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Opinion Article

Next-Generation Sequencing *vs.* RT-PCR in Detecting Emerging Viral Pathogens: A Diagnostic Paradigm Shift?

Eleanor V. Clarke*

Department of Molecular Diagnostics, University of Oxford, Nuffield Department of Medicine, Oxford, United Kingdom

DESCRIPTION

The COVID-19 pandemic has starkly highlighted both the strengths and limitations of our existing diagnostic infrastructure. At the centre of global detection efforts has Been Reverse Transcription Polymerase Chain Reaction (RT-PCR), a well-established technique that became synonymous with pandemic response. However, as new viral threats continue to emerge and diversify, questions are increasingly being asked about whether RT-PCR alone can meet the diagnostic demands of the future. Enter Next-Generation Sequencing (NGS) a powerful, high-throughput technology capable of analysing entire viral genomes with remarkable speed and resolution. Once restricted to academic research due to its cost and complexity, NGS is now emerging as a practical tool for pathogen surveillance, variant detection and even frontline diagnostics. The debate between RT-PCR and NGS is not just technical it represents a fundamental discussion about the direction of infectious disease diagnostics in the 21st century.

RT-PCR has long been favoured for its sensitivity, specificity and rapid turnaround for detecting known viral targets. It functions by amplifying specific genetic sequences of pathogens, making it ideal for situations where the virus is already identified and its genome is well-characterized. The method is relatively cost-effective, scalable and compatible with automated platforms, which explains its dominance in routine diagnostics and mass screening protocols. However, RT-PCR is inherently limited by design. It can only detect viruses that it has been programmed to recognize. In scenarios involving novel, mutating, or co-infecting pathogens, RT-PCR may yield false negatives or miss important genomic changes, such as mutations affecting primer binding sites. This was notably observed with variants of SARS-CoV-2, where mutations in spike protein genes occasionally affected test sensitivity.

This is where NGS presents a compelling advantage. Unlike RT-PCR, NGS is agnostic in approach it does not require prior knowledge of the pathogen. It can sequence entire viral genomes or even analyse metagenomics samples containing a mixture of host and microbial nucleic acids. This makes it ideally suited for detecting emerging or unexpected pathogens, identifying co-infections and performing variant characterization in a single test. Several public health successes illustrate the transformative potential of NGS. For instance, NGS enabled the rapid identification and genomic tracking of SARS-CoV-2 variants such as Delta and Omicron, informing vaccine updates and containment strategies. More recently, NGS played a role in detecting zoonotic spill over events involving viruses like Hendra, Nipa and various influenza subtypes cases where traditional RT-PCR would likely have failed without a pre-existing assay.

Yet, despite these advantages, NGS is not without its challenges. The technology requires sophisticated equipment, strong bioinformatics infrastructure and highly trained personnel. Costs, while decreasing, are still substantially higher than RT-PCR for single-target detection. Turnaround time may also be longer, depending on the workflow, which makes NGS less suitable for urgent bedside decisions, especially in low-resource or high-volume settings. There are also regulatory and quality assurance hurdles to overcome. Standardizing NGS workflows, from sample preparation to data interpretation, remains complex. Unlike RT-PCR, which delivers binary results, NGS produces vast datasets requiring interpretation, often involving uncertain clinical significance.

Despite these challenges, the future of viral diagnostics likely lies in a complementary model. RT-PCR will remain the frontline tool for rapid, high-throughput screening of known pathogens. But NGS will increasingly serve as a second-tier or confirmatory tool, particularly valuable for genomic surveillance, outbreak investigation and understanding pathogen evolution. The integration of both platforms can create a more resilient and adaptive diagnostic ecosystem. Several initiatives in high-income countries, including the UK's COG-UK consortium, the CDC's SPHERES program in the US and GENOTEND in France, have demonstrated how coupling RT-PCR with NGS can enhance public health responsiveness. These hybrid approaches ensure that while RT-PCR handles volume, NGS provides depth a combination necessary in an era of rapid viral evolution and global interconnectedness.

Correspondence to: Eleanor V. Clarke, Department of Molecular Diagnostics, University of Oxford, Nuffield Department of Medicine, Oxford, United Kingdom, E-mail: eleanclarke@ndm.uk

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CONCLUSION

The question is no longer whether NGS will replace RT-PCR, but rather how the two technologies can coexist synergistically. RT-PCR offers speed and scalability for known threats; NGS provides undestanding, flexibility and discovery power for unknown ones. Together, they form a dual-front defines against current and future viral outbreaks. For health systems preparing for the next pandemic or even the next variant the smart integration of NGS and RT-PCR is not a luxury, but a necessity. Policymakers, laboratories and clinicians must invest in infrastructure and training to ensure that both technologies can be deployed effectively when the next viral threat emerges. The future of pathogen detection is not about choosing one tool over the other it's about building a diagnostic arsenal that is agile, accurate and ready for the unknown.