics include PCR, Real-Time PCR (qPCR), Multiplex PCR, NGS, LAMP, and CRISPR-based diagnostic methods. These techniques not only accelerate diagnostic processes but also provide critical information about the genetic structure of infectious agents, contributing to treatment planning.

2.1. Molecular Diagnostic Methods

PCR is considered one of the cornerstones of molecular diagnostics and has been a revolutionary development in the diagnosis of infectious diseases. Developed by Kary Mullis in 1983, this technique allows for the rapid amplification of a specific segment of target DNA millions of times, enabling the detection of even extremely small amounts of genetic material [4]. This high sensitivity has made PCR an indispensable tool in the diagnosis of infectious diseases. Today, PCR is used to detect a wide range of microorganisms, including viruses, bacteria, fungi, and parasites, and is regarded as the "gold standard" in clinical microbiology laboratories [16].

The principle of PCR involves targeting a specific region of DNA or RNA and amplifying this region using primers, allowing for the detection of the pathogen in a laboratory setting (Figure 1). One of the main reasons for the widespread use of this method in infectious diseases is its ability to detect even very small amounts of pathogenic DNA or RNA [17]. PCR provides highly sensitive and specific results, especially in the early stages of viral infections such as HIV, Hepatitis B (HBV), and Hepatitis C (HCV) [18,19]. Similarly, for bacterial infections like tuberculosis, Group B streptococcus, and Methicillin-resistant Staphylococcus aureus, PCR offers faster and more reliable results compared to traditional culture methods [20].
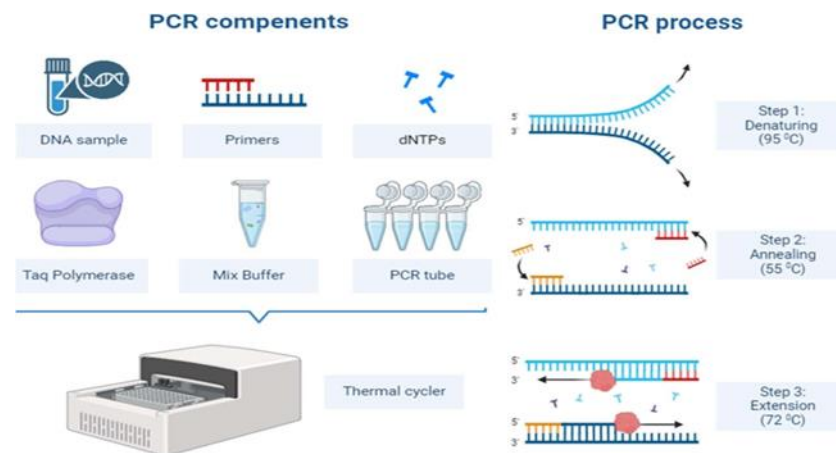
Figure 1. Working principle of polymerase chain reaction.

Over time, advanced variants of PCR have made significant progress in the diagnosis of infectious diseases. For example, Real-Time PCR (qPCR) performs DNA amplification while simultaneously measuring the amount of amplified DNA in real time. By using a fluorescent signal to monitor the amplification process, qPCR indicates how much target DNA is being replicated, allowing for both qualitative and quantitative analysis. This technology plays a crucial role in determining disease burden and monitoring response to treatment. qPCR offers high sensitivity and specificity, making it a powerful tool for pathogen detection [21]. During the COVID-19 pandemic, it was the most widely used method for detecting SARS-CoV-2 [22]. Additionally, because qPCR can detect multiple pathogens simultaneously, it plays an important role in identifying co-infections [23]. Multiplex PCR is a PCR technique that allows the simultaneous amplification of multiple target DNA regions. This method enables the detection of more than one pathogen in the same sample. By using multiple pairs of primers, the genetic material of various pathogens can be amplified in the same reaction tube [24]. This approach offers a significant advantage, especially in respiratory infections where multiple viruses or bacteria may be present simultaneously [25]. Additionally, multiplex PCR can detect antimicrobial resistance genes at the same time, playing a crucial role in managing patient treatment [26].

Digital PCR (dPCR) is a newer and more advanced form of PCR, in which the sample is partitioned into thousands of micro-reactions to enhance quantitative accuracy. dPCR allows for the more sensitive and precise detection of nucleic acids present in low copy numbers. As a result, it is used for detecting rare mutations or low levels of pathogens [27].

Despite the widespread use of PCR in infectious diseases, it has some limitations. For instance, this method only indicates the presence of DNA or RNA, which does not always signify an active infection. Additionally, PCR testing is prone to technical errors that can result in false positive or negative results. Therefore, it is important to support PCR results with clinical findings.

2.1.1. Applications of PCR in the Diagnosis of Infectious Diseases

PCR has become an indispensable tool in modern clinical microbiology laboratories due to its wide range of applications in diagnosing infectious diseases [28]. One of the major advantages of PCR is its ability to produce results quickly, with a typical PCR process completed within just a few hours, which is crucial for the rapid diagnosis of infections. Additionally, its high sensitivity allows for the detection of even very small amounts of pathogenic DNA, setting it apart from other diagnostic methods. It is widely used in the diagnosis of viral, bacterial, fungal, and parasitic infections, providing significant advantages in the clinical management of infections [17,29].

2.1.1.1. Detection of Viral Infections

PCR has been a groundbreaking method in the diagnosis of viral infections. Traditional serological methods can only yield positive results at specific stages of an infection, whereas PCR technology can detect the virus's genetic material at much earlier stages, thereby speeding up the diagnostic process [30].

PCR technology is indispensable for the detection of HBV and HCV. The detection of HCV RNA using PCR can be performed in the early stages of infection, allowing for the rapid identification of these viruses, which have the potential to become chronic. Moreover, PCR enables the measurement of viral load, which is useful for monitoring the progression of the disease and the response to antiviral treatment [31,32].

PCR also plays a crucial role in the diagnosis of Human Immunodeficiency Virus (HIV) infection. It can detect the virus's RNA even during the pre-seroconversion period, providing an opportunity to initiate antiretroviral therapy (ART) early. The quantitative applications of PCR are essential for monitoring viral load in HIV patients, allowing for rapid intervention in cases of treatment failure or disease progression [33,34].

During the COVID-19 pandemic, PCR became one of the most widely used methods for detecting SARS-CoV-2. The amplification of RNA from nasopharyngeal swab samples using reverse transcription PCR (RT-PCR) emerged as the most common approach for diagnosing the disease. The high sensitivity of RT-PCR allowed for the detection of not only symptomatic cases but also asymptomatic carriers, thus playing a significant role in controlling the spread of the pandemic [5,35,36].

2.1.1.2. Detection of Bacterial Infections

One of the main advantages of PCR in the diagnosis of bacterial infections is its ability to provide results much faster than traditional culture methods. The speed and accuracy offered by PCR are crucial for clinical outcomes, especially in serious bacterial infections that require rapid treatment [37].

Sepsis is one of the most serious examples of bacterial infections that require rapid intervention. PCR allows for the quick identification of pathogens causing sepsis, enabling the prompt initiation of appropriate antibiotic treatment. While diagnosis using traditional blood culture methods can take several days, PCR can complete the process within hours. This has a direct impact on the course of the disease and the patient's chances of survival [38].

In the diagnosis of chronic bacterial infections such as tuberculosis (TB), the speed and sensitivity provided by PCR make a significant difference. While culturing Mycobacterium tuberculosis, the causative agent of tuberculosis, using traditional methods can take weeks, PCR can diagnose tuberculosis within a few hours [39,40]. Moreover, PCR has the ability to detect antibiotic resistance genes, making it an important guide in treatment planning. For instance, the rapid identification of strains resistant to drugs such as rifampicin and isoniazid allows for the swift and effective management of the treatment process [41,42].

2.1.1.3. Detection of Fungal Infections

PCR also makes a significant contribution to the diagnosis of fungal infections. In particular, the ability of PCR to provide rapid and sensitive results in diagnosing invasive fungal infections allows for timely initiation of antifungal treatments. The detection of clinically important fungi such as Candida and Aspergillus species can take weeks with traditional methods, whereas PCR enables

diagnosis within a few hours. This is especially crucial for immunocompromised patients, as early detection of invasive fungal infections in this group significantly reduces mortality rates [43].

Moreover, one of the broad applications of PCR is the identification of antifungal resistance genes. In cases where pathogens such as Candida albicans and Aspergillus fumigatus have developed resistance to antifungal treatments, PCR-based tests enable the rapid detection of resistant isolates, guiding the clinical treatment process [44,45].

2.1.1.4. Detection of Parasitic Infections

The effectiveness of PCR in diagnosing parasitic infections is particularly evident in cases where traditional microscopic diagnostic methods are inadequate. Since PCR can directly detect parasite DNA, it is especially effective in identifying asymptomatic or low-intensity infections.

For example, in the diagnosis of malaria caused by Plasmodium species, PCR can provide much more sensitive results compared to traditional

microscopy methods. The specific detection of malaria species such as Plasmodium falciparum is important for clinical management, and PCR can expedite this process [46]. Additionally, the identification of drug-resistant parasites is also possible with PCR [47].

In parasitic diseases such as leishmaniasis, PCR greatly facilitates the diagnostic process. In cases where microscopic diagnosis is challenging, such as with cutaneous and visceral leishmaniasis, PCR can amplify the pathogen's genomic material to provide a rapid and accurate diagnosis [48,49].

2.2. Loop-mediated Isothermal Amplification (LAMP)

In recent years, alternative molecular diagnostic methods have been developed in addition to the advantages offered by PCR. These methods overcome some of the limitations of PCR, providing faster, more cost-effective, and portable solutions. One such innovative method is LAMP. LAMP is a rapid and sensitive molecular diagnostic technique that can amplify DNA or RNA at a constant temperature. Unlike PCR, the LAMP method does not require a thermal cycler and is carried out under isothermal conditions, meaning at a single constant temperature. Typically operating between 60-65°C, LAMP delivers quick results and is notable for its ease of use in field settings (Figure 2) [50].
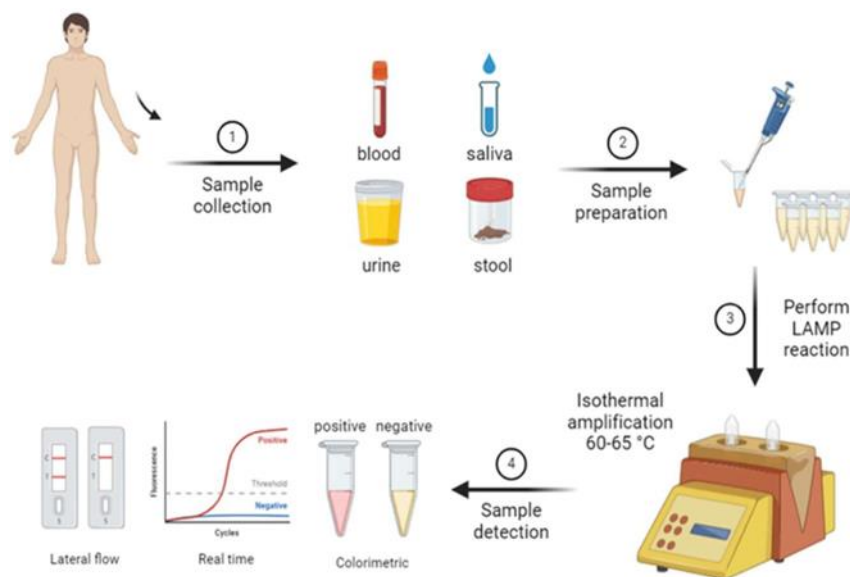

Figure 2. Principle of Loop-Mediated Isothermal Amplification (LAMP) Technique.

2.2.1. Principles of the LAMP Method

Since LAMP performs the amplification process at a constant temperature, it does not require complex and expensive thermal cyclers. This feature provides a significant advantage for use in

developing regions and fieldwork. The principle of LAMP involves the binding of four to six different primers to the target DNA sequence to initiate amplification, followed by the cyclic replication of DNA using DNA polymerase [50]. Primer design is a critical step in ensuring the specificity of LAMP; therefore, primers must be carefully selected and specific to the target DNA.

During the LAMP reaction, the amplification of the target DNA results in the accumulation of magnesium pyrophosphate, a distinct byproduct. This accumulation creates a turbidity in the reaction tube that can be observed with the naked eye, allowing for visual evaluation of the results. Additionally, LAMP reactions can be monitored using fluorescent dyes or color changes, enabling users to easily interpret the outcomes [51].

2.2.2. Advantages and Applications of LAMP

One of the main advantages of LAMP is its ability to deliver rapid results. While PCR typically requires a process lasting 1-2 hours, LAMP results can usually be obtained within 30 minutes to 1 hour. Moreover, since LAMP is an isothermal method, it does not require the cyclic temperature changes needed in PCR. This makes LAMP suitable for use in field conditions and easily applicable with portable devices [52].

LAMP is more advantageous than PCR in terms of cost. The technique does not require thermal cyclers and can be performed with simple equipment, making it particularly appealing in regions with limited resources. Additionally, the ability to visually observe the byproducts produced during the LAMP reaction eliminates the need for expensive detection devices. These characteristics make LAMP an ideal option for low-cost, portable, and rapid diagnostic tests, especially for detecting outbreaks and tropical infections [53,54].

LAMP can be used for the detection of a wide range of pathogens. It has been effectively applied in diagnosing infections that are common in tropical regions, such as malaria, dengue, and Zika virus. In the detection of Plasmodium species, the causative agent of malaria, LAMP provides much faster and more reliable results compared to traditional microscopic methods.

RNA viruses like dengue and Zika can also be easily detected with LAMP following a reverse transcription step [55,55,57].

During the COVID-19 pandemic, LAMP attracted significant attention. It was used as an alternative to PCR for detecting the SARS-CoV-2 virus, and its ability to deliver results in a shorter time contributed to the widespread adoption of rapid diagnostic tests. LAMP could be used to detect the virus in both symptomatic and asymptomatic individuals, and its low cost made it a preferred choice for mass screening tests. These characteristics have made LAMP an important diagnostic tool for managing pandemics, especially in regions with limited resources [58,59,60].

2.2.3. Comparison of LAMP with Other Molecular Methods

Compared to PCR, LAMP offers significant advantages due to its simpler technology and operation under isothermal conditions. LAMP allows for rapid results while providing a similar level of sensitivity and specificity to PCR. Moreover, unlike PCR, it does not require complex laboratory equipment, making it easier to implement in the field. The low cost and speed of LAMP make it particularly valuable in resource-limited regions and during outbreaks requiring urgent diagnosis [51,61].

However, LAMP does have some limitations. The primer design for LAMP is quite complex, and the selection of appropriate primers plays a critical role in the success of the method. Additionally, while LAMP is typically optimized for the detection of a single pathogen, PCR has the capability to detect multiple targets simultaneously. Therefore, LAMP is generally used for the detection of single-pathogen infections, and in clinical samples containing multiple pathogens, PCR may be more advantageous than LAMP [51,62].

2.3. Next-Generation Sequencing (NGS) and Metagenomic Approaches

Following the success of PCR in diagnosing infectious diseases, another significant advancement in molecular diagnostics came with the introduction of NGS technologies in clinical applications.

NGS revolutionized the field of genome sequencing by enabling the simultaneous sequencing of numerous DNA and RNA molecules. This technology allows for the high-capacity, sensitive, and rapid analysis of genetic material, breaking new ground in the molecular diagnosis of infectious diseases. In addition to providing comprehensive genome sequencing, NGS has also facilitated transcriptomic, epigenomic, metagenomic, and other omics studies [63,64].

The fundamental principle of NGS is its ability to read billions of base sequences in parallel. This technology allows for the analysis of the genetic material of specific pathogens as well as the detection of various infectious agents such as viruses, bacteria, and parasites. It also offers high efficiency in identifying genetic variations and mutations, and even discovering new pathogen species [65]. One of the key features that sets NGS apart from other molecular methods is its ability to analyze the entire microbiome with a single test. However, the implementation of NGS is a costly process that requires technical infrastructure, which limits its widespread use in clinical laboratories. Nonetheless, the development of portable NGS devices and the reduction in costs are expected to make this technology more widely accessible in the near future [66].

2.3.1. The Use of NGS in Diagnosis and Treatment

NGS technology plays a significant role not only in detecting pathogens but also in identifying mutations, antimicrobial resistance genes, and virulence factors. For example, the detection and monitoring of resistance genes in pathogens such as carbapenem-resistant Klebsiella pneumoniae, a bacterium carrying antibiotic resistance, is possible with NGS technology [67]. This is crucial for controlling hospital-acquired infections and tracking the spread of antibiotic resistance. Additionally, NGS enables the epidemiological monitoring of pathogens and understanding their spread dynamics, which plays a critical role in planning public health measures.

Especially during outbreaks, the genetic information provided by NGS offers rapid and comprehensive analysis, contributing to the shaping of public health policies. The interest in using this technology during the COVID-19 pandemic was particularly notable for tracking genetic mutations and different variants of the virus. NGS became an essential tool for understanding the evolution of SARS-CoV-2 and the spread of variants in different regions. Additionally, this information played a critical role in vaccine development efforts and public health measures [68]. The emergence of variants such as Delta, Beta, and Omicron highlighted the vital importance of NGS in monitoring mutations.

2.3.2. Metagenomic Approaches and Clinical Applications

One of the innovations brought by NGS technologies is the use of metagenomic approaches in clinical applications. Metagenomics allows for the sequencing of all microbial genomes present in environmental samples or disease tissues, enabling the analysis of complex microbial communities without the need to detect individual pathogens. This technique is particularly useful for identifying pathogens that cannot be cultured by traditional methods or are previously unknown. Clinical metagenomics serves as an important diagnostic tool for rare pathogens or newly emerging disease agents [69].

Metagenomic approaches offer a non-hypothesis-driven screening method. This allows for the sequencing and analysis of all microbial genomes present in a sample without prior knowledge of the infection's cause. Such an approach provides a significant advantage in resolving complex or unknown infections. For example, metagenomic analysis is one of the most effective methods for diagnosing conditions like meningitis or sepsis when the cause cannot be determined. Additionally, it plays a crucial role in outbreak management and individual patient care by providing information on the origins and transmission pathways of diseases [69,70,71].

Metagenomic applications provide important insights not only into infectious diseases but also into immune responses and pathogen-host interactions by studying the human microbiome. For example, metagenomic analyses in infectious diseases can reveal how pathogens and host defense

mechanisms interact, thereby aiding in the development of more personalized treatment approaches [72].

2.3.3. The Future of NGS and Metagenomics

The widespread use of NGS in clinical applications is currently limited due to high costs and technical infrastructure requirements. However, this situation is beginning to change with the rapid development of portable NGS devices. The advancement of portable NGS technology and the reduction in sequencing costs are significant developments that will enable more widespread use of this technology in clinical laboratories [66].

In the future, as NGS and metagenomic approaches become more cost-effective and faster, they are expected to become standard methods for diagnosing infectious diseases. The role of these technologies will especially grow in the fields of personalized medicine and pathogen monitoring, providing effective results even in more complex clinical samples.

In addition to its advantages in diagnosing infectious diseases, NGS has important applications in areas such as monitoring viral genomic variations, identifying antimicrobial resistance genes, and tracking gene transfer between pathogens. This plays a crucial role in controlling outbreaks and determining treatment strategies [65].

2.4. CRISPR-Based Diagnostic Methods

Another innovative approach is diagnostic methods based on CRISPR-Cas technology. Although CRISPR-Cas technology is primarily known as a gene-editing tool, it has also been successfully used for the specific detection of genetic material. This method stands out for its ability to deliver rapid, sensitive, and specific results [73]. CRISPR-based diagnostic platforms such as SHERLOCK, DETECTR, FELUDA, CONAN, and VaNGuard have been successfully applied in diagnosing viral infections like COVID-19 [74].

In the future, CRISPR-based diagnostic systems are expected to develop further and become more widespread. Additionally, the impact of CRISPR technology is increasing in personalized medicine and the diagnosis of genetic diseases. The flexibility offered by CRISPR-Cas systems will allow

this technology to be integrated into new diagnostic platforms and find a broader range of applications.

3. Future Perspectives and Challenges

3.1. The Future Role of Molecular Diagnostic Methods

In the future, the integration of artificial intelligence (AI) and machine learning (ML) technologies into the analysis of molecular diagnostic data will make diagnostic processes faster and more accurate. Especially in the analysis of NGS and metagenomic data, where large amounts of data are generated, AI and ML algorithms can contribute in the following ways:

• Automation of Data Analysis: By using artificial intelligence in the analysis of large-scale genetic data, it will be possible to rapidly detect and classify pathogens. This significantly reduces diagnostic time and minimizes human error.

• Detection of Resistance Genes and Mutations: AI-assisted analyses increase the accuracy in identifying antibiotic resistance and genetic variations of pathogens, enabling more precise results. This allows for the rapid determination of appropriate treatment options.

3.2. The Role of Artificial Intelligence and Machine Learning

The use of molecular diagnostic methods in clinical laboratories enables diagnostic processes to be carried out more rapidly and accurately. In the future, advanced technologies such as PCR, NGS, and CRISPR are expected to become more widespread and accessible. As these technologies become standard practices in healthcare, significant advancements are anticipated in the following areas:

• Widespread Use of Rapid Diagnostic Tests: The development of portable and low-cost molecular diagnostic devices will enable the expansion of rapid diagnostic tests in the field and rural areas. This provides a critical advantage, particularly for the early detection of outbreaks and controlling the spread of infectious diseases.

• Personalized Medicine Applications: Molecular diagnostic technologies will support personalized medicine approaches, such as genetic profiling and the identification of individual disease risks, allowing for more effective treatment planning.

3.3. Clinical and Ethical Challenges

The widespread use of molecular diagnostic methods also brings certain clinical and ethical challenges.

• Cost and Infrastructure Requirements: The need for high-cost equipment and technical infrastructure limits the use of these technologies in low- and middle-income countries. In developing countries, innovative solutions should be developed to reduce costs and promote the widespread use of portable devices.

• Detection of Resistance Genes and Mutations: Comprehensive genetic analyses such as NGS can result in the acquisition of sensitive information about individuals' genetic data. This raises ethical concerns and issues related to data security. Therefore, legal regulations should be developed to ensure the protection and ethical use of genetic data.

3.4. Development of Portable and Low-Cost Diagnostic Devices

In recent years, the development of low-cost molecular diagnostic systems, such as portable PCR and NGS devices, has made it possible to conduct diagnostics in field conditions. These innovations will play a significant role, especially in monitoring and controlling infectious disease outbreaks. In the future, the widespread adoption of such devices and further reductions in costs are expected.

3.5 The Future of CRISPR-Based Diagnostic Systems

The use of CRISPR technology for diagnostic purposes holds great potential, particularly for the rapid and sensitive detection of DNA and RNA targets. In the future, CRISPR-based diagnostic systems are expected to further develop and become more widespread. These innovative diagnostic methods could have a significant impact in the following areas:

• Personalized Medicine: Diagnostic and treatment approaches tailored to patients' genetic profiles could be offered.

• New Diagnostic Platforms: CRISPR-based systems can enable the development of portable and low-cost diagnostic devices, providing ideal solutions for rapid diagnostics in field conditions.

Conclusions

Molecular diagnostic methods have significantly advanced the diagnosis and management of infectious diseases, offering rapid, sensitive, and specific detection capabilities that surpass traditional techniques. The emergence of technologies such as PCR, NGS, and CRISPR has transformed clinical microbiology by enabling the early identification of pathogens, detection of antimicrobial resistance, and monitoring of genetic variations. As these methods continue to evolve, they are becoming more accessible and practical, with the development of portable, low-cost diagnostic devices expanding their use in resource-limited settings. However, the high costs and infrastructure requirements associated with these technologies, along with the need for skilled personnel, still pose challenges, particularly in low- and middle-income countries. Innovative solutions to reduce costs and simplify the technology are essential to overcoming these barriers and promoting global health equity.

Future directions in molecular diagnostics will likely involve the integration of artificial intelligence and machine learning for the automated analysis of complex datasets generated by technologies such as NGS and metagenomics. This approach could significantly enhance the accuracy and speed of diagnostic processes, while also aiding in the identification of resistance genes and novel pathogens. Moreover, ethical considerations related to genetic data privacy and the use of sensitive information must be addressed through the establishment of appropriate regulatory frameworks. As CRISPR-based diagnostics mature, they could further revolutionize the field, providing personalized medicine solutions and rapid, field-deployable testing for emerging infectious diseases. Together, these advancements hold the promise of more effective disease control, improved patient outcomes, and the potential to transform global health practices.

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