

Recent Advances and Future Trends in the Diagnosis of Viral Infections: A Review

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ABSTRACT

Diagnosis of viral infections has been completely transformed with the development of sophisticated technologies that enable rapid, sensitive and specific detection. Molecular techniques have now supplemented, and in many cases replaced, traditional methods (e.g., viral culture and serology). This has made PCR (polymerase chain reaction) and its variants a cornerstone of viral diagnostics because of their high accuracy together with the possibility to detect infections in an early stage. Microfluidic facilities have largely been developed in recent years with testing tools, such as Reverse Transcription Polymerase Chain Reaction (RT-PCR) for rapid diagnosis, using isothermal amplification and Next-Generation Sequencing (NGS) platforms to cover the comprehensive genomic detection of viruses. Novel molecular approaches include development of CRISPR-based diagnostic tools, biosensors and point-of-care tests, allowing timely results to be obtained in settings lacking the resources (including skilled personnel). In addition, the confluence of artificial intelligence with digital health technologies is streamlining diagnostics and enabling real-time disease surveillance. However, barriers including high costs and limited accessibility still exist, along with obstacles like viral mutations that can influence the accuracy of diagnostic tests. Trends in viral diagnostics are moving forward towards the development of affordable, portable, and multiplex diagnostics platforms for detecting multiple pathogens all at once. The global collaboration due to the invention of new technologies will as well advance early diagnosis, management tools and control of viral infections worldwide.

Keywords: Biosensors, CRISPR-based diagnostics, Molecular diagnostics, Next-Generation Sequencing (NGS), Point-of-Care Testing (POCT), Polymerase Chain Reaction (PCR), Viral infections.

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INTRODUCTION

Viral infections are caused by various types of viruses. Viruses are diverse structures that range in size from 20 to 900 nanometers and come in different shapes, consisting of genetic material that can be either single or double-stranded, and may contain either positive- or negative-sense Deoxyribonucleic Acid (DNA) or Ribonucleic Acid (RNA), all encased in a layer made of proteins, glycoproteins, or lipids. Viruses lack the ability to generate energy, do not grow on their own, and have a simple structure. Consequently, they can only replicate within living cells (host cells) that are compatible with a specific type of virus. When a virus penetrates a cell (Figure 1), it releases and incorporates its genetic material into the host cell's genome, taking control of the cell's functions necessary for its replication. Additionally, some infected cells replicate themselves while also producing copies

of the virus's genome. When the immune system of the host organism identifies a virus, it initiates a specific response. One of these responses in mammalian species involves the production of specific antibodies that assist in neutralizing the virus and the infected cells. Nonetheless, the host's organism is not always capable of defending itself. It is important to remember, however, that all viruses evolve rapidly and are disseminated through the global movement of living organisms (Rojek *et al.*, 2017).

Viral diseases have long been a major threat to global public health, impacting clinical medicine, epidemiology and socioeconomic stability (Zhang and Ren, 2025, Gong *et al.*, 2025). In the last decade, several major outbreaks of viruses have been witnessed worldwide, emphasizing the urgent need for rapid and efficient diagnostic tools (Li and Ren, 2020). The emergence of new viruses such as SARS-CoV-2 which caused the COVID-19 pandemic and the re-emergence of known pathogens (e.g., Ebola virus and Zika virus) have not only led to widespread morbidity and mortality, but also to strain on healthcare systems, disruption of global supply chains and profound social and economic consequences (Baker *et al.*, 2022). In such cases, early viral diagnosis is essential. Many viruses are zoonotic, emphasizing the importance of animal health and zoonotic surveillance (Chen *et al.*, 2021).



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HISTORICAL PERSPECTIVE ON VIRAL DIAGNOSTICS

From conventional culture-based techniques to cutting-edge molecular technologies, the field of viral diagnostics has undergone substantial change. Initially, immunofluorescence tests, electron microscopy, and virus isolation in cell cultures were the main methods used for virus detection. These techniques were fundamental to virology but had significant drawbacks. These traditional methods relied on viable viral samples, required highly qualified workers and specialized laboratory equipment, and frequently took several days to weeks to get results. These methods lacked the speed required for prompt clinical decision-making, despite their historical significance and accuracy, particularly in situations with limited resources or when outbreaks were spreading quickly (Dronina *et al.*, 2021; Gradisteanu *et al.*, 2022). As viral infections started to pose a major worldwide threat in the second half of the 20th century, the shortcomings of these diagnostic techniques became more and more obvious.

The advent of Polymerase Chain Reaction (PCR) in the 1980s marked a watershed in the development of diagnostics. This ground-breaking method made it feasible to amplify particular DNA or RNA sequences, which allowed for the very sensitive and specific detection of minute amounts of viral nucleic acids straight from patient samples. The transition from culture-dependent diagnosis to genotype-based identification was revolutionary (Borkakoty *et al.*, 2024). In addition to speeding up the diagnostic process, this invention set the stage for the molecular methods that now rule the diagnostic field. The necessity for quick and accurate diagnostic techniques was brought to light by later outbreaks, such as the 2002 SARS epidemic, the 2009 H1N1 influenza pandemic, and most recently the COVID-19 pandemic.

Real-time PCR, automated systems, and point-of-care diagnostic platforms have become widely used as a result of these worldwide occurrences, which greatly spurred research and financing in diagnostic development. The historical shift from conventional to contemporary diagnostic techniques highlights how important innovation is in addressing changing public health risks.

DIAGNOSTIC TECHNIQUES

Reverse Transcriptase PCR (RT-PCR)

Rapid, sensitive, and scalable diagnostic methods are desperately needed since viral infections continue to be a major global public health concern. Because of its superior analytical performance, Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) is still the gold standard for RNA virus identification among currently available techniques. According to a systematic evaluation, COVID-19 RT-PCR assays had pooled sensitivity of 89% and specificity of 98.9%. Depending on the assay design and target genes, the limits of detection ranged from 10 to 100

copies per reaction (Rojek *et al.*, 2017; Zhang and Ren, 2025). Furthermore, comparative clinical investigations demonstrated the value of non-invasive sampling techniques by finding saliva-based RT-PCR sensitivity of 83.2%, which is comparable to the 85.1% sensitivity shown with nasopharyngeal swabs (Gong *et al.*, 2025).

These results emphasize advancements in specimen collection while supporting RT-PCR's ongoing essential role in the diagnosis of respiratory viral infections.

Real-Time Quantitative PCR (qPCR)

By enabling the simultaneous detection and quantification of viral nucleic acids, Real-Time Quantitative PCR (qPCR) has significantly increased the usefulness of molecular virology. Commercial qPCR assays are very dependable for treatment monitoring in HIV therapy because they can measure plasma viral load down to 20 copies/mL with inter-laboratory variance below 5% (Li and Ren, 2020). Similar to this, extensive prospective studies on Hepatitis B Virus (HBV) infection showed that serum HBV DNA levels greater than 20,000 IU/mL were significantly linked to higher risks of cirrhosis and hepatocellular carcinoma, making viral load assessment a crucial prognostic indicator (Baker *et al.*, 2022). As a result, qPCR is now essential for both long-term illness treatment and diagnosis.

Multiplex PCR Panels

Another significant development is the ability to detect several viral infections simultaneously in a single reaction thanks to multiplex PCR systems. For common respiratory viruses, such as influenza, respiratory syncytial virus, adenovirus, and coronaviruses, clinical evaluation of respiratory multiplex panels has demonstrated sensitivity ranging from 89.4% to 99.0% and specificity exceeding 97% (Chen *et al.*, 2021). Additionally, implementation studies showed that quick multiplex testing lowered the time it took to decide whether to isolate a patient by around 21 hr and decreased median antibiotic use from 8 days to 6 days (Dronina *et al.*, 2021). These findings suggest that syndromic multiplex testing enhances hospital workflow efficiency and antibiotic stewardship in addition to improving diagnostic accuracy.

Real-Time Quantitative PCR (qPCR)

An extremely sensitive next-generation nucleic acid measurement Technique is Digital PCR (dPCR). dPCR allows for absolute quantification without the use of standard curves by dividing samples into thousands of microreactions. 94% of faint positive or ambiguous samples that were overlooked by traditional RT-PCR were found by dPCR in studies involving SARS-CoV-2 specimens (Gradisteanu *et al.*, 2022). Droplet digital PCR significantly outperformed conventional qPCR in precision in HIV reservoir studies, with coefficients of variation < 2% (Borkakoty *et al.*, 2024). Because of these benefits, dPCR is particularly useful for

research applications, residual disease surveillance, and low viral load infections.

Loop-Mediated Isothermal Amplification (LAMP)

Loop-Mediated Isothermal Amplification (LAMP) and other isothermal amplification methods are becoming more and more common since they do not require thermal cyclers. Pooled sensitivity of 92.1% and specificity of 95.8%, with typical turnaround times of about 30 min, were reported in a meta-analysis of RT-LAMP for SARS-CoV-2 diagnosis (Böger *et al.*, 2021). LAMP-based solutions are especially helpful for

point-of-care testing and resource-constrained environments because of their speed, mobility, and cheap equipment needs.

CRISPR-Based Diagnostics

Viral detection has undergone a paradigm shift thanks to CRISPR-based molecular diagnostics. Nucleic acid amplification and CRISPR-mediated target recognition are combined by platforms like DETECTR and SHERLOCK to produce quick and extremely specific findings. In less than 40 min, a clinical evaluation of the DETECTR test for SARS-CoV-2 reported 95% sensitivity and 100% specificity (Corman *et al.*, 2020).

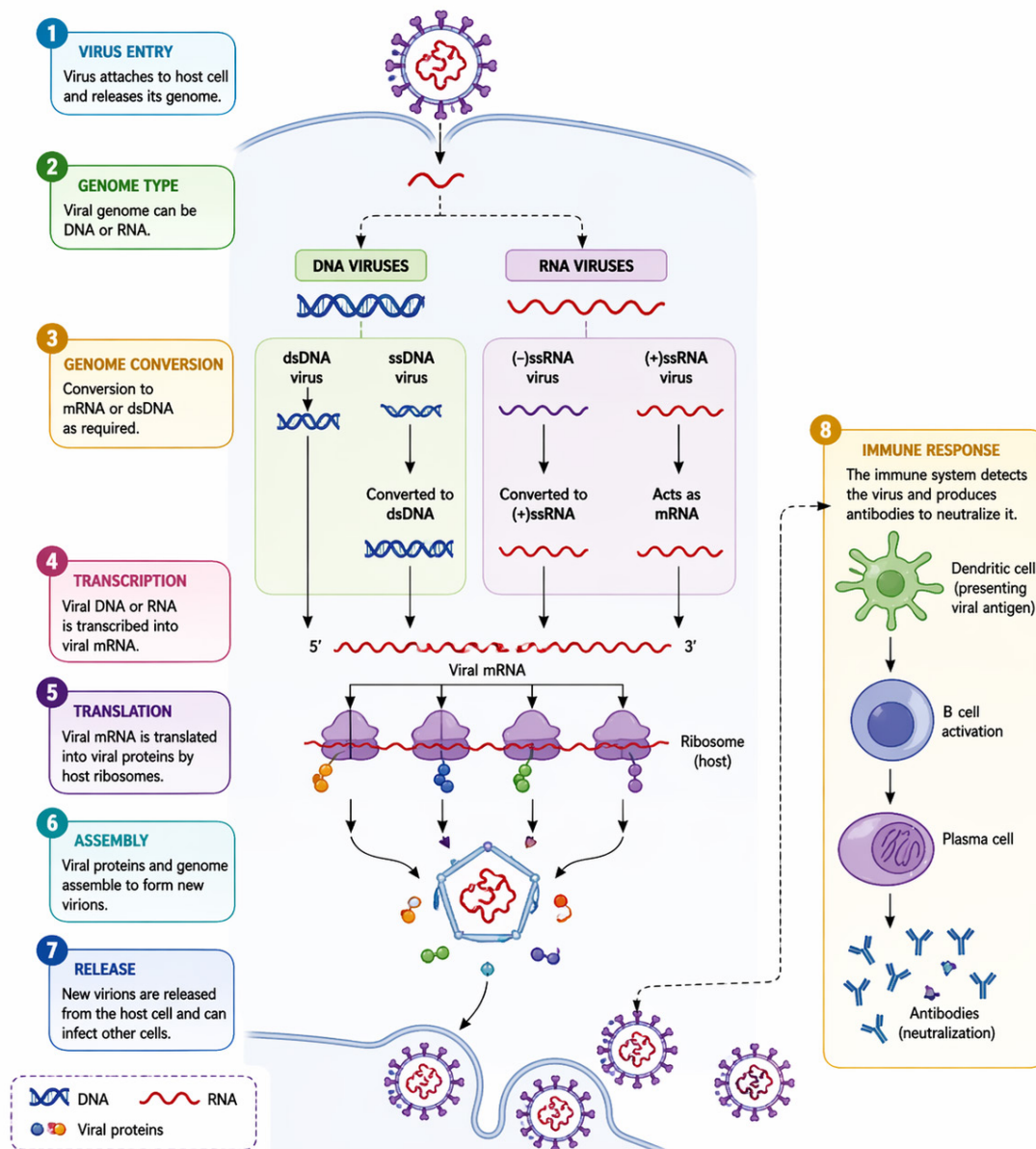


Figure 1: The virus binds to particular receptors on the host cell through surface proteins such as hemagglutinin . 2. Once within the cell, the virus releases its genetic material. 3. Within the host cell, the viral genome replicates. 4. Viral mRNA is produced by transcription. 5. Host ribosomes transform viral mRNA into proteins. 6. While DNA viruses first transcribe RNA and retroviruses transform RNA into DNA, RNA viruses can operate as mRNA immediately. 7. New virions are created when viral components come together and are released to infect more cells. 8. In response, the host immune system creates antibodies that fight the infection.

In the meanwhile, under experimental settings, SHERLOCK technology showed that it could identify Zika and Dengue viral RNA at quantities as low as 2 attomolar (Butler-Laporte *et al.*, 2021). According to these findings, CRISPR diagnostics could soon be crucial instruments for quick, decentralized viral testing.

Next Generation Sequencing (NGS)

By enabling thorough genomic characterisation of infections, Next-Generation Sequencing (NGS) has revolutionized viral epidemiology. Global sequencing initiatives found and monitored variants including Alpha, Delta, and Omicron in real time during the COVID-19 pandemic. Sequencing-informed public health measures decreased forward transmission clusters by 17%, according to a UK genomic surveillance study (Stevens *et al.*, 2017). Additionally, 13% of instances of meningitis and encephalitis that had not yet been confirmed had unexpected viral etiologies, indicating the clinical use of metagenomic NGS in complex illnesses (Chen *et al.*, 2006). These results demonstrate the growing use of NGS in precision diagnosis and epidemic control.

Nanopore Sequencing

Viral genomics has been further advanced by nanopore sequencing, which provides real-time and portable sequencing. Field-deployed nanopore devices produced viral genome data in less than a day during the West African Ebola outbreak, but centralized laboratory sequencing took one to two weeks (Renaud *et al.*, 2019). Nanopore processes allowed for same-day lineage typing in 6 to 8 hr in SARS-CoV-2 surveillance programs (Rogers *et al.*, 2015). Such quick genetic turnaround is very beneficial for infection control and outbreak response.

Biosensor Diagnostics

As quick, decentralized testing methods, biosensor-based diagnostics are increasingly gaining popularity. In clinical tests, a graphene-based electrochemical biosensor for SARS-CoV-2 antigen detection reached a limit of detection of 1 fg/mL (Dong *et al.*, 2021). Although sensitivity fluctuates between 55% and 85% depending on symptom onset and viral load, rapid antigen lateral flow tests have demonstrated pooled specificity above 99% (Strain *et al.*, 2013). These technologies offer affordable and easy-to-use solutions for home testing and bulk screening.

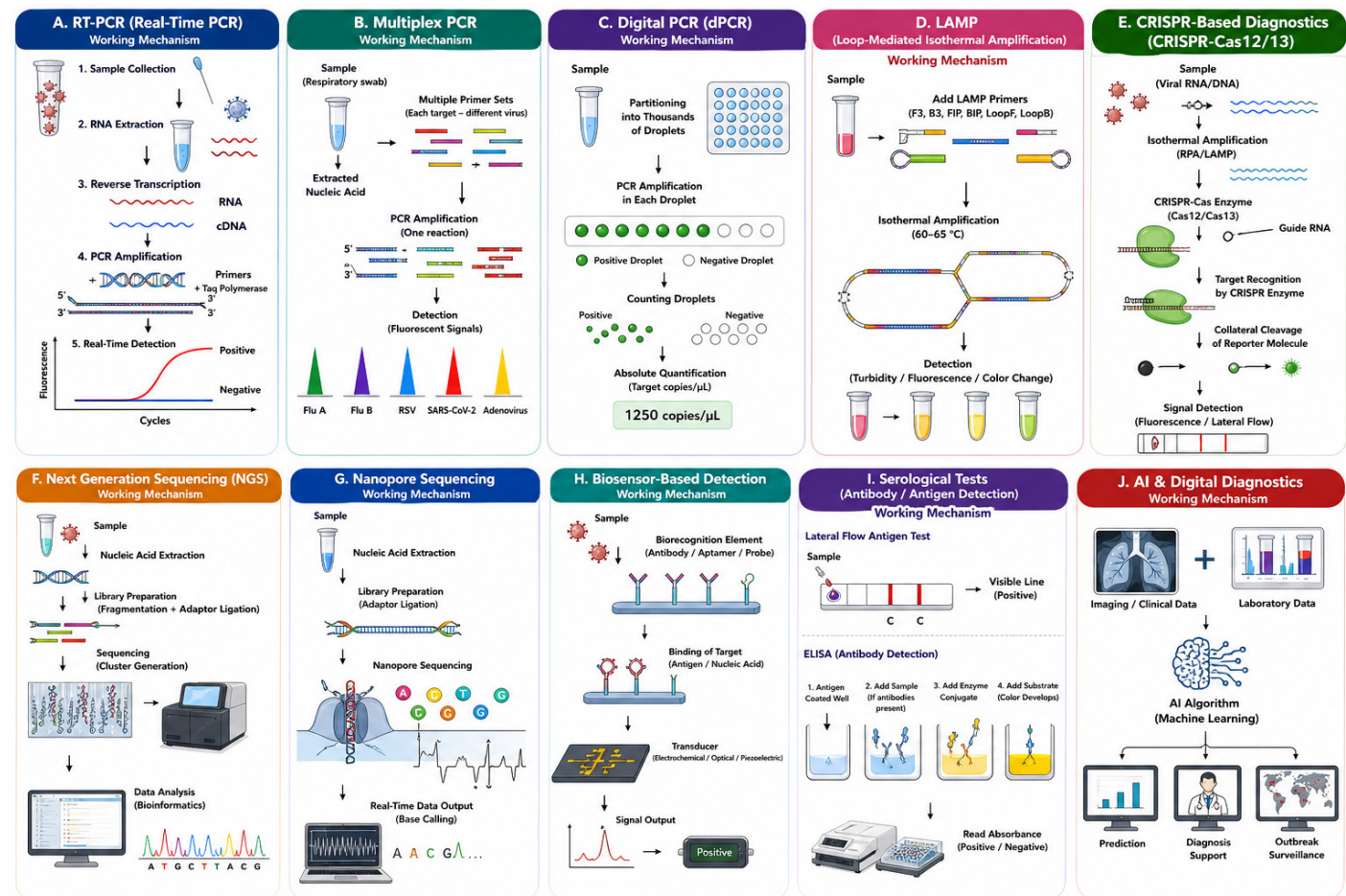


Figure 2: Schematic representation of the working mechanisms of advanced viral diagnostic techniques including RT-PCR, multiplex PCR, digital PCR, LAMP, CRISPR-based assays, next-generation sequencing, nanopore sequencing, biosensor-based detection, serological tests, and AI-assisted diagnostics used for rapid and accurate identification of viral infections.

Table 1: Comparative Analysis of Viral Diagnostic Techniques.

Technique	Sensitivity / Performance	Time to Result	Cost	Portability	Major Advantages	Major Limitations	Citations
RT-PCR	Very high sensitivity and specificity; reference standard for viral diagnosis	2-6 hr	High	Low	Accurate, widely validated	Requires laboratory setup and trained personnel	(Kubina and Dziedzic, 2020, Kevadiya <i>et al.</i> , 2021)
qPCR	High sensitivity with quantitative viral load monitoring	2-4 hr	High	Low	Useful for treatment monitoring	Expensive instrumentation	(Kubina and Dziedzic, 2020, Yüce <i>et al.</i> , 2021)
Multiplex PCR	High sensitivity for simultaneous multi-pathogen detection	1-4 hr	High	Low	Detects several viruses in one assay	Higher assay cost	(Sheridan, 2020, Drain, 2022)
Digital PCR	Superior precision for low viral load detection	3-5 hr	Very High	Low	Absolute quantification	Costly and limited availability	(Peeling <i>et al.</i> , 2022, Mina <i>et al.</i> , 2020)
LAMP	High sensitivity with rapid amplification	20-45 min	Moderate	High	No thermal cycler needed	Primer complexity	(Augustine <i>et al.</i> , 2020, Vandenberg <i>et al.</i> , 2021)
CRISPR Diagnostics	High sensitivity and specificity with rapid detection	<1 hr	Moderate	High	Portable and specific	Emerging commercialization stage	(Augustine <i>et al.</i> , 2020, Vandenberg <i>et al.</i> , 2021)
Antigen Rapid Tests	Moderate sensitivity, high specificity	10-30 min	Low	Very High	Fast, home/community screening	Lower sensitivity in asymptomatic cases	(Brümmer <i>et al.</i> , 2021, Gwinn <i>et al.</i> , 2019)
NGS	Very high analytical power; detects variants and unknown viruses	24-72 hr	Very High	Low	Whole-genome surveillance	Expensive and bioinformatics intensive	(Yüce <i>et al.</i> , 2021, Hansen <i>et al.</i> , 2021)
Point-of-Care NAAT	High sensitivity in near-patient setting	15-60 min	Moderate-High	High	Rapid molecular diagnosis	Cartridge/platform costs	(Mina <i>et al.</i> , 2020, Roberts <i>et al.</i> , 2021)
AI-Assisted Diagnostics	Improves interpretation speed and triage accuracy	Minutes	Moderate	High	Automation and decision support	Requires quality datasets	(Sheridan, 2020, Roberts <i>et al.</i> , 2021)

Artificial Intelligence in Viral Diagnosis

Viral diagnostics are rapidly incorporating Artificial Intelligence (AI) for predictive analytics and automated interpretation. In identifying viral pneumonia, meta-analyses of AI-assisted chest computed tomography models for COVID-19 revealed pooled sensitivity of 94% and specificity of 88% (Lee *et al.*, 2021). Additionally, AUROC scores for influenza prediction ranged from 0.82 to 0.91 using machine learning models that combined laboratory markers and symptoms (Broughton *et al.*, 2020). These results imply that AI can significantly increase viral diagnosis speed, consistency, and decision assistance. Every method is displayed in Figure 2 (Gootenberg *et al.*, 2018; Aggarwal *et al.*, 2022; Wilson *et al.*, 2019; Quick *et al.*, 2016; Bull *et al.*, 2020; Seo *et al.*, 2020; Dinnes *et al.*, 2021; Wynants *et al.*, 2020; Shillan *et al.*, 2019).

CONCLUSION

From traditional culture-based and serological techniques to extremely advanced molecular and digital technologies, viral infection diagnosis has come a long way. Despite their historical

significance, traditional diagnostic methods were frequently constrained by lengthy turnaround times, poor sensitivity, and reliance on specialized laboratory equipment. On the other hand, the speed, sensitivity, specificity, and dependability of viral detection have greatly increased thanks to contemporary methods including RT-PCR, qPCR, multiplex PCR, digital PCR, Loop-Mediated Isothermal Amplification (LAMP), CRISPR-based assays, and Next-Generation Sequencing (NGS) (Table 1).

Because of its great analytical accuracy and broad clinical acceptance, RT-PCR continues to be the gold standard among these techniques for routine diagnosis. By making it possible to monitor virus loads and evaluate treatment responses, quantitative PCR has increased the role of diagnostics. While digital PCR provides better accuracy for low viral load illnesses and research purposes, multiplex PCR panels have simplified syndromic testing by simultaneously identifying numerous pathogens. For point-of-care settings, isothermal amplification techniques like LAMP offer quick and affordable testing. CRISPR-based diagnostics are a new class of quick, portable, and

highly precise assays that hold great promise for decentralized healthcare delivery.

Additionally, outbreak research, mutation tracking, and surveillance of new viruses have been transformed by genomic technologies like NGS and nanopore sequencing. Due to their ability to detect viral variations and transmission dynamics in real time, these technologies were crucial during the COVID-19 pandemic. Rapid antigen testing and biosensor-based devices have also made community screening and at-home testing more accessible through accessible, user-friendly platforms. Additionally, through automated image interpretation, predictive analytics, and decision-support systems, artificial intelligence has started to change diagnostic operations.

Even with these significant advancements, there are still a number of obstacles to overcome, such as high implementation costs, technical complexity, unequal access in settings with limited resources, regulatory obstacles, and decreased assay performance due to viral mutations. Future viral diagnostics should therefore prioritize automation, affordability, portability, multiplex capacity, and worldwide accessibility. It is anticipated that integrating molecular testing with wearable biosensors, cloud-based surveillance, digital health systems, and AI-driven analytics will result in more intelligent and adaptable diagnostic ecosystems.

In conclusion, early disease identification, prompt treatment, outbreak preparedness, and global health security will all be greatly impacted by the ongoing development of viral diagnostic technology. To guarantee that these diagnostic advancements benefit people everywhere, investment in research, fair distribution, and international cooperation will be crucial.

ABBREVIATIONS

AI: Artificial Intelligence; **AUROC:** Area Under the Receiver Operating Characteristic Curve; **COVID-19:** Coronavirus Disease 2019; **CRISPR:** Clustered Regularly Interspaced Short Palindromic Repeats; **dPCR:** Digital Polymerase Chain Reaction; **DNA:** Deoxyribonucleic Acid; **HBV:** Hepatitis B Virus; **HIV:** Human Immunodeficiency Virus; **LAMP:** Loop-Mediated Isothermal Amplification; **NAAT:** Nucleic Acid Amplification Test; **NGS:** Next-Generation Sequencing; **PCR:** Polymerase Chain Reaction; **POCT:** Point-of-Care Testing; **qPCR:** Quantitative Polymerase Chain Reaction; **RNA:** Ribonucleic Acid; **RT-PCR:** Reverse Transcription Polymerase Chain Reaction; **SARS:** Severe Acute Respiratory Syndrome; **SARS-CoV-2:** Severe Acute Respiratory Syndrome Coronavirus 2.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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