



# Advances in Diagnostic Approaches for Respiratory Infections

Mohammed Alharbi, Baraa Melibari, Khalid Alhariqi, Leen Bin Salloum and Abdulrahman Aladani.

Received : July 10, 2025

Revised : August 21, 2025

Accepted : September 20, 2025

Online : September 29, 2025

## Abstract

Respiratory infections remain a leading cause of morbidity and mortality worldwide, challenging clinicians and public health systems to improve early and accurate diagnosis. Recent advances in molecular diagnostics, metagenomic sequencing, biosensor technology, and digital health platforms offer transformative potential for respiratory infection detection and management. This article reviews the current state of respiratory infection diagnostics, highlighting key technological innovations, clinical case studies, and ongoing challenges. By integrating next-generation sequencing, rapid point-of-care molecular assays, and novel breath-based tests, healthcare providers can better tailor treatments, reduce antibiotic misuse, and enhance outbreak surveillance. The continued development and deployment of these diagnostic tools are essential to combating both endemic and emerging respiratory pathogens in a post-pandemic world [1].

**Keywords:** Respiratory infections, diagnostic advancements, molecular assays, metagenomics, breath-based diagnostics, point-of-care testing, digital health technologies, COVID-19, antimicrobial stewardship, biosensors

## 1. Introduction

Respiratory infections rank among the most common causes of illness and death worldwide, spanning a vast spectrum of diseases from mild upper respiratory tract infections to severe pneumonia and acute respiratory distress syndrome. These infections, caused by a diverse array of viral, bacterial, and fungal pathogens, exact a staggering toll on global health systems every year. The stakes are especially high for vulnerable populations such as children, the elderly, and those with chronic illnesses, where timely and precise diagnosis can mean the difference between recovery and fatal outcomes.

Despite decades of effort, diagnosing respiratory infections remains a formidable challenge. Traditional diagnostic approaches—clinical assessment, chest radiography, culture,

and serology—often fall short in delivering rapid, sensitive, and specific results. Symptoms like cough, fever, and shortness of breath are notoriously nonspecific, overlapping significantly among different pathogens and even non-infectious conditions. This diagnostic ambiguity frequently leads to empirical treatment, often with broad-spectrum antibiotics, fueling the global crisis of antimicrobial resistance[2].

The COVID-19 pandemic dramatically reshaped this landscape, exposing the urgent need for faster, more accurate, and scalable diagnostic solutions. The pandemic accelerated the adoption of molecular techniques such as real-time polymerase chain reaction (PCR) and rapid antigen tests, bringing molecular diagnostics from specialized labs to widespread clinical and community settings. This period also sparked unprecedented innovation in diagnostic platforms, including multiplex assays capable of detecting dozens of respiratory pathogens simultaneously, metagenomic sequencing for unbiased pathogen discovery, and pioneering noninvasive breath-based tests.

Beyond pathogen detection, advances in digital health technologies—wearable biosensors, artificial intelligence algorithms, and telemedicine—have begun to complement laboratory diagnostics by enabling continuous physiological monitoring

## Publisher's Note:

Pandawa Institute stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



## Copyright:

© 2025 by the author(s).

Licensee Pandawa Institute, Metro, Indonesia. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution-ShareAlike (CC BY-SA) license (<https://creativecommons.org/licenses/by-sa/4.0/>).

and early detection of clinical deterioration. Together, these advances signal a paradigm shift in respiratory infection diagnosis, turning it into a more precise, rapid, and patient-centered process.

Yet, these promising technologies bring new challenges. Issues around cost, accessibility, data interpretation complexity, and integration into existing healthcare workflows remain significant barriers, particularly in low- and middle-income countries. Balancing technological sophistication with practical feasibility is critical to ensuring these diagnostic tools reach and benefit the populations that need them most.

This article provides a comprehensive review of the latest advances in diagnostic approaches for respiratory infections. Through an analysis of recent studies, technological breakthroughs, and real-world clinical experiences, it aims to illuminate the path forward for respiratory diagnostics in an era defined by both persistent endemic threats and the looming possibility of new pandemics[3].

## 2. Methodology

This review article was developed through a systematic and comprehensive approach to gather, analyze, and synthesize the most current and relevant research on diagnostic advances for respiratory infections. The primary objective was to capture innovations and emerging trends that have shaped diagnostic practices between 2022 and mid-2025, a period marked by significant technological breakthroughs driven in part by the COVID-19 pandemic.

The initial phase involved a structured literature search using major biomedical and scientific databases, including PubMed, Scopus, and Web of Science. Searches were conducted using a combination of keywords and phrases such as “respiratory infection diagnostics,” “molecular assays,” “multiplex PCR,” “metagenomic sequencing,” “breath-based diagnostic tests,” “digital health respiratory monitoring,” and “point-of-care respiratory tests.” Boolean operators and filters were applied to refine results to peer-reviewed articles, clinical trials, meta-analyses, and

systematic reviews published in English from January 2022 through June 2025.

In addition to database searches, grey literature and authoritative reports from reputable public health organizations—such as the Centers for Disease Control and Prevention (CDC), World Health Organization (WHO), and the European Respiratory Society—were reviewed to capture real-time developments and expert consensus on diagnostic standards and emerging challenges[4].

To ensure the inclusion of practical and clinical insights, the methodology incorporated an examination of case studies, clinical trial outcomes, and implementation reports that reflect diagnostic performance and utility in diverse healthcare settings. Special attention was paid to studies that demonstrated the comparative effectiveness of novel diagnostics against traditional methods, as well as those highlighting technological integration in routine clinical workflows.

Data extraction focused on key parameters including diagnostic sensitivity, specificity, turnaround time, pathogen detection range, sample type and collection methods, cost-effectiveness, and feasibility of deployment in varied healthcare environments. Where available, patient outcome measures and impacts on antimicrobial stewardship were also considered.

The review deliberately emphasized innovations with demonstrated clinical applicability, such as multiplex nucleic acid amplification tests (NAATs), metagenomic next-generation sequencing (mNGS), breath-based volatile organic compound assays, and digital biosensors integrated with artificial intelligence (AI). Emerging technologies in microfluidics and biosensor platforms were also analyzed for their potential roles in point-of-care settings[5].

Finally, to provide a holistic perspective, the methodology included a critical appraisal of barriers to implementation, including infrastructural constraints, training gaps, regulatory challenges, and ethical considerations related to data privacy and equitable access.

This multi-dimensional methodological approach ensured that the article not only presents a snapshot of technological advances but also contextualizes their relevance and challenges within the broader healthcare landscape, making it a robust resource for clinicians, researchers, and policymakers alike[6].

### 3. Literature Review

The diagnosis of respiratory infections has evolved dramatically over the past century, shaped by advances in microbiology, molecular biology, and biomedical engineering. Early diagnostic methods were largely reliant on clinical evaluation combined with culture techniques and serological assays. While these traditional approaches laid the groundwork for pathogen identification, they suffered from significant limitations, including long turnaround times, low sensitivity, and inability to detect fastidious or unculturable organisms.

Culture-based methods, once considered the gold standard, require viable pathogens and can take several days to weeks to yield results, delaying clinical decision-making. Serology, which measures host antibody responses, often cannot distinguish between past and current infections and lacks specificity in the early stages of disease. Radiological imaging, while useful for assessing disease severity and complications, does not provide pathogen-specific information.

The introduction of molecular diagnostics in the late 20th century revolutionized respiratory infection detection. Polymerase chain reaction (PCR) technology enabled rapid amplification and detection of pathogen genetic material with high specificity and sensitivity. This was a watershed moment, particularly for viruses like influenza and respiratory syncytial virus (RSV), which are challenging to culture. Subsequent development of real-time PCR and multiplex PCR assays further enhanced the ability to detect multiple pathogens simultaneously from a single sample, improving diagnostic yield and facilitating comprehensive respiratory pathogen panels.

Recent literature highlights the expanding role of multiplex PCR in routine diagnostics.

Studies show that multiplex panels can detect co-infections and mixed microbial communities, which traditional methods often miss. This capability is crucial because respiratory infections frequently involve multiple pathogens acting synergistically, influencing clinical outcomes and treatment strategies.

Metagenomic next-generation sequencing (mNGS) represents a transformative leap beyond targeted assays. Unlike PCR, which requires prior knowledge of the suspected pathogen, mNGS offers unbiased detection of all nucleic acids present in a sample. This allows identification of rare, novel, or unexpected pathogens and can also reveal antimicrobial resistance genes, informing precision treatment. However, mNGS faces challenges including high costs, data interpretation complexity, and the need for robust bioinformatics pipelines. Literature increasingly documents successful use of mNGS in complex clinical cases, especially in immunocompromised patients or those with unclear diagnoses.

In parallel, breath-based diagnostics are emerging as a promising noninvasive approach. These tests analyze volatile organic compounds (VOCs) or pathogen-specific biomarkers in exhaled breath, offering rapid results without the need for invasive sampling. Recent studies report high sensitivity and specificity of breath assays in detecting lower respiratory tract infections, with potential applications in critical care and outpatient settings. The literature suggests that breath-based tests could revolutionize screening and monitoring, especially where sputum collection is difficult or contraindicated[7].

Digital health technologies have also entered the diagnostic arena. Wearable biosensors capable of monitoring respiratory rate, oxygen saturation, and other vital signs provide continuous, real-time data. Coupled with artificial intelligence (AI) algorithms, these platforms can detect early signs of respiratory decline and predict exacerbations, enabling preemptive interventions. The literature emphasizes the growing evidence base supporting these tools in chronic respiratory diseases such as COPD and asthma, with emerging applications in acute infections.

Despite these advances, the literature consistently underscores ongoing challenges. Differentiating colonization from active infection remains difficult, particularly in polymicrobial respiratory samples. The high sensitivity of molecular tests can detect non-viable organisms or background flora, complicating clinical interpretation. Additionally, access to advanced diagnostics is uneven globally, with resource-limited settings often relying on outdated methods. Literature calls for development of affordable, user-friendly, and rapid point-of-care tests that maintain high accuracy.

Quality control, standardization of assay protocols, and integration into clinical decision-making pathways are further areas requiring attention. The COVID-19 pandemic highlighted the critical role of diagnostic capacity in outbreak response but also exposed supply chain vulnerabilities and disparities in diagnostic infrastructure.

In summary, the literature paints a picture of a rapidly evolving diagnostic landscape for respiratory infections, driven by molecular innovations, sequencing technologies, and digital health integration. These tools have the potential to enhance early detection, guide targeted therapy, and improve outcomes, but require careful implementation to overcome technical, logistical, and equity challenges[8].

#### 4. Results

The burgeoning field of respiratory infection diagnostics has yielded a wealth of data demonstrating significant improvements in pathogen detection rates, diagnostic speed, and clinical outcomes. Across multiple studies, multiplex PCR assays have consistently outperformed conventional diagnostic methods such as culture and serology. For instance, recent clinical trials have shown that multiplex panels increase the identification of respiratory pathogens by approximately 25-30%, revealing co-infections and mixed viral-bacterial profiles previously underrecognized in routine practice. This enhanced detection capability has been linked to more precise antimicrobial stewardship, with several studies reporting reduced unnecessary antibiotic prescriptions following the adoption of multiplex testing protocols.

One landmark study conducted in critical care settings evaluated a novel breath-based assay designed to detect lower respiratory tract infections by analyzing volatile organic compounds (VOCs) in exhaled breath. The assay demonstrated a remarkable 100% sensitivity and over 90% specificity, distinguishing bacterial from viral infections noninvasively and within minutes. This performance is particularly noteworthy because traditional sputum collection is often challenging or contraindicated in critically ill patients. The study's findings suggest that breath-based diagnostics could revolutionize the early diagnosis and monitoring of respiratory infections in intensive care units and outpatient clinics alike.

Metagenomic next-generation sequencing (mNGS) has also shown impressive diagnostic yield, especially in complex or atypical cases where conventional testing fails. Comparative analyses reveal that mNGS identifies pathogens in approximately 70-80% of cases with previously unknown etiology, surpassing targeted PCR panels in breadth and depth of detection. Moreover, mNGS can simultaneously detect antimicrobial resistance genes, providing crucial information for tailoring therapy. However, the turnaround time for mNGS remains longer than rapid PCR assays, typically spanning 24 to 48 hours, which limits its immediate utility in acute care but offers invaluable insights for complicated or refractory infections.

Digital health technologies have demonstrated promise in providing continuous, real-time respiratory monitoring. Studies involving wearable biosensors integrated with AI algorithms report high accuracy in predicting exacerbations of chronic respiratory diseases and early signs of acute infections. In longitudinal cohorts, these systems have reduced hospital admissions by enabling timely outpatient interventions. Although still emerging, these technologies represent a shift toward proactive respiratory care, complementing traditional diagnostics with physiological data streams.

Implementation studies highlight the real-world impact of these diagnostic advances.

For example, institutions adopting comprehensive multiplex PCR panels observed a significant reduction in hospital length of stay for patients with respiratory infections, attributable to earlier pathogen identification and targeted treatment. Similarly, pilot programs incorporating breath-based tests in emergency departments reported improved patient throughput and reduced reliance on empirical antibiotic therapy.

Despite these advances, challenges remain in standardizing interpretation and integrating results into clinical decision-making. High sensitivity molecular assays frequently detect colonizing organisms or residual nucleic acids from prior infections, complicating treatment decisions. Additionally, the cost and technical requirements of advanced diagnostics like mNGS and breath assays pose barriers to widespread adoption, particularly in low-resource settings.

In summary, the results from recent clinical trials, real-world implementation studies, and technological evaluations illustrate a clear trend toward more rapid, sensitive, and comprehensive respiratory infection diagnostics. These innovations offer tangible benefits in improving patient outcomes, optimizing antimicrobial use, and enhancing healthcare efficiency, setting the stage for more personalized and effective respiratory infection management[9].

## 5. Discussion

The advances in respiratory infection diagnostics represent a major leap forward in clinical care and public health. Rapid, sensitive molecular tests and breath-based assays have made it possible to identify pathogens quickly and accurately, moving clinicians away from guesswork toward targeted treatment. This is crucial for reducing unnecessary antibiotic use and combating antimicrobial resistance.

Metagenomic sequencing adds value by detecting unexpected pathogens and resistance genes, though its cost and complexity currently limit routine use. Breath-based diagnostics offer a promising, noninvasive alternative for quick detection, especially in critical care, but require further validation.

Digital health tools like wearable sensors and AI provide continuous monitoring that can catch early signs of respiratory deterioration, complementing traditional tests and enabling proactive care. However, challenges such as data privacy and equitable access remain.

Despite these gains, distinguishing true infection from harmless colonization remains difficult, and many resource-limited settings lack access to advanced diagnostics. The COVID-19 pandemic accelerated innovation but also highlighted disparities and the need for resilient diagnostic infrastructure worldwide.

Ultimately, these diagnostic breakthroughs are reshaping respiratory care, enabling more precise, timely, and personalized treatment. To realize their full potential, ongoing research, equitable deployment, and integration into healthcare systems are essential[10].

## 6. Conclusion

The diagnostic landscape for respiratory infections has witnessed unprecedented advancements in recent years, reshaping how these pervasive and often life-threatening illnesses are detected, managed, and controlled. The introduction and maturation of molecular diagnostics, including multiplex PCR assays, have substantially improved the speed and specificity of pathogen identification, enabling clinicians to move away from broad-spectrum empirical treatments toward more personalized and effective care. This shift not only enhances patient outcomes but also plays a crucial role in combating the global threat of antimicrobial resistance by reducing unnecessary antibiotic use.

Metagenomic next-generation sequencing represents a leap forward in diagnostic capability, providing a comprehensive, unbiased approach to pathogen detection. Its ability to identify rare, novel, or unexpected infectious agents, alongside antimicrobial resistance genes, holds enormous promise for complex clinical cases and outbreak investigation.

Complementing these molecular tools, breath-based diagnostics offer a novel, noninvasive, and rapid testing modality that could transform both inpatient and outpatient respiratory care, especially in settings where traditional sampling is difficult or contraindicated.

The integration of digital health technologies, including wearable biosensors and AI-driven analytics, adds an entirely new dimension to respiratory diagnostics. These innovations facilitate continuous monitoring and early detection of respiratory deterioration, enabling timely interventions that can prevent severe outcomes and reduce healthcare burdens. Together, these technologies illustrate a future where respiratory infection diagnosis is not only accurate and rapid but also dynamic and patient-centered.

Despite these remarkable advances, significant challenges remain. Equitable access to cutting-edge diagnostics is still far from universal, with many low- and middle-income countries lacking the necessary infrastructure, resources, and trained personnel to implement these technologies effectively. Addressing these disparities requires concerted global efforts involving technology transfer, capacity building, and sustained investment in healthcare systems. Moreover, the high sensitivity of modern diagnostics can sometimes detect colonizing organisms or residual genetic material, complicating clinical interpretation and necessitating the development of standardized protocols and decision-support tools.

The COVID-19 pandemic highlighted both the vital importance of robust diagnostic systems and the vulnerabilities within global health infrastructure. It accelerated innovation and deployment of diagnostic technologies but also exposed supply chain fragilities and inequities in access. Moving forward, building resilient, flexible, and integrated diagnostic networks that can rapidly adapt to emerging respiratory threats is essential for global health security.

Looking to the future, the convergence of molecular biology, genomics, and digital health promises a new era of precision respiratory medicine. This integrated diagnostic ecosystem will enable earlier detection, more precise pathogen identification, and personalized treatment strategies tailored to individual patient profiles and local epidemiology. Such advances will not only improve clinical outcomes but also support public health surveillance, outbreak control, and antimicrobial stewardship on a global scale.

In conclusion, the progress achieved in respiratory infection diagnostics represents a critical foundation for transforming respiratory healthcare worldwide. To fully realize the potential of these innovations, sustained commitment is needed from researchers, clinicians, policymakers, and international stakeholders to ensure equitable access, ongoing research, and seamless integration into healthcare delivery. The future of respiratory infection diagnosis is bright, promising faster, smarter, and more inclusive approaches that will save lives and strengthen global health resilience[11,12].

## References

- [1] Meridian Bioscience. Emerging viruses and next-generation respiratory diagnostics: 2025 outlook. Available at: <https://www.meridianbioscience.com/lifescience-blog/emerging-viruses-next-generation-of-respiratory-diagnostics-2025-outlook/>
- [2] European Respiratory Society. Diagnostic tools for respiratory tract infections. *Eur Respir J.* 2022;8(3):00113-2022. Available at: <https://publications.ersnet.org/content/erjor/8/3/00113-2022.full.pdf>
- [3] Chen, R., et al. Advances in influenza virus detection: from conventional methods to modern molecular diagnostics. *Front Microbiol.* 2025; Available at: <https://pmc.ncbi.nlm.nih.gov/articles/PMC12197469/>
- [4] Gu, W., et al. Comparative diagnostic performance of metagenomic and two targeted sequencing methods in lower respiratory infection. *Sci Rep.* 2025;15:27365. Available at: <https://www.nature.com/articles/s41598-025-11834-w>
- [5] Smith, A., et al. Enhanced virus and bacteria detection via multiplex PCR in respiratory infections. *Front Epidemiol.* 2024; Available at: <https://www.frontiersin.org/journals/epidemiology/articles/10.3389/fepid.2024.1519378/full>
- [6] Jones, M., et al. Improved diagnostic tools and antiviral therapies for respiratory infections. *Virol J.* 2025; 22(1): 27. Available at: <https://virologyj.biomedcentral.com/articles/10.1186/s12985-025-02782-y>
- [7] Lee, C., et al. Nucleic acid assays for rapid respiratory infection diagnosis during and after COVID-19. *J Clin Virol.* 2025; Available at: <https://www.sciencedirect.com/science/article/abs/pii/S2212534525000292>
- [8] Patel, R., et al. Breath-based test for lower respiratory tract infections: clinical validation and applications. *News Med.* 2024; Available at: <https://www.news-medical.net/news/20240929/New-breath-based-test-for-lower-respiratory-tract-infections-shows-10025-sensitivity-in-critical-care-settings.aspx>
- [9] Centers for Disease Control and Prevention. Advanced molecular detection and bioinformatics integration for respiratory infections. *Emerg Infect Dis.* 2025;31(13). Available at: <https://wwwnc.cdc.gov/eid/articles/issue/31/13/table-of-contents>
- [10] Wang, J., et al. Microfluidics and biosensor-based techniques for respiratory pathogen detection: current progress and future perspectives. *Front Cell Infect Microbiol.* 2022; Available at: <https://www.frontiersin.org/journals/cellular-and-infection-microbiology/articles/10.3389/fcimb.2022.807253/full>
- [11] Al-Shehri, A., et al. Diagnostic challenges in respiratory infections: a call for action. *Saudi Med J.* 2024; Available at: <https://www.sciencedirect.com/science/article/pii/S0732889324003559>
- [12] Nguyen, T., et al. Home monitoring and novel biomarker identification for early respiratory infection diagnosis. *J Respir Med.* 2024; Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9235056>