Diagnosis Techniques and Molecular Research

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Abstract

Diagnosis Techniques and molecular research revolutionize clinical microbiology by providing more sensitive, rapid, and specific approaches to pathogens. Traditional methods rely on phenotypic expression and biochemical products that are gradually being replaced by molecular techniques, including Polymerase Chain Reaction (PCR), which are emerging as the tool of early detection. PCR's development from single-round PCR to real-time PCR has greatly enhanced the efficiency, sensitivity, and cost-effectiveness of molecular diagnostics. Molecular methods are now integral in viral, bacterial, parasitic, and fungal infection diagnosis, as well as in viral load monitoring and resistance testing in virology and bacteriology. PCR allows for viruses rapid detection such as HIV, HCV, and CMV to improve patient outcomes by guiding timely interventions and monitoring treatment effectiveness. Moreover, molecular diagnostics provide reliable identification of fastidious bacteria and antibiotic resistance detection to address challenges that traditional culture methods face in bacteriology. Furthermore, broad-range PCR use has broadened applications in identifying previously undetected pathogens, although PCR-based genotyping is transforming the detection of infections like HPV and bacterial resistance. Molecular diagnostics in public health offer precise pathogen identification and enable efficient disease response. For instance, limitations including the inability to distinguish viable pathogens from non-viable nucleic acids and the need for standardization in molecular tests remain with its advancements. The future of molecular diagnostics lies in the new technologies integration including microarrays and automation, which will further streamline the diagnostic process while reducing costs.

Keywords: Molecular Diagnostics, PCR Techniques, Infectious Disease Detection, Pathogen Identification, Diagnostic Innovation, Epidemiology and Microbiology

1. Introduction

The identification of various infectious agents enables us to focus on the traditional methods that are dependent on antigens' phenotypic expression or products of biochemical to molecular methodologies (Gerace et al., 2022). However, these molecular approaches are gradually being adopted in clinical microbiology laboratories, mostly to identify and characterize viral infections and diagnose diseases caused by pathogens. Furthermore, their effectiveness must be supported by stringent validation and robust quality control measures, while their rapid turnaround time, high sensitivity, and specificity make them attractive (Ain et al., 2024). Polymerase Chain Reaction (PCR) technology has emerged as the primary molecular detection method in clinical microbiology. Initially, this involved single-round or nested PCR with gel electrophoresis for detection

(Kashnikov et al., 2024). Moreover, advancements such as the automation of DNA or RNA automation, detection, extraction, and amplification processes have significantly enhanced the efficiency and cost-effectiveness of molecular laboratories due to the introduction of real-time PCR. Technologies like DNA microarrays or chips are expected to further expand the utility of molecular diagnostics in this field (Xu et al., 2024).
Key applications like virology, in which molecular techniques are employed for genotyping, resistance testing, routine viral detection, and viral load quantification. In bacteriology, these methods facilitate resistance testing, rapid detection of fastidious bacteria, and the diagnosis of bacterial infections post-antibiotic administration (Ryu et al., 2023). Molecular approaches have also advanced the diagnosis of parasitic and fungal infections, such as early infection and early detection in neutropenic patients. Additional uses are the biosecurity agent's identification, epidemiological studies, and control of infection is expressed in Tble 1(Robertson, 2020).

Table 1. Examples of Molecular Applications Beyond Microorganism Identification

Test	Examples	References
Viral Load Monitoring	Cytomegalovirus, Epstein-Barr virus, hepatitis B and C, HIV	(Guterres, 2023)
Bacterial Resistance	Vancomycin-resistant enterococci, MRSA, extended-spectrum beta-lactamase (ESBL) producers like *K. pneumoniae*, *M. tuberculosis*	(Nagshetty et. al., 2021)
Broad-Range PCR	Infective endocarditis, bacterial meningitis	(Burban et. al., 2024)
Viral Genotyping	Human papillomavirus, hepatitis B and C, HIV	(Galati et. al., 2024)

2. Virology Applications

Historically, diagnosing viral infections has been challenging due to the costs, time requirements, and specialized skills necessary for cell culture systems that are compounded by their low sensitivity and decline in growth rates in the media of artificial (Fernández et al., 2020). PCR technology has significantly enhanced the detection of various viruses. For instance, PCR now enables non-invasive Herpes simplex virus (HSV) DNA detection within cerebrospinal fluid with 95% sensitivity by avoiding invasive brain biopsies (Olie et al., 2024). Similarly, PCR has improved the diagnosis of viral meningitis caused by enteroviruses or HSV to offer faster results as compared to traditional culture methods. Moreover, molecular methods such as PCR and non-PCR technologies aid in the detection of bloodborne infections like hepatitis C virus (HCV) and HIV to provide critical diagnostic insights that serological tests cannot. Applications extend to detecting intrauterine infections like cytomegalovirus (CMV) and rubella using PCR analysis of amniotic fluid (Leber et al., 2024). Respiratory viruses, including SARS-CoV and avian influenza, are also efficiently identified using PCR-based multiplex assays, which streamline diagnostics and reduce hospitalization and unnecessary antibiotic use. Molecular diagnostics for viral diarrheal diseases, such as rotavirus and norovirus, have also advanced by offering higher sensitivity and quicker results than traditional methods (Zaczek-Moczydlowska et al., 2021).

2.1. Treatment Monitoring

Monitoring viral load is integral to managing chronic viral infections by utilizing advanced PCR techniques like real-time PCR and competitive PCR. These methods allow precise measurement of viral DNA or RNA loads through aiding in treatment efficacy assessment and resistance early detection (Lu et al., 2021). For instance, HIV viral load testing is essential to assess antiretroviral therapy and predict disease progression with newer ultrasensitive tests achieving detection limits as low as 50 copies/mL (Meng et. al., 2021).

2.2. Viral Load Testing

Testing for viral load is integral in monitoring therapeutic responses in chronic hepatitis C virus and hepatitis B virus infections. Additionally, there is a 75% probability of achieving SVR for patients with undetectable HCV RNA after 12 weeks of therapy. However, if viral RNA persists but shows a 100-fold reduction in viral load, then there is still a 33% likelihood of achieving SVR. Similarly, HBV DNA levels are monitored in carriers with active liver disease to evaluate the need for antiviral therapies like interferon-alpha or lamivudine to track their effectiveness (Fung et al., 2022). Furthermore, HBV viral load increase serves as a lamivudine-resistant viral mutation. Cytomegalovirus (CMV) infections pose significant risks to bone marrow and organ transplant recipients, as well as individuals with HIV (Azimi et al., 2020). Quantitative PCR-based viral load testing has become the gold standard for early detection of CMV to surpass traditional culture methods in sensitivity. This enables preemptive therapy before clinical symptoms emerge and improves patient outcomes. The viral loading is explained in Fig. 1 (Meganck et al., 2021).

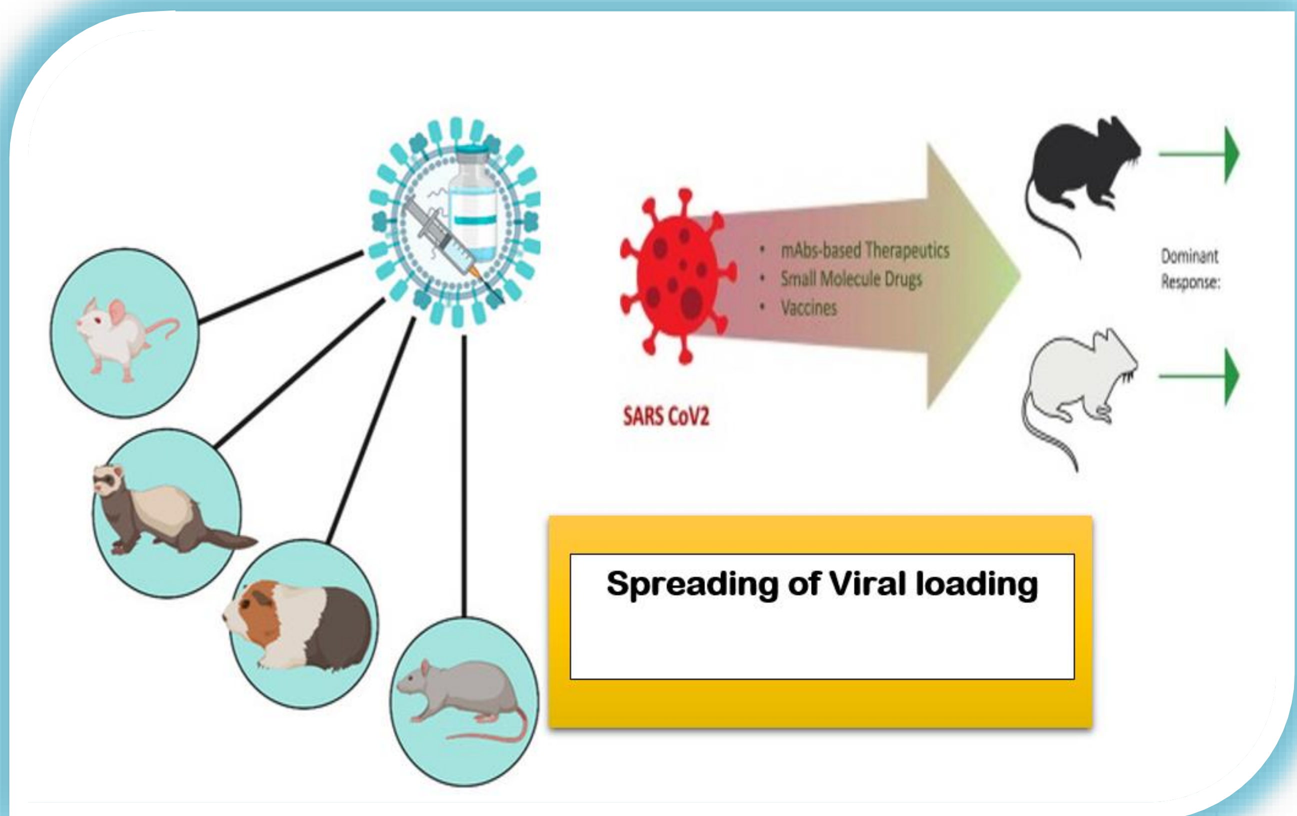

Fig. 1 Representation of Viral Loading

2.3. Viral Genotyping and Resistance Testing

Genotyping and resistance testing play crucial roles in managing chronic viral infections. For HIV, genotypic resistance testing complements viral load assessments to guide antiretroviral therapy by detecting mutations of drug resistance. Resources like the Stanford HIV Drug Resistance Database assist in analyzing resistance profiles (Blassel et al., 2021). In HCV infections, genotyping is pivotal because the six genotypes influence treatment success rates. HBV treatment with lamivudine necessitates monitoring for mutations in the polymerase gene, including YMDD variants, which develop frequently during prolonged therapy (Ramesh et al., 2021). These mutations are identified through rising viral loads and confirmed with sequencing of the active site of the polymerase genes. Additionally, mutants of HBV pre-core causing active liver disease can only be identified through genotypic analysis. Human papillomavirus (HPV) genotyping has transformed cervical cancer screening. Molecular methods detect high-risk HPV genotypes by supplementing traditional Pap smears. FDA-approved tests like the Digene Hybrid Capture 2 assay target high-risk HPV genotypes using RNA probes and antibody detection of DNA-RNA hybrids (Abdulhamit et al., 2023). Molecular testing aids in risk assessment for cervical cancer and optimizes follow-up intervals for women with normal Pap smears but negative high-risk HPV results. Consequently, genotyping's role in prevention will expand further with the introduction of HPV genotype 16 vaccines (Shah et al., 2020).

3. Molecular Advancements in Bacteriology

3.1. Fastidious Bacteria

Infection diagnosis caused by fastidious bacteria has advanced significantly with molecular detection techniques. Non-culture-based methods such as PCR, transcription-based amplification, and ligase chain reaction circumvent delays associated with traditional culture by facilitating early treatment and public health interventions (Hammou et al., 2020). Molecular diagnostics

for sexually transmitted infections, particularly C. trachomatis and N. gonorrhoeae, have increased laboratory-confirmed cases due to their superior sensitivity. These methods enable the use of noninvasive specimens like first-catch urine and self-collected vaginal swabs to enhance compliance and accessibility (Jaya et al., 2022). For example, PCR-based testing for C. trachomatis from urine samples matches the sensitivity and specificity of invasive specimens. Although, testing for N. gonorrhoeae in women shows lower sensitivity with urine and vaginal swabs which provide a reliable alternative (Meyer and Buder, 2020). Different methods of Infection diagnosis are sketched in Figure 2.

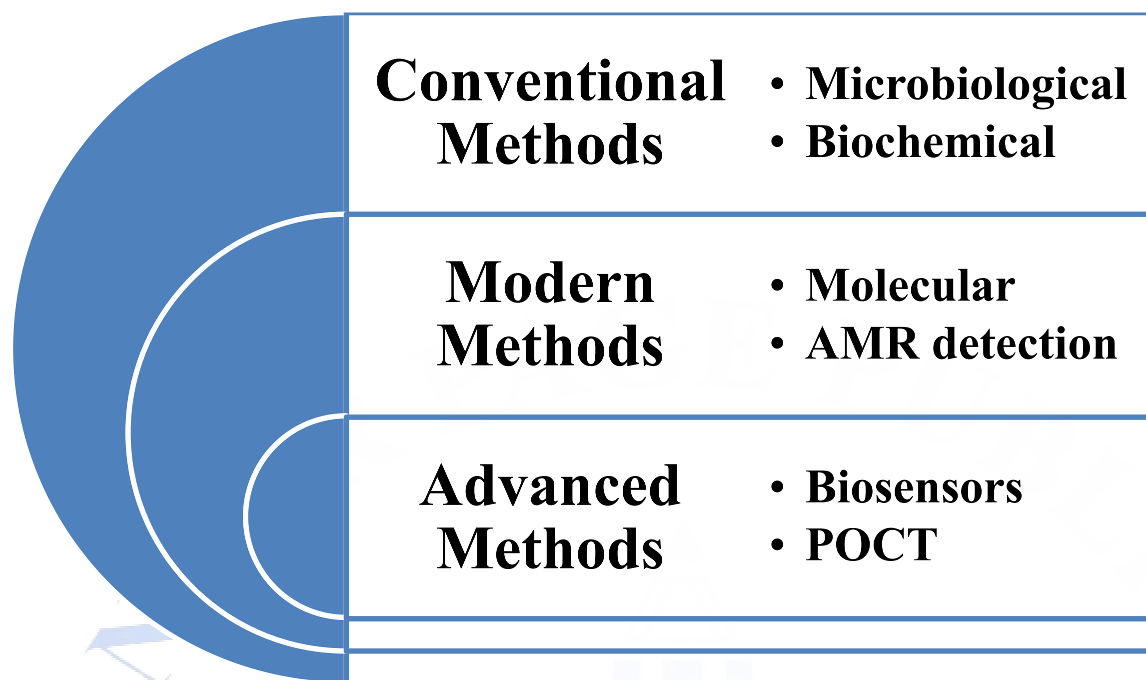

Fig. 2 Expression of Infection diagnosis

Molecular methods offer additional advantages in remote areas in which dry swabs preserve DNA better during transport compared to traditional culture samples. Furthermore, these techniques can screen for multiple pathogens like C. trachomatis, N. gonorrhoeae, and Mycoplasma species, using a single sample by streamlining diagnostic workflows (Moradi et al., 2024). The field of mycobacteriology has significantly benefited from molecular methods, though it is worth noting that conventional culture remains more sensitive than molecular detection for Mycobacterium tuberculosis in many cases. This sensitivity difference is attributed to challenges in effectively extracting DNA from bacterial cells (Paul et al., 2020). Certain bacteria remain uncultivable or extremely difficult to culture in routine laboratories, or pose significant risks to laboratory personnel. Examples include Tropheryma whipplei is responsible for Whipple's disease, which was traditionally diagnosed through histopathology and electron microscopy, often post-mortem (Lathe et al., 2023). PCR now allows detection from noninvasive specimens, enabling diagnosis of conditions like neuro-Whipple's disease and endocarditis. Similarly, molecular methods facilitate the diagnosis of diseases caused by difficult-to-culture bacteria. The rapid diagnosis of bacterial infections has also seen significant advancements, particularly for life-threatening conditions like meningococcal disease (Bhowmik, 2023). PCR methods offer same-day detection from sterile specimens that outperform traditional culture in both speed and sensitivity. Additionally, PCR enables the genoserogrouping of Neisseria meningitidis serogroups B and C, informing vaccination decisions (Hoang et al., 2022).

The application of molecular methods to antibiotic resistance detection is transformative, circumventing challenges associated with phenotypic variability. For bacteria like methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE), molecular techniques provide rapid and reliable resistance detection (Zakaria et al., 2023). Extended-spectrum β-lactamases detection requires specialized tests since routine susceptibility testing often fails to identify resistance. In addition, molecular detection of mutations within the β-lactamase gene confirms ESBL presence and facilitates resistance monitoring across healthcare facilities globally, while phenotypic methods remain prevalent (Geleta et al., 2024). Traditional resistance detection methods rely on additional culture incubation by delaying diagnosis and increasing community transmission risks. Molecular approaches like multiplex PCR targeting the rpoB and hsp65 genes enable the same-day detection

of most MDR strains. However, comprehensive resistance testing may require multi-gene or full-gene sequencing, which is better suited for advanced technologies like microarrays (Nomi et al., 2024).

3.2. Mycology and Parasitology

Molecular testing, though less commonly employed for eukaryotic infections, proves valuable in specific clinical scenarios. Pneumocystis jiroveci, formerly known as Pneumocystis carinii, causes severe pneumonia in immunosuppressed individuals. Detection traditionally relies on microscopy of respiratory specimens, with methods (Calderaro et al., 2022). Immunofluorescence offers greater sensitivity because it is costlier and requires specialized infrastructure. PCR demonstrates higher sensitivity, particularly in non-HIV patients and its specificity is limited due to the organism's ubiquity as a commensal. Similarly, 18S rRNA gene PCR is utilized for detecting Aspergillus spp. in neutropenic hematology patients by addressing challenges that can be posed through poor culture sensitivity and difficulty obtaining biopsy specimens (Azar et al., 2020). Frequent application of Aspergillus PCR can expedite diagnosis, though its precise impact on improving management outcomes remains uncertain. For parasitological diagnoses, molecular techniques offer advantages given that most parasites are not routinely cultured, making microscopy and serology less sensitive. For instance, Toxoplasma gondii is detectable via PCR from amniotic fluid for fetal infection and cerebrospinal fluid for toxoplasma encephalitis (Robert et al., 2021).

3.3. Broad-Range PCR

Broad-range PCR diverges from targeted PCR by employing primers for conserved genetic regions to enable the detection of diverse organisms. Amplified products are sequenced and matched against extensive online databases such as GenBank, EMBL, or RIDOM. This technique is metaphorically a "molecular petri dish" that has identified organisms like Bartonella henselae in bacillary angiomatosis and Tropheryma whipplei in Whipple’s disease (Agarwal et al., 2022). It is particularly useful in diagnosing complex conditions such as infective endocarditis or bacterial meningitis, especially when conventional cultures fail due to prior antibiotic use. Broad-range PCR also played a pivotal role in identifying the SARS-CoV virus during its outbreak (Jalandra et al., 2020). Broad-based primers detected novel viral sequences by leading to the development of specific SARS-CoV PCR assays within weeks. Broad-range PCR has limitations such as the risk of amplifying contaminant DNA and reliance on potentially inaccurate database sequences. Misinterpretation due to shared or erroneous sequences is a concern, particularly for rare pathogens (Vashisht et al., 2023). The demonstrative form of broad-range PCR is explained in Fig. 3.

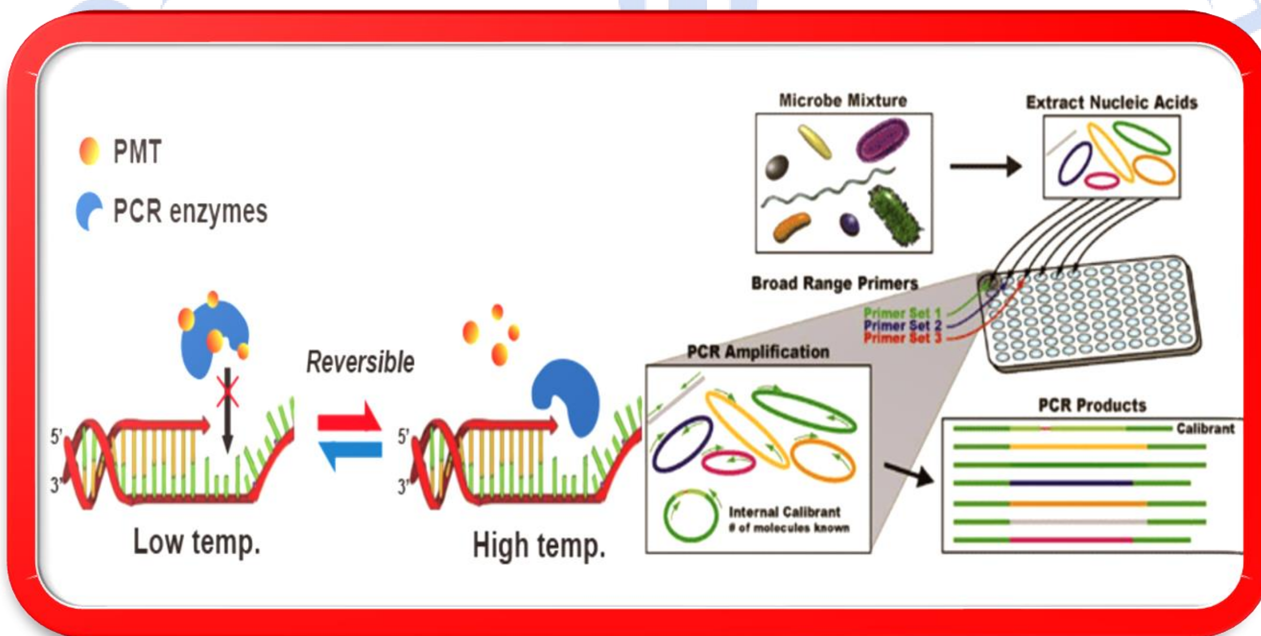

Fig.
3

Fig.03.Demonstration of Broad-Range PCR

3.4. Public Health Aspects

Molecular diagnostics significantly enhance the management of contagious diseases by facilitating rapid and precise etiological identification. The SARS-CoV outbreak demonstrated the utility of molecular tools, in which PCR helped distinguish SARS

from other respiratory infections by enabling timely quarantine measures (Datta et al., 2021). Today, PCR-based diagnostic kits ensure preparedness for any resurgence of SARS-CoV. Similarly, molecular methods accelerate the detection of diarrheal viruses like noroviruses and bloodborne pathogens such as hepatitis B and C, enabling swift containment measures. Bacterial pathogens of public health concern, including Bordetella pertussis, Mycobacterium tuberculosis, and Neisseria meningitidis, benefit from molecular diagnostics by combining conventional and advanced testing to curb transmission effectively (Laxton et al., 2023).

3.5. Molecular Epidemiology

Molecular epidemiology has transformed outbreak investigations through precise pathogen tracking using genotypic analysis. Phenotypic methods often falter due to variable marker expression while traditional genotyping techniques like multilocus enzyme electrophoresis are labor-intensive (Ramadan, 2022). In contrast, multilocus sequence typing (MLST) enables unambiguous strain typing by comparing nucleotide sequences of housekeeping genes. These sequences are matched against global databases, such as those for N. meningitidis, aiding in the identification and global tracking of virulent clones. For tuberculosis, genotyping methods like MIRU typing are suggested for outbreak evaluation in communities and healthcare facilities (Byrne et al., 2020).

3.6. Biosecurity

Biological warfare agents are often invisible and can remain asymptomatic for several days, and are highly infectious, with minute quantities capable of impacting large populations. Among the available technologies, real-time PCR is particularly well-suited for this purpose because traditional methods may lack precision and are slow or require specialized expertise. However, the release of DNA from spores is a prerequisite for PCR, which is challenging without interfering with the process (Sidstedt et al., 2020). Recent advancements now allow for the disruption of spores and the completion of PCR within 15 minutes using innovative hardware. Additionally, portable, battery-operated machines utilizing TaqMan® real-time PCR can perform the entire process in just 30 minutes. Microarray technologies also hold significant promise in this field, though they currently face limitations due to the sample preparation required for microfluidic devices (Chandnani et al., 2023).

4. Molecular Techniques limitations:

Molecular techniques cannot entirely replace conventional approaches for certain infectious outbreaks, although they have many benefits. Numerous routine tests in the field of clinical microbiology are fast and cost-effective (Vasala et al., 2020). Innovations in traditional techniques, such as rapid antigen tests providing results within minutes and current automated culture methods for faster identification and susceptibility testing, can ensure their continued relevance. PCR tests are restricted to detecting organisms whose DNA matches the primers used. Consequently, achieving broad coverage would require affordable and user-friendly microarray tools, which are not widely presented (Cathryn et al., 2022).

4.1. Incorrect Positive and Incorrect Negative Outcomes

The application of molecular techniques in routine analysis is also limited by the possibility of false negatives or positives. However, laboratory contamination and extensive lab facilities are necessary to physically separate areas for reagent preparation, specimen handling, and product detection to decrease false positives (Adedokun et al., 2024). This requires well-trained personnel and strict adherence to protocols. Additionally, replacing dTTP with dUTP during amplification and treating preassembled reactions with uracil N-glycosylase (UNG) to destroy dUTP-containing amplicons can prevent contamination. Disposable equipment and personal protective gear further reduce intersample contamination risks (Wei et al., 2024). Despite stringent practices, challenges may still arise with reagents. Poorly designed primers can result in incorrect positive outcomes by amplifying unintended microorganisms. The discovery of new organisms or sequences may reduce the specificity of these tests over time because primers are typically designed from sequences in international databases (Church et al., 2020).

Some clinical specimens, including sputum or feces, may contain substances that degrade nucleic acids or inhibit PCR enzymes. Hence, inhibitor checks should be performed on every sample to ensure that a negative result is not due to inhibition (Nisafani et al., 2020). Internal amplification controls, such as spiked non-human pathogen DNA (e.g., equine herpesvirus) or human β-globin gene detection which can verify successful extraction and absence of inhibitors. However, the latter method's variable human DNA levels in samples may affect sensitivity. When inhibitors are detected by dilution of the DNA extract to treatment with reagents like GeneReleaser, or adding PCR facilitators such as bovine serum albumin can help (Liu et al., 2023).

4.2. Absence of Standardization in Molecular Testing

Molecular diagnostics face challenges due to the widespread use of in-house PCR tests, which vary significantly between laboratories. Although commercial tests exist for diseases like HIV, hepatitis B and C, Chlamydia trachomatis, and Neisseria gonorrhoeae, rare diseases are unlikely to have commercially available PCR kits (Hsieh et al., 2022). Variations in primer

selection, amplification methods (e.g., single-round, nested, or real-time PCR), and detection techniques complicate comparisons of sensitivity and specificity, as explained in the Table.2 (Joseph et al., 2023).

Table. 2 Differentiating Infection from Disease

Variants of infections	Description
PCR Limitation	PCR cannot distinguish between viable pathogens and non-viable nucleic acids.
Predictive Value for Certain Diseases	For diseases like invasive meningococcal infections, DNA detection from sterile sites has high predictive value.
Colonization Infection	In conditions like Pneumocystis jiroveci pneumonia (PCP), detection may indicate colonization, not active infection.
Viral Shedding	Viruses can shed intermittently without causing disease.
Role of Quantitative PCR	Enhances predictive value by correlating higher viral loads with disease severity.
Culture Methods	It offers higher specificity than PCR.
CMV	Viral load thresholds and progression rates can predict clinical disease.

5. Future Trends in Molecular Methods

PCR and sequencing have revolutionized pathogen identification and the study of developing infectious illnesses. Furthermore, molecular techniques reveal that over 30 bacterial species are forming uncultivable states within unfavorable surroundings, which is challenging the application of Koch's postulates (Manoil et al., 2024). These technologies now enable the study of pathogen evolution, host susceptibility, and the development of DNA/RNA banks for genes linked to pathogenicity. Microarray and gene chip technologies offer benefits such as miniaturization, automation, and rapid analysis of genetic information (Xu et al., 2024). Platforms like Affymetrix® GeneChip® microarrays can simultaneously identify pathogens, virulence factors, and drug resistance markers. Additionally, their application in clinical diagnostics is expanding, though primarily research-oriented due to high costs (Fichtner et al., 2022). Economic pressures are driving the automation and cost reduction of molecular diagnostics. Systems like Roche's MagNA Pure LC automate nucleic acid extraction are process up to 32 samples in an hour with contamination-prevention features (Jallow et al., 2021). Additional automated platforms like QIAGEN's BioRobot systems and Corbett Robotics' CAS-1200™ DNA Sample Setup streamline PCR setup and improve throughput. Questions regarding DNA persistence, colonization versus active infection, and the normal presence of microbial DNA in sterile sites require further investigation. Until molecular diagnostics can quickly and affordably analyze multiple genetic markers, conventional methods will continue to play a vital role in clinical microbiology (Lewinski et al., 2023).

6. Conclusion

In conclusion, molecular techniques are significantly advancing the microbiology field by providing faster, more accurate, and more sensitive methods to diagnose a wide range of infectious diseases. However, technological evolution including PCR, real-time PCR, and microarrays, revolutionized viral, bacterial, fungal, and parasitic infection detection to improve diagnostic accuracy and the results speed which is crucial for timely treatment and infection control. Moreover, these molecular methods have proven to be indispensable in fields such as virology, bacteriology, and epidemiology in enhancing our pathogen behavior, resistance patterns, and transmission dynamics. Nonetheless, molecular diagnostics will remain complementary to traditional approaches to integrate with them to form a robust framework for global health management.

References

1. Abdulhamit, Ç. A. L. I., Aslan, R., Çelik, C., & Ahmet, A. L. İ. M. (2023). FDA-Approved Molecular Tests Used to Define Human Papillomavirus (HPV) Infections which Cause Cervix Cancer. Cumhuriyet Medical Journal, 45(1), 1-11.
2. Adedokun, G., Alipanah, M., & Fan, Z. H. (2024). Sample preparation and detection methods in point-of-care devices towards future at-home testing. Lab on a Chip.
3. Agarwal, A., Rübsam, A., Zur Bonsen, L., Pichi, F., Neri, P., & Pleyer, U. (2022). A comprehensive update on retinal vasculitis: etiologies, manifestations and treatments. Journal of Clinical Medicine, 11(9), 2525.
4. Ain, Q. U., Nazir, R., Nawaz, A., Shahbaz, H., Dilshad, A., Mufti, I. U., & Iftikhar, M. (2024). Machine Learning Approach towards Quality Assurance, Challenges and Possible Strategies in Laboratory Medicine. Journal of Clinical and Translational Pathology, 4(2), 76-87.
5. Azar, M. M., Gaston, D. C., Kotton, C. N., & Malinis, M. F. (2020). Emerging microbiology diagnostics for transplant infections: on the cusp of a paradigm shift. Transplantation, 104(7), 1358-1384.
6. Azimi, T., Tavakolian, S., Goudarzi, H., Pourmand, M. R., & Faghihloo, E. (2020). Global estimate of phenotypic and genotypic ganciclovir resistance in cytomegalovirus infections among HIV and organ transplant patients; A systematic review and meta-analysis. Microbial pathogenesis, 141, 104012.
7. Bhowmik, A. (2023). Role of Diagnostic procedures in managing human Bacterial infections: A comprehensive overview. Archives of Hematology Mini Reviews and Reviews, 8(1), 008-019.
8. Blassel, L., Zhukova, A., Villabona-Arenas, C. J., Atkins, K. E., Hué, S., & Gascuel, O. (2021). Drug resistance mutations in HIV: new bioinformatics approaches and challenges. Current opinion in virology, 51, 56-64.
9. Burban, A., Słupik, D., Reda, A., Szczerba, E., Grabowski, M., & Kołodzińska, A. (2024). Novel diagnostic methods for infective endocarditis. International Journal of Molecular Sciences, 25(2), 1245.
10. Byrne, A. S., Goudreau, A., Bissonnette, N., Shamputa, I. C., & Tahlan, K. (2020). Methods for detecting mycobacterial mixed strain infections–a systematic review. Frontiers in genetics, 11, 600692.
11. Calderaro, A., Buttrini, M., Farina, B., Montecchini, S., De Conto, F., & Chezzi, C. (2022). Respiratory tract infections and laboratory diagnostic methods: a review with a focus on syndromic panel-based assays. Microorganisms, 10(9), 1856.
12. Cathryn, R. H., Kumar, S. U., Younes, S., Zayed, H., & Doss, C. G. P. (2022). A review of bioinformatics tools and web servers in different microarray platforms used in cancer research. Advances in protein chemistry and structural biology, 131, 85-164.
13. Chandnani, K., Rajput, N., Jadav, T., Pillai, M., Dhakne, P., Tekade, R. K., & Sengupta, P. (2023). Technological advancement and current standing of microfluidic chip based devices for targeted analysis of biomarkers. Microchemical Journal, 195, 109532.
14. Church, D. L., Cerutti, L., Gürtler, A., Griener, T., Zelazny, A., & Emler, S. (2020). Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory. Clinical microbiology reviews, 33(4), 10-1128.
15. Datta, M., Singh, D. D., & Naqvi, A. R. (2021). Molecular diagnostic tools for the detection of SARS-CoV-2. International Reviews of Immunology, 40(1-2), 143-156.
16. Fernández, L., Cima-Cabal, M. D., Duarte, A. C., Rodriguez, A., García, P., & García-Suárez, M. D. M. (2020). Developing diagnostic and therapeutic approaches to bacterial infections for a new era: implications of globalization. Antibiotics, 9(12), 916.
17. Fichtner, M., Edström, K., Ayerbe, E., Berecibar, M., Bhowmik, A., Castelli, I. E., ... & Weil, M. (2022). Rechargeable batteries of the future—the state of the art from a BATTERY 2030+ perspective. Advanced Energy Materials, 12(17), 2102904.
18. Fung, S., Choi, H. S., Gehring, A., & Janssen, H. L. (2022). Getting to HBV cure: The promising paths forward. Hepatology, 76(1), 233-250.
19. Galati, L., Chiantore, M. V., Marinaro, M., & Di Bonito, P. (2024). Human Oncogenic Viruses: Characteristics and Prevention Strategies—Lessons Learned from Human Papillomaviruses. Viruses, 16(3), 416.
20. Geleta, D., Abebe, G., Alemu, B., Workneh, N., & Beyene, G. (2024). Mechanisms of Bacterial Drug Resistance with Special Emphasis on Phenotypic and Molecular Characterization of Extended Spectrum Beta-lactamase. New Microbiologica, 47(1), 1-14.
21. Gerace, E., Mancuso, G., Midiri, A., Poidomani, S., Zummo, S., & Biondo, C. (2022). Recent advances in the use of molecular methods for the diagnosis of bacterial infections. Pathogens, 11(6), 663.

22. Guterres, A. (2023). Viral load: We need a new look at an old problem?. Journal of Medical Virology, 95(8), e29061.
23. Hammou, R. A., Benhassou, M., Bessi, H., & Ennaji, M. M. (2020). Scientific Advances in the Diagnosis of Emerging and Reemerging Viral Human Pathogens. Emerging and Reemerging Viral Pathogens, 93-120.
24. Hoang, M. T. V., Irinyi, L., Hu, Y., Schwessinger, B., & Meyer, W. (2022). Long-reads-based metagenomics in clinical diagnosis with a special focus on fungal infections. Frontiers in Microbiology, 12, 708550.
25. Hsieh, K., Melendez, J. H., Gaydos, C. A., & Wang, T. H. (2022). Bridging the gap between development of point-of-care nucleic acid testing and patient care for sexually transmitted infections. Lab on a Chip, 22(3), 476-511.
26. Jalandra, R., Yadav, A. K., Verma, D., Dalal, N., Sharma, M., Singh, R., ... & Solanki, P. R. (2020). Strategies and perspectives to develop SARS-CoV-2 detection methods and diagnostics. Biomedicine & Pharmacotherapy, 129, 110446.
27. Jallow, A., Xie, H., Tang, X., Qi, Z., & Li, P. (2021). Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control. Comprehensive reviews in food science and food safety, 20(3), 2332-2381.
28. Jaya, Z. N., Mapanga, W., Van Niekerk, B., Dlangalala, T., Kgarosi, K., Dzobo, M., ... & Mashamba-Thompson, T. P. (2022). Mapping evidence of self-sampling to diagnose sexually transmitted infections in women: a scoping review. Diagnostics, 12(8), 1803.
29. Joseph, L., Raca, G., & Stringer, K. F. (2023). Molecular Techniques/Molecular Diagnostics for Surgical Pathologists: An Overview. Molecular Surgical Pathology, 1-68.
30. Kashnikov, A. Y., Epifanova, N. V., & Novikova, N. A. (2024). Molecular Genetic Methods for Detection and Genotyping of Human Sapoviruses. Biology Bulletin Reviews, 14(6), 750-757.
31. Lathe, R., Schultek, N. M., Balin, B. J., Ehrlich, G. D., Auber, L. A., Perry, G., ... & Haas, J. G. (2023). Establishment of a consensus protocol to explore the brain pathobiome in patients with mild cognitive impairment and Alzheimer's disease: Research outline and call for collaboration. Alzheimer's & Dementia, 19(11), 5209-5231.
32. Laxton, C. S., Peno, C., Hahn, A. M., Allicock, O. M., Perniciaro, S., & Wyllie, A. L. (2023). The potential of saliva as an accessible and sensitive sample type for the detection of respiratory pathogens and host immunity. The Lancet Microbe, 4(10), e837-e850.
33. Leber, A. L. (2024). Maternal and congenital human cytomegalovirus infection: laboratory testing for detection and diagnosis. Journal of Clinical Microbiology, 62(4), e00313-23.
34. Lewinski, M. A., Alby, K., Babady, N. E., Butler-Wu, S. M., Bard, J. D., Greninger, A. L., ... & Nolte, F. (2023). Exploring the utility of multiplex infectious disease panel testing for diagnosis of infection in different body sites: a joint report of the Association for Molecular Pathology, American Society for Microbiology, Infectious Diseases Society of America, and Pan American Society for Clinical Virology. The Journal of Molecular Diagnostics.
35. Liu, Y., Kumblathan, T., Tao, J., Xu, J., Feng, W., Xiao, H., ... & Le, X. C. (2023). Recent advances in RNA sample preparation techniques for the detection of SARS-CoV-2 in saliva and gargle. TrAC Trends in Analytical Chemistry, 165, 117107.
36. Lu, S., Lin, S., Zhang, H., Liang, L., & Shen, S. (2021). Methods of respiratory virus detection: advances towards point-of-care for early intervention. Micromachines, 12(6), 697.
37. Manoil, D., Parga, A., Bostanci, N., & Belibasakis, G. N. (2024). Microbial diagnostics in periodontal diseases. Periodontology 2000.
38. Meganck, R. M., & Baric, R. S. (2021). Developing therapeutic approaches for twenty-first-century emerging infectious viral diseases. Nature medicine, 27(3), 401-410.
39. Meng, Z., Guo, S., Zhou, Y., Li, M., Wang, M., & Ying, B. (2021). Applications of laboratory findings in the prevention, diagnosis, treatment, and monitoring of COVID-19. Signal transduction and targeted therapy, 6(1), 316.
40. Meyer, T., & Buder, S. (2020). The laboratory diagnosis of Neisseria gonorrhoeae: current testing and future demands. Pathogens, 9(2), 91.
41. Moradi, F., Nasoohian, N., Ghorbanian, N., & Fooladfar, Z. (2024). Recent advances in laboratory detection of Chlamydia trachomatis using gold (Au) nanoparticle-based methods; another evolution of nanotechnology in diagnostic bacteriology. Microchemical Journal, 111373.
42. Nagshetty, K., Shilpa, B. M., Patil, S. A., Shivannavar, C. T., & Manjula, N. G. (2021). An overview of extended spectrum beta lactamases and metallo beta lactamases. Advances in Microbiology, 11(01), 37.
43. Nisafani, A. S., Kiely, G., & Mahony, C. (2020). Workers' technostress: A review of its causes, strains, inhibitors, and impacts. Journal of Decision Systems, 29(sup1), 243-258.

44. Nomi, Z. A., Mumtaz, M., Shahbakht, R. M., Rajput, S. A., & Zeeshan, M. (2024). Molecular Insights into Zoonotic Diseases: Understanding the Interface Between Animals and Humans. *Biological Times*, *3(*2), 32-33.
45. Olie, S. E., Andersen, C. Ø., van de Beek, D., & Brouwer, M. C. (2024). Molecular diagnostics in cerebrospinal fluid for the diagnosis of central nervous system infections. Clinical Microbiology Reviews, 37(4), e00021-24.
46. Paul, R., Ostermann, E., & Wei, Q. (2020). Advances in point-of-care nucleic acid extraction technologies for rapid diagnosis of human and plant diseases. Biosensors and bioelectronics, 169, 112592.
47. Ramadan, A. A. (2022). Bacterial typing methods from past to present: A comprehensive overview. Gene Reports, 29, 101675.
48. Ramesh, D., Vijayakumar, B. G., & Kannan, T. (2021). Advances in nucleoside and nucleotide analogues in tackling human immunodeficiency virus and hepatitis virus infections. ChemMedChem, 16(9), 1403-1419.
49. Robert, M. G., Brenier-Pinchart, M. P., Garnaud, C., Fricker-Hidalgo, H., & Pelloux, H. (2021). Molecular diagnosis of toxoplasmosis: recent advances and a look to the future. Expert Review of Anti-infective Therapy, 19(12), 1529-1542.
50. Robertson, I. D. (2020). Disease control, prevention and on-farm biosecurity: the role of veterinary epidemiology. Engineering, 6(1), 20-25.
51. Ryu, H., Abdul Azim, A., Bhatt, P. J., Uprety, P., Mohayya, S., Dixit, D., ... & Narayanan, N. (2023). Rapid diagnostics to enhance therapy selection for the treatment of bacterial infections. Current Pharmacology Reports, 9(4), 198-216.
52. Shah, U. J., Nasiruddin, M., Dar, S. A., Khan, M. K. A., Akhter, M. R., Singh, N., ... & Haque, S. (2020). Emerging biomarkers and clinical significance of HPV genotyping in prevention and management of cervical cancer. Microbial pathogenesis, 143, 104131.
53. Sidstedt, M., Rådström, P., & Hedman, J. (2020). PCR inhibition in qPCR, dPCR and MPS—mechanisms and solutions. Analytical and bioanalytical chemistry, 412(9), 2009-2023.
54. Vasala, A., Hytönen, V. P., & Laitinen, O. H. (2020). Modern tools for rapid diagnostics of antimicrobial resistance. Frontiers in cellular and infection microbiology, 10, 308.
55. Vashisht, V., Vashisht, A., Mondal, A. K., Farmaha, J., Alptekin, A., Singh, H., ... & Kolhe, R. (2023). Genomics for emerging pathogen identification and monitoring: Prospects and obstacles. BioMedInformatics, 3(4), 1145-1177.
56. Wei, C., Fu, D., Ma, T., Chen, M., Wang, F., Chen, G., & Wang, Z. (2024). Sensing Patches for Biomarker Identification in Skin-Derived Biofluids. Biosensors and Bioelectronics, 116326.
57. Xu, J., Chun, H., Wang, L., Mei, H., Chen, S., & Huang, X. (2024). DNA microarray chips: Fabrication and cutting-edge applications. Chemical Engineering Journal, 155937.
58. Zaczek-Moczydlowska, M. A., Beizaei, A., Dillon, M., & Campbell, K. (2021). Current state-of-the-art diagnostics for Norovirus detection: Model approaches for point-of-care analysis. Trends in Food Science & Technology, 114, 684-695.
59. Zakaria, N. D., Hamzah, H. H., Salih, I. L., Balakrishnan, V., & Abdul Razak, K. (2023). A review of detection methods for vancomycin-resistant enterococci (VRE) genes: from conventional approaches to potentially electrochemical DNA biosensors. Biosensors, 13(2), 294.