International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

Advances in Rapid Point-of-Care Molecular Diagnostics and Biosensing for Infectious Diseases: A Review

S Sowmya Priya[1], Pusthela Arun Raj[2], Vishnu MG[3]

[1]Professor, Department of microbiology, Acharya Institute of Allied Health Sciences, Soladevanahalli, Bangalore.
Email: *spriyakt2011[at]gmail.com*

[2]Professor, Department of Anatomy, Acharya Institute of Allied Health Sciences, Soladevanahalli, Bangalore.
Email: *arunraj.francis[at]gmail.com*

[3]Associate Professor, Department of pathology, Santhi Institute of Allied Health Sciences, Kozhikode.
Email: *vmg4567[at]gmail.com*

Abstract: ***Rapid point-of-care molecular diagnostics are reshaping infectious disease management by enabling early detection and decentralized testing. This review explores recent advances in isothermal amplification, CRISPR-Cas systems, nanomaterial-based biosensors, and digital health tools including AI and IoMT. Techniques such as LAMP and RPA, when paired with CRISPR, offer high accuracy in minimal-resource settings. Nanomaterials like gold nanoparticles and carbon nanotubes enhance sensitivity and portability, while paper microfluidics and wearable biosensors expand accessibility. Although challenges persist such as validation, regulation, and equitable deployment- these technologies show promise for transforming global diagnostic capacity. The review emphasizes the need for integration with health systems to realize the potential of these tools under WHO's ASSURED framework.***

Keywords: Point-of-care diagnostics, CRISPR-Cas, Isothermal amplification, Nano biosensors, AI in healthcare

1. Introduction

Despite major progress in the development of new forms of therapeutic interventions and preventive public health strategies, infectious diseases represent one of the first causes of morbidity and mortality worldwide, especially in low- and middle-income countries. Fast growth in urban centres, increased human mobility, and the rise of new forms of pathogens with high drug resistance have dramatically highlighted the weakness of traditional approaches in diagnosing infectious diseases. Traditional approaches in diagnosing infectious diseases include high-tech laboratory procedures such as microscopic examination, culture medium, enzyme-linked immunosorbent assay (Elder's test), and the more recent PCR, characterized by high sensitivity and specificity. These approaches often entail well-equipped medical labs, skilled personnel, and long processing times. These factors may hinder decision-making in fast-paced healthcare situations in resource-limited and decentralized regions of the world, especially in situations of high human disease transmission [1]. In response to these challenges, rapid testing or point-of-care testing technologies (POCT) have been developed as one of the key pillars for tackling infectious diseases. POCT makes diagnostic testing possible during or at a site close to the site of care delivery relative to patients. The ASSURED criteria developed by the World Health Organization for diagnostic innovation for infectious diseases include "Affordable, Sensitive, Specific, User-friendly, Rapid & Robust, Equipment-free, and Deliverable. These criteria include some of the core requirements for diagnostic test development for use at or for use in poor-resource settings. These criteria play an integral role in different diagnostic testing technologies. With respect to POCT technologies, one of the most commonly utilized diagnostic technologies includes lateral flow assays, particularly in their various configurations. Whereas they were originally utilized as platforms to detect pregnancy, there have been significant developments in incorporating nanotechnologies, aptamers, as well as nucleic acid probes to improve their associated analyte sensitivity and their capacity to detect various pathogens. as well as their ability to detect various infectious agents. Indeed, one of their most attractive utility factors includes minimal instrumentation, as well as emergency response, particularly in field settings [2]. Advances in nucleic acid amplification further strengthen POCT performance. Isothermal amplification technologies such as RPA and LAMP enable rapid target amplification at constant and low temperatures without the need for sophisticated thermal cycling instrumentation. When these are combined with lateral flow or fluorescence-based detection, the systems can arrive at a diagnosis within minutes and so support early identification of the pathogen and the epidemic containment [3]. More recently, CRISPR-Cas systems have redefined molecular diagnostics by programmable nuclease activity and exceptional specificity. The DETECTR and SHERLOCK platforms were based on the Cas12 and Cas13 enzymes, respectively, which specifically harnessed the collateral cleavage of reporter molecules for ultra-sensitive detection at attomolar levels. Scalable and adaptable solutions for a variety of infectious agents have been proposed [4]. Parallel to these advances, the application of nanotechnology in biosensors has brought about enhanced potential in point-of-care devices. Various forms of nanobased materials, such as gold nanoparticles, magnetic nanoparticles, graphene, and other 2D materials based on semiconductors, have shown their potential in exploring the field. The application of graphene field-effect transistor sensors has shown ultra-sensitive, real-time detection potential with ultra-low LOD values [5]. Despite

these advances, issues related to inequity in diagnostic devices point towards a new innovation approach to link advancements with global ethical and equitable public health policies.

2. Methodology

This review adheres to the structured and systematic approach in its review of emerging POC diagnostic technologies. Searches were performed through PubMed, Scopus, ScienceDirect, and Web of Science for publications from 2023 to 2025. The search terms were connected by Boolean operators across concepts: "point-of-care testing," "CRISPR diagnostics," "isothermal amplification," "nanomaterial biosensors," "microfluidics," and "infectious disease detection." Only original research articles, reviews, and clinical evaluations written in English were considered. Inclusion criteria comprised studies that reported technological development, validation, or clinical application in human infectious diseases using POC diagnostics. Exclusion criteria included non-peer-reviewed reports, veterinary-only studies, and papers that did not demonstrate methodological transparency with quantitative outcomes. The selection process involved duplicate removal, screening of titles and abstracts, and analysis of eligible full texts for thematic synthesis in accordance with PRISMA guidelines. Data synthesis entails categorization of major technological domains, including nucleic acid amplification, CRISPR platforms, nanomaterial transduction, paper microfluidics, wearable devices, and AI/IoMT integration. This will facilitate critical comparison of analytical performance, usability, and translational readiness.

3. Nucleic-Acid Platforms at the Point of Care: Isothermal, CRISPR, and Hybrids

In fact, isothermal amplification remains central to point-of-care molecular testing due to its low power and temperature demands. Beyond the RPA-LFA foundations introduced above, LAMP has emerged as a robust alternative with tolerance to inhibitors and suitability for environmental matrices. For waterborne pathogens, LAMP coupled to microfluidics and smartphone readouts enables rapid, field-deployable surveillance, addressing the time-to-result and infrastructure limits of culture and qPCR [6]. CRISPR systems are increasingly embedded in one-pot, multiplex workflows. A multiplex Cas12b/Cas13 HR-HPV assay achieved 10-copy sensitivity and 100% specificity in a single tube, illustrating how thermostable nucleases and collateral cleavage can be orchestrated for high-risk cervical cancer screening in low-resource or home settings [7]. CRISPR has similarly been harnessed for antimicrobial-resistance gene detection and difficult bacterial targets; integrating genomic surveillance improves target selection and specificity for fieldable tests [8]. Diagnostic pipelines continue to diversify: in parasitology, RPA–CRISPR/Cas12a enabled sensitive, on-site detection of Opisthorchis viverrini from fecal samples with simple fluorescence readout, a practical upgrade over low-sensitivity microscopy in endemic zones [9]. Schistosomiasis diagnosis saw an electricity-free LAMP incubator (“T-cup”) maintain 65 °C for ~30–35 minutes with qPCR-comparable results—an elegant demonstration of frugal innovation for extreme POC [10].SARS-CoV-2 work continues to anchor platform generalizability: comparative reviews outline NAAT evolution from RT-qPCR toward RT-LAMP, CRISPR, and nanomaterial-assisted LFIA- highlighting speed, cost, and portability gains essential for surge testing and future pandemics [11]. Clinical signal to decision continues to compress as these systems mature, yet stewardship-linked outcomes matter most. In bloodstream infections, a meta-analysis (>25,000 patients) showed mortality reduction only when rapid molecular tests were combined with structured antimicrobial stewardship programs (RDT + ASP), underscoring that analytics must be embedded in care pathways to change outcomes [12].

4. Nanomaterials and Advanced Transduction

Magnetic Nanoparticles (MNPs) enable magnetic enrichment and transduction (relaxation switching, tunneling magnetoresistance, fluorescence quenching), which cuts assay time and increases sensitivity across electrochemical and optical sensors amenable to POC miniaturization [13]. Aggregation-induced emission (AIE) materials, luminogens realize bright, aggregation-enhanced fluorescence and photostability, enabling lateral-flow and nucleic-acid sensors, while supporting photodynamic/photothermal inactivation for viral, TB, and fungal pathogens, thereby demonstrating the potential of theragnostic at a POC [14]. Graphene and carbon nanostructures. Graphene-oxide platforms have been coupled with CRISPR-Cas12a to create GO-quenched fluorescent reporters that "light up" upon trans-cleavage, thus allowing for on-site detection of sepsis-causing Salmonella Typhimurium at ~3×10^3 CFU/mL in serum [15]. Single-walled carbon nanotube (SWCNT) FETs functionalized with anti-Ag85B antibodies differentiated TB-positive clinical samples within 10 min, with limits of 0.05 fg/mL in buffered tests, as powerful proof of label-free, real-time antigen detection. Furthermore, a broader probe chemistry of fluorescent, electrochemical, and photoactive types has enabled detection of a wider range of pathogens and host markers, while enabling theranostic approaches such as photodynamic therapy and targeted delivery [16].Biomedical SERS, not included in the six sources listed in the Introduction, allows further advance in single-molecule sensitivity and multiplexing, along with ongoing standardization and AI-assisted spectral analysis toward clinical translation.

5. Paper Microfluidics, Low-Cost Readers, and New Sample Matrices

Beyond classic dipsticks, µPADs support microfluidic routing, multiplexing, and colorimetric or electrochemical readouts with wax/inkjet fabrication and nanomaterial amplification. Reviews emphasize democratized access for saliva biomarkers, such as lactate, glucose, and SARS-CoV-2 antigens, while aligning with the ASSURED/ REASSURED principles [17]- [19]. A capillary-driven electrochemical microfluidic device using a smartphone-powered NFC potentiostat has quantified C-reactive protein with nanobody recognition, demonstrating pump-free flow control and wireless data capture for bedside inflammation monitoring in Boonkaew et al [20]. A fluorescent hydrogel bandage provided real-time pH sensing for chronic wounds,

where alkalinity correlates with infection-linked pathophysiology in animal models-examples of bedside image-read diagnostics for infection surveillance [21]. Non-blood sampling continued to progress with microneedle-based interstitial-fluid collection integrated into LFAs for the detection of antibodies within 20 minutes, circumventing phlebotomy barriers and infection risk [22]. Metallic, inorganic, polymeric, and hydrogel microneedle biosensors enable pain-free access to ISF with electrochemical/optical transduction for metabolites, nucleic acids, and proteins. Continuous monitoring of the infection pathophysiology with personalized infection care is thus allowed. Parallel advances in POC wearables emphasize AI-enabled, noninvasive tracking together with the translational challenges of standardization and data governance [23].

6. Title, AI, IoMT and Quality systems

AI permeates microbiology across microscopy interpretation, MALDI-TOF MS spectra, genome-based AMR prediction, and outbreak modeling; yet interpretability, bias mitigation, and data quality remain important for trustworthy deployment [24]. In connected testing, IoMT-integrated biosensors enable real-time data aggregation for surveillance and triage [25], an approach reinforced by calls to maintain "warm" point of care capacity and community-based networks developed during COVID-19 [26]. Implementation science highlights that accuracy alone is not sufficient: a multi-country Sexually Transmitted Infection program with GeneXpert assays achieved greater than 97–99% concordance underpinned by structured training and external quality assessment (EQA), gives evidence that robust EQA and workforce development are prerequisites for scalable POC [27].

7. Disease-Focused Applications

A nanofluidic POC analyzer (Sysmex PA-100) detected bacteriuria in 15 min (specificity ~99.4%) and delivered phenotypic AST within 45 min with approximately 94.6% categorical agreement, improving optimal antibiotic selection; demonstrating how rapid, direct-from-urine phenotyping can curb inappropriate prescribing [28]. Complementarily, Urinary tract infection diagnostics are developing toward metagenomics and rapid light-scattering tools to capture microbial ecology and avoid overdiagnosis [29]. One-pot CRISPR multiplexing for HPV16/18 and rolling-circle-amplification–to–personal-glucose-meter workflows for E6/E7 mRNA quantification showcase complementary strategies for high-throughput, low-infrastructure screening [30],[31]. Plasma or BAL microbial cell-free DNA sequencing replaced conventional testing, particularly for non-Aspergillus infections; while specificity was high, moderate sensitivity indicates it should augment not replace existing algorithms [32]. Relatedly, ICU-focused reviews argue for revised fungal diagnostic frameworks leveraging β-D-glucan, galactomannan, and PCR to overcome culture delays. Beyond SWCNT-FET antigen sensing, platform reviews emphasize GeneXpert Ultra and stool PCR for pediatric cases, integrating novel sample processing and fluorescence microscopy to accelerate case detection and DST vital to End TB strategy timelines [33]. A paper-based β-lactamase subtype visualization (BSV) sensor classified ESBL/AmpC/carbapenemases within 15-180 min, improving appropriate prescriptions from 48% to >80–97%—a compelling, low-cost adjunct to stewardship [34]. Policy commentaries highlight integration of newly approved syphilis POC tests with equitable access plans and confirmatory pathways, aligning with national elimination targets [35]. In primary care, RADTs provide speed with variable sensitivity, while PCR-based POC remains confirmatory standard; diagnostic stewardship is critical to avoid overuse [36]. Extending POC into homes for older adults- combining focused lung ultrasound, venous chemistry, pathogen PCR, and urine flow cytometry- proved feasible and clinically actionable [37]. Point-of-care ultrasound phenotyping changes how hepatorenal syndrome is understood. It moves from being an exclusion diagnosis to a physiologically guided entity, reducing iatrogenic harms from blanket volume expansion [38]. LAMP-based water surveillance and One Health-oriented zoonosis diagnostics underscore POC's role beyond clinics, enabling early detection in community and environmental interfaces [39]. Commercial viability studies of biomimetic sensors (molecular imprinting, aptamers) identify stability, specificity, multiplexing, and regulatory validation as success predictors, while also reaffirming LFA's outsized role under REASSURED for cost-sensitive deployment [40].

Table 1: Comparison of Point-of-Care Diagnostic Technologies

Technology	Target Analyte	Detection Principle
LAMP	DNA/RNA	Isothermal amplification
RPA	DNA/RNA	Recombinase-mediated isothermal amplification
CRISPR-Cas (Cas12/13)	DNA/RNA	Collateral cleavage–based signal amplification
Lateral Flow Assays (LFA)	Antigen / Nucleic acid	Immunochromatography / probe hybridization
Gold Nanoparticle Biosensors	DNA, protein, antigen	Optical/electrochemical signal enhancement
Magnetic Nanoparticle Sensors	DNA, protein	Magnetic enrichment and transduction
Graphene / CNT FET Sensors	Antigen, protein	Label-free electrical signal modulation
Paper Microfluidics	Antigen, metabolites	Capillary-driven microfluidics
Wearable / Microneedle Sensors	ISF biomarkers	Electrochemical / optical sensing
AI-IoMT Integrated Systems	Multi-modal data	Algorithmic pattern recognition

8. Conclusion and Future Perspectives

Rapid and POC molecular diagnostics are redefining infectious disease management through the integration of molecular precision, nanotechnology, and digital intelligence. The convergence of CRISPR-Cas systems, isothermal amplification, and smart biosensing materials has drastically shortened time-to-result while maintaining clinical-grade sensitivity. Emerging trends such as AI-assisted data analytics, IoMT-based connectivity, and wearable biosensors promise continuous monitoring and proactive outbreak surveillance. However, equitable deployment remains the greatest challenge—necessitating cost-effective manufacturing, global quality control

frameworks, and ethical policies that prioritize low-resource settings. Future research should emphasize multiplexed, self-calibrating systems, frugal innovations for extreme POC environments, and harmonized regulatory standards. Ultimately, the next generation of molecular diagnostics must bridge technology with accessibility to achieve universal diagnostic equity and strengthen pandemic preparedness.

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Author Profile

S Sowmya Priya received her Bachelor's degree in Microbiology, Biochemistry, and Chemistry and a Master's degree in Microbiology from Osmania University, Hyderabad, in 2009 and 2011, respectively. She began her academic career as a Lecturer at RR Institute of Allied Health Sciences, and subsequently joined Acharya Institute of Allied Health Sciences, Bengaluru, where she served as an Assistant Professor and is currently working as a professor, actively engaged in teaching, scholarly research, and institutional academic initiatives.

Pusthela Arun Raj received his B.Sc. and M.Sc. degrees in Biotechnology and Medical Anatomy from Sri Ramakrishna Degree College and Sri Venkateshwara Institute of Medical Sciences in 2006 and 2010, respectively. He began his academic career at KLE's Dental College, where he worked from 2011 to 2013. Subsequently, he served at Narayana Hrudayalaya, Bangalore, from 2013 to 2016. From 2016 to 2021, he was associated with Santhiram Medical College, Andhra Pradesh, contributing extensively to teaching and academic activities. Since 2022, he has been working as a Professor of Anatomy at the Acharya Institute of Allied Health Sciences, Bangalore, where he continues to engage in teaching, research, and academic development.

Vishnu M. G. received his Bachelor's and Master's degrees in Medical Laboratory Technology from Calicut University, Kerala, India. He is currently working as an Associate Professor at Santhi Institute of Allied Health Sciences, Kozhikode, Kerala with over 7 years of teaching experience. He is a PhD scholar at Meenakshi Academy of Higher Education and Research, Chennai.