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REVIEW

Research Progress on Point-of-Care Testing Technology for Mycoplasma Pneumonia

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Abstract: *Mycoplasma pneumoniae* (MP) is a significant respiratory pathogen in children, often causing refractory and severe pneumonia. Sensitive, rapid, and portable diagnostic tools are crucial for guiding clinical management. Although traditional methods like culture, ELISA, and PCR are widely used, they suffer from drawbacks such as lengthy turnaround times, complex procedures, or reliance on laboratory equipment. Consequently, *Point-of-care testing* (POCT) technologies, valued for their speed, portability, and ease of use, have emerged as a key research focus for MP diagnosis. This review systematically summarizes advancements in POCT platforms, covering: (1) immunological methods; (2) molecular biology methods; and (3) biosensor technologies. Their sensitivity, specificity, and clinical performance are comparatively analyzed. Colloidal gold immunochromatography delivers results within 15 minutes but exhibits limited sensitivity. Molecular methods like LAMP and CRISPR-coupled systems achieve single-copy detection limits via isothermal amplification and gene editing, with processing times under 1 hour. Biosensors enable high-sensitivity automated detection through integrated signal amplification and microfluidics. Despite these advantages, POCT development faces challenges including cost-sensitivity tradeoffs, standardization barriers, and sample matrix interference. Future directions encompass multi-modal detection, AI-assisted interpretation, multiplex pathogen screening, and dynamic drug resistance gene monitoring. These innovations will expand POCT device deployment in primary care and home settings. This will ultimately improve effectiveness in controlling respiratory infections.

Keywords: microbiology, microbiological detection, mycoplasma pneumonia, point-of-care testing, CRISPR-cas system

Introduction

Mycoplasma pneumoniae (MP) is a common cause of pediatric community-acquired pneumonia.[1] While capable of causing respiratory symptoms independently, its synergistic interactions with other pathogens can significantly complicate the disease course.[2] Recent research highlights mixed infections of MP with other pathogens as a key factor in disease aggravation. These coinfections increase treatment difficulty via immunomodulatory mechanisms. The synergy in coinfections speeds up lung lesion progression, leading to *refractory Mycoplasma pneumoniae pneumonia* (RMPP) and *severe Mycoplasma pneumoniae pneumonia* (SMPP).[3] Consequently, MP infections accompanied by other pathogens predispose patients to refractory RMPP and SMPP.[4] Consequently, MP infections accompanied by other pathogens—particularly common respiratory viruses such as adenovirus, influenza virus, and *respiratory syncytial virus* (RSV)—are strongly associated with progression to refractory RMPP and SMPP. The overlapping clinical presentations in these co-infections create diagnostic ambiguity, often delaying targeted therapy. This underscores the critical need for rapid and specific POCT, which can promptly identify MP at the bedside, guide timely antimicrobial intervention, and help mitigate the risk of severe disease progression.

Mycoplasma pneumoniae pneumonia (MPP) is a respiratory infection causing lung inflammation, characterized by a gradual onset of fever, headache, sore throat, and an initially dry cough that often progresses to a persistent, hacking or

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paroxysmal cough. Typical radiographic findings include bronchopneumonia, atelectasis, or nodular infiltrates on chest imaging.[5] Severe cases may have extrapulmonary complications and respiratory failure. Early diagnosis with PCR or serology is key for proper treatment. Macrolides are preferred for children, with tetracyclines or fluoroquinolones as alternatives for severe cases or high resistance. MP infections are hard to distinguish clinically due to overlapping symptoms and limited rapid testing. Delayed treatment can worsen lung damage, increase complications, and mortality, especially in immunocompromised or elderly patients.[6]

Point-of-care testing (POCT) is a diagnostic approach utilizing portable analytical instruments and corresponding reagents to rapidly obtain test results. This method offers key advantages: (1) Flexibility: It minimizes constraints on testing time and location while reducing reliance on specialized laboratory technicians, enabling on-site detection of target analytes; (2) Accessibility: Devices are compact, user-friendly, and cost-effective, making them adaptable to resource-limited settings such as emergency departments or remote clinics; (3) Efficiency: Results are delivered rapidly with minimal sample processing, maintaining high accuracy for critical decision-making.[7]

Various POCT platforms targeting MP employ diverse methodologies with distinct performance characteristics. Consequently, the advancement and adoption of POCT technology have transformative potential for clinical diagnostics, food safety inspections, and environmental monitoring.[8] This review summarizes recent POCT applications for MP detection, systematically detailing the working principles, performance strengths and limitations, and current implementation scenarios of each method.

Research Progress of Emerging POCT Technology

This section reviews the advancements in POCT platforms, covering immunological methods, molecular biology methods, and biosensor technologies. The structural and operational principles of five representative platforms—GICA, SAI, FIA, CRISPR-Cas systems, and biosensor-coupled technologies—are schematically compared in Figure 1.

POCT Technology Based on Immunological Methods

Immunological detection of MP uses antibody–antigen interactions. In ELISA, a secondary antibody with enzyme binds to the primary antibody on the target antigen, catalyzing a substrate reaction that changes color. The color intensity indicates the concentration of the antigen or antibodies, providing a rapid readout for MP identification.[9] These techniques are extensively adopted in clinical diagnostics for early screening and epidemiological research due to their operational simplicity and high throughput.

Colloidal Gold Immunochromatography Assay (GICA)

Colloidal gold immunochromatography assay (GICA) is a rapid detection technique leveraging antigen–antibody specificity and the unique optical properties of gold nanoparticles, which arise from localized surface plasmon resonance.[10] The approach uses gold nanoparticles to label antibodies. During testing, the sample moves on a membrane, dissolving the labeled antibodies. Target antigens bind to these antibodies, forming complexes that move to the test line and are captured, creating a red band. Unbound antibodies move to the control line, binding with specific antibodies to produce a color change, confirming the assay. Results can be assessed visually or via reflectance spectroscopy. The process takes 10–15 minutes. Sensitivity varies with factors like antibody affinity and gold stability, and detection thresholds differ for various analytes.

GICA employs paired monoclonal antibodies for MP detection: one conjugated to colloidal gold as the detection probe, and another immobilized on the test line as the capture antibody. When MP antigens bind to the gold-labeled antibody, the complex migrates chromatographically to the test line.[11] Capture by the immobilized antibody triggers gold nanoparticle accumulation, generating a visible red band that indicates potential pathogen presence.

GICA rapid testing is limited by low sensitivity, poor anti-interference, and lack of quantification, hindering its use in precision medicine. Future work should focus on overcoming these issues through new materials and signal amplification, and antibody engineering to enhance POCT technology's capabilities.

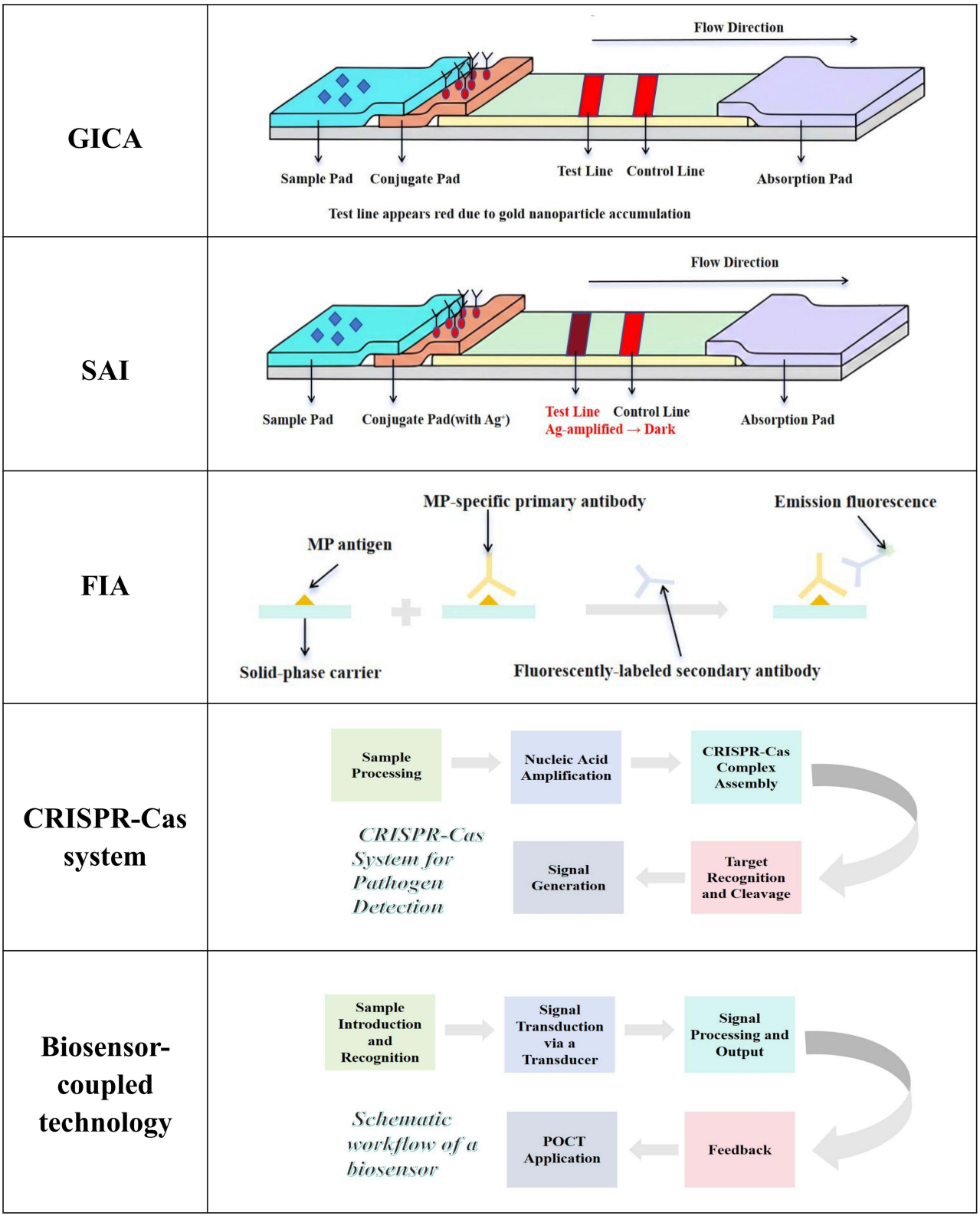

Figure 1 Comparison of Structural and Principe Diagrams of Five Common POCT Technologies. GICA: Based on the accumulation of gold nanoparticles for colorimetric detection, suitable for rapid qualitative testing. SAI: Introduces silver ions on the basis of colloidal gold to amplify the signal and improve sensitivity. FIA: Utilizes fluorescently labeled secondary antibodies for quantitative detection via fluorescence signal. CRISPR-Cas system: Combines nucleic acid amplification with the CRISPR system to achieve highly specific pathogen detection. Biosensor-coupled technology: Integrates recognition, transduction, and processing modules, suitable for real-time monitoring and multi-parameter detection.

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Silver Amplification Immunochromatography (SAI)

This technology employs antigen–antibody specificity and metallic silver amplification. During testing, MP antigens bind to antibodies on a carrier, forming a complex. Silver ions are then reduced to metallic silver, forming a dense silver shell that amplifies signal intensity, allowing antigen detection via color change or optical methods. Silver enhancement converts trace antigens into detectable signals, improving sensitivity in detecting MP in clinical samples.[12]

Silver amplification-based ICAs are highly sensitive and rapid for MP diagnosis, with improved detection limits. They show >90% sensitivity and >99% specificity in respiratory infections, with 94.1% agreement with PCR. They outperform serological tests and antigen assays by reducing turnaround time to 20 minutes, but their use in nosocomial pneumonia and stability with high volumes need further study. Overall, they could improve MP diagnostic pathways and support resistance management.[13]

Ishiguro et al assessed a silver amplification assay for MP detection in 170 pediatric patients' throat swabs, achieving 85.2% sensitivity, 99.1% specificity, and 94.1% accuracy in 20 minutes. The assay is valuable for clinical use within 5 days of infection but has lower sensitivity than PCR. It's a fast, simple diagnostic tool, yet PCR is superior for high-sensitivity needs.[14] This technology offers rapid, sensitive POCT through signal amplification, crucial for primary care and epidemic screening. However, PCR is still required for high-precision diagnostics due to its limitations. Future advancements may include CRISPR integration or multiplex test strip development.

Fluorescence Immunoassay (FIA)

Fluorescence immunoassay (FIA) uses fluorescent probes to detect targets with high sensitivity through antigen–antibody interactions. It offers specific, rapid, and matrix-resistant detection, suitable for clinical diagnostics and POCT. FIA is crucial for early disease diagnosis and biomarker screening in immunology research and diagnostics.[15]

Gao et al compared ELISA and IFA for diagnosing MP infection in 180 patients. They found ELISA and IFA positive rates at 15.6% and 10.0%, respectively, with a combined rate of 18.4%. Agreement between methods was low. Gender and age were significant factors for discrepancies. Combining assays is recommended to enhance sensitivity for high-risk groups.[16]

Cauwels et al evaluated a *fluorescent microsphere immunoassay* (FMIA) for detecting M. hyopneumoniae antibodies. FMIA demonstrated superior early sensitivity versus three commercial ELISAs. With adjusted cutoffs, specificity was comparable, supporting its dual utility for high-efficiency screening and confirmatory testing in swine herd health management.[17]

Fluorescence immunoassay technology has potential in human medicine for MP detection and rapid diagnosis. Optimizing fluorophore engineering and immunoassay protocols can enhance pathogen detection, supporting clinical decisions. This approach may improve access in healthcare settings and aid global MP control. Fluorescence immunochromatography is a rapid screening tool, but its accuracy varies and requires correlation with clinical manifestations and confirmatory methods. Future improvements may balance sensitivity and convenience.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA combines immunology with enzyme technology for sensitive detection using antigen–antibody recognition and enzyme amplification, with detection limits down to 0.01 to 0.1 ng/mL. It offers high sensitivity, specificity, and stable results, ideal for screening and diagnostics. It's essential in immunology, expanding applications in diagnosis and research.[18]

Yacoub created an ELISA for MP serodiagnosis, using affinity-purified rabbit antibodies pre-adsorbed against Mycoplasma and respiratory commensals. Optimizing buffers and blocking agents eliminated non-specific binding, achieving a 0.1 ng/mL detection limit and CV < 5%, distinguishing asymptomatic carriers from active infections.[19] The assay provides a robust serological tool for epidemiological surveillance and co-infection differential diagnosis.

Despite serving as a near-point-of-care technology for quantitative analysis, ELISA's clinical utility remains constrained by equipment dependency and prolonged turnaround times exceeding 4 hours. Future advancements require rapid test kits with nanomaterial-enhanced sensitivity, microfluidic chips integrating sample preprocessing and multiplex

detection, alongside AI-driven interpretation systems to enable portable, cost-effective applications in primary care and home settings.

POCT Technology Based on Nucleic Acid Testing

Nucleic acid detection technologies leverage the principle of base-pairing complementarity inherent in nucleic acid molecules. These techniques employ specifically designed probes or primers that efficiently hybridize with target sequences. This process, followed by signal amplification or sequence amplification, enables highly sensitive and specific detection of pathogens. Such technologies not only accurately identify pathogen species but also allow for the quantitative assessment of infection levels. Consequently, they hold significant value in various applications, including early pathogen diagnosis, genotyping, and drug resistance testing.[20]

Isothermal Amplification Technology

Loop-mediated isothermal amplification (LAMP) enables rapid nucleic acid amplification under constant temperature conditions by leveraging strand-displacing DNA polymerase.[21] LAMP, a rapid detection method without thermal cycling, was optimized for MP detection using primers designed from NCBI sequences. The P1 primer set showed superior performance at 65°C in 30 minutes, with fluorescence detectable within 5 minutes. Validation confirmed its 100% concordance with qPCR and a detection limit of 10 copies/reaction. The method is suitable for POCT and home-based testing due to its high specificity and rapid turnaround.

Recent research optimizes isothermal amplification POCT technologies for MP infections to enhance detection speed, sensitivity, and clinical utility. Meta-analysis confirms that *nucleic acid sequence-based amplification* (NASBA) achieves 77% sensitivity and 98% specificity, demonstrating high reliability though PCR replacement requires further validation.[22] Real-time synchronous amplification detection targeting ribosomal RNA exhibits significantly higher positive rates in pediatric pneumonia samples than IgM ELISA, with 0.97 kappa agreement versus real-time PCR and a detection limit of 10^1–10^7 CFU/mL, enabling early diagnosis.[23] Among rapid nucleic acid detection platforms, the Coyote series outperforms Ustar with 100% diagnostic accuracy and full-process time under 30 minutes.[24] Real-time fluorescence multiple cross displacement amplification combined with fluorescent primers achieves 43 fg/μL sensitivity without cross-reactivity, completing analysis within 1 hour ideal for resource-limited settings.

Portable detectors with heating modules are developed for field use. Primer design algorithms and reaction conditions are optimized for LAMP specificity and efficiency. These advances improve LAMP's application in *Mycoplasma pneumoniae* POCT, but validation and cost reduction are key for adoption.[25]

Real-Time Quantitative PCR Technology

Real-time quantitative PCR technology employs primers and fluorescent probes targeting specific *Mycoplasma pneumoniae* genes. During amplification, Taq DNA polymerase hydrolyzes probes via its 5'→3' exonuclease activity, releasing fluorescent signals; alternatively, intercalating dyes such as SYBR Green I bind double-stranded DNA to emit fluorescence. This enables simultaneous monitoring of fluorescence dynamics and quantitative analysis based on cycle threshold values, achieving high-sensitivity detection and precise pathogen nucleic acid quantification. The technique offers exceptional specificity, efficiency, and capacity for identifying drug-resistant mutations, with a total detection time of 60–90 minutes.[26]

A novel MIRA-qPCR technology was developed for rapid MP detection, reducing time to 40 minutes while maintaining sensitivity and specificity. It has a detection limit of 10 copies/reaction and no cross-reactivity. Clinical validation confirms its superior sensitivity and 100% concordance with commercial kits. This method offers rapid, precise operation without thermal cycling, providing an efficient POCT solution for MP infections, especially important post-pandemic.

MIRA-qPCR technology offers stable and reproducible MP detection with low inter-batch variation and high concordance rates. Its streamlined workflow suits primary healthcare facilities, broadening POCT access for respiratory[27] infections. The tech cuts costs and shortens processing time, potentially reducing healthcare costs. Traditional qPCR is hindered by equipment needs, inhibitor issues, primer cross-reactivity, quantification inconsistencies,

and high expenses. Future improvements with lyophilized reagents and microfluidic automation could bolster its utility in pandemic readiness and clinical care.

POCT Technology Based on Rapid Serological Testing

Rapid serological tests for MP detect antibodies binding to immobilized antigens on a carrier, using labeled secondary antibodies for signal amplification and visualization. These tests identify IgM or IgG antibodies within 15–30 minutes, suitable for early infection screening and POCT.[28]

Rapid assays detect MP-specific IgM in serum/plasma within 15 mins, indicating active infection 7 days post-infection. Initial negatives should be retested after 7 days to avoid false negatives. Limitations include early false negatives due to low IgM levels, false positives from rheumatoid factor or polyclonal IgM, semi-quantitative results, and reagent instability requiring controlled storage.[29] Future advancements should prioritize high-affinity monoclonal antibodies for specificity, quantum dot nanomaterials for sensitivity, microfluidic multiplexing for co-detection of respiratory pathogens, AI-driven titer quantification models, and lyophilized reagents for ambient-temperature stability to enable primary care and home testing.

POCT Technology Based on Biosensor Technology

Biosensor-based POCT uses electrochemical transduction for ultra-sensitive detection of pathogens, while CRISPR-Cas diagnostics offer rapid, highly accurate detection through Cas12a trans-cleavage activity. Innovations in Cas12a efficiency and thermal stability, along with microfluidic multiplexed detection, are advancing towards field-deployable platforms. Future technologies aim to combine CRISPR with electrochemical systems and AI for faster, more accurate diagnostics, supporting primary care and outbreak management.[30]

Electrochemical Biosensor Technology

Electrochemical biosensors detect MP by combining biorecognition elements with electrochemical signals. Probes on electrodes form a biosensing interface, and MP DNA hybridization alters charge distribution and electron transfer, producing electrochemical signals that indicate pathogen levels. Nanomaterials on electrodes increase sensitivity by expanding surface area and speeding up electron transfer. Signal amplification reduces detection limits, and standard curves convert signals to MP concentrations, enabling rapid, sensitive detection within 30 minutes using minimal equipment.[31]

Electrochemical biosensors offer high sensitivity, miniaturization, and cost efficiency in POCT. They combine enzyme-based and antibody-based platforms for precise quantification of metabolic markers in bodily fluids. Performance benchmarks include glucose sensitivity at 0.019 mmol/L, lactate linearity from 1 to 20 mmol/L, and uric acid detection at 0.33 μmol/L. Antibody-based platforms detect pathogens rapidly, with malaria biomarker PfHRP2 in 5 minutes and cardiac troponin I sensitivity at 10 pg/mL. Nanomaterials enhance signal stability and PSA sensitivity to 2.7 pg/mL. Despite challenges, interdisciplinary approaches enable chronic disease monitoring and pathogen screening. This technology is valuable for healthcare in resource-limited regions and has clinical potential.[27]

POCT Technology Assisted by the CRISPR-Cas System for Detection

The CRISPR-Cas system detects MP by leveraging sequence-specific CRISPR RNA to target conserved genomic regions of the pathogen. Upon Cas complex formation, the target nucleic acid is precisely recognized and cleaved. Concurrently, collateral cleavage activity is activated, degrading fluorophore-quencher-labeled reporter molecules and generating measurable signals. This cascade enables rapid, highly sensitive pathogen diagnosis.[32]

PaCD, a CRISPR-Cas12a-based platform, detects four respiratory pathogens in a multiplex system. It integrates amplification and CRISPR-Cas12a in one tube, enhancing specificity with optimized components. A 3D-printed device with heating and blue-light modules performs nucleic acid amplification and fluorescence detection in 30 minutes. Clinical validation shows high sensitivity and concordance with next-generation sequencing, with no cross-reactivity. The platform is compatible with various samples, easy to use, portable, and consistent, offering a rapid diagnostic solution for resource-limited settings.[33]

This study presents a CRISPR/Cas9-based biosensor for rapid POCT of MP, combining RPA and CRISPR/Cas9 in a two-stage process. RPA amplifies target genes at 25°C, producing biotin-labeled amplicons. The Cas9/sgRNA complex then recognizes targets, binding gold nanoparticle probes to generate visible red signals at the test line. The biosensor has a detection limit of three copies and a 100% accuracy rate in clinical validation with 123 samples. It operates at room temperature without the need for temperature control equipment, enabling acute respiratory infection screening in resource-limited settings. Future work includes the use of lyophilized reagents and process integration.[30]

Biosensor-Coupled Technology

The study created a POCT technology for MP detection, combining recombinase polymerase amplification with CRISPR-Cas12a to bypass the need for specialized equipment. It uses a dual-signal system for both real-time fluorescence kinetics and visual interpretation with immunochromatographic strips, achieving 100% concordance with quantitative PCR in testing 49 clinical samples. This technology provides a rapid, accurate, and user-friendly diagnostic platform suitable for resource-limited settings, with clinical translational potential.[34]

The study created a rapid MP detection system combining microfluidics and fiber optic sensing, avoiding complex equipment. A microfluidic chip with Y-shaped channels and dual-mode immunoagglutination probes was designed. These probes used carboxyl-modified polystyrene microspheres conjugated with antibodies, which aggregated upon target antigen binding. A fiber optic sensor captured real-time changes in scattering intensity, enabling dynamic monitoring of agglutination kinetics. The platform detected MP in 90 seconds with 50 pg/mL sensitivity, integrating sample pretreatment and signal detection, and eliminating centrifugation steps. The innovation is the in-situ fiber optic sensing module, establishing a label-free, high-throughput portable platform for pathogen POCT.[35]

Clinical Application and Evaluation of POCT Technology

A comprehensive comparison of the sensitivity, specificity, turnaround time, cost, and equipment requirements for the major POCT platforms discussed is presented in Table 1. Furthermore, their practical characteristics, including typical samples, key advantages, and major limitations, are detailed in Table 2. As shown in Table 1, molecular methods such as real-time qPCR and CRISPR-Cas systems offer very high sensitivity, whereas immunoassays like GICA prioritize rapid turnaround and low cost at the expense of lower sensitivity. Table 2 further elucidates that while techniques like LAMP and biosensor-coupled technologies are suitable for field use, they face challenges related to standardization and cost.

Table 1 Comparative Summary of Key POCT Technologies for MP Detection

Detection Technology	Target	Sensitivity	Specificity	Turnaround Time	Approx. Cost per Test	Equipment Requirement
GICA	Ag	Medium	Medium	Short	Low	None
SAI	Ag	Medium	High	Short	Low-Medium	Reader (opt.)
FIA	Ab/Ag	High	High	Medium	Medium-High	Fluorometer
ELISA	Ab/Ag	High	High	Long	Medium	Plate reader
LAMP	DNA	High	High	Medium	Medium	Heater
Real-time qPCR	DNA	Very High	High	Medium-Long	High	PCR system
Rapid IgM Test	IgM	Medium	Medium	Short	Low	None
CRISPR-Cas System Detection	DNA	Very High	Very High	Medium	High	Portable detector
Biosensor-coupled technology	DNA/Ag	Very High	High	Very Short-Medium	High	Integrated detector

Notes: Target: Ag, antigen; Ab, antibody; IgM, immunoglobulin M. Sensitivity/Specificity Ratings: "Low", "Medium", "High", and "Very High" are qualitative summaries based on a synthesis of peer-reviewed literature comparing these technologies for MP detection. Turnaround Time: Includes hands-on and incubation/amplification time. "Short" typically <30 min, "Medium" 30–120 min, "Long" >2 hours. Approximate Cost: Costs are estimates and vary by region, manufacturer, and scale. Equipment Requirement: "None" indicates results can be interpreted visually. Other entries specify the core device needed for test execution or readout.

Table 2 Practical Characteristics, Advantages, and Limitations of POCT Technologies for MP Detection

Detection Technology	Typical Sample	Key Advantages	Major Limitations
GICA	Nasopharyngeal swab, sputum	Rapid (<30 min), low-cost, user-friendly, no equipment needed, room-temperature stable	Lower sensitivity, qualitative/semi-quantitative only, prone to subjective interpretation.
SAI	Nasopharyngeal swab, sputum	Enhanced sensitivity over GICA, rapid, visual or reader-based quantitation.	Higher cost than GICA, silver enhancement step may add complexity.
FIA	Serum (for Ab), nasopharyngeal swab/ sputum (for Ag)	High sensitivity and specificity, potential for quantitative results, multiplexing capability.	Requires expensive fluorometer, higher cost per test, trained personnel often needed.
ELISA	Serum (primary for Ab), sputum (Ag)	High throughput, excellent sensitivity and specificity, quantitative, well-standardized.	Long turnaround time, laboratory-bound, requires plate washer and reader, skilled technician.
LAMP	Nasopharyngeal swab, sputum, BALF	Isothermal amplification, high sensitivity, rapid, suitable for field use with simple heater.	High risk of amplicon contamination, complex primer design, may lack standardized commercial kits.
Real-time qPCR	Nasopharyngeal swab, sputum, BALF	Gold standard sensitivity and specificity, quantitative, detects active infection.	Requires sophisticated thermal cycler and lab infrastructure, high cost, skilled personnel, longer time-to-result.
Rapid IgM Test	Serum, whole blood (fingerstick)	Rapid indication of current/recent infection, POCT suitable, low cost.	Cannot distinguish active from past infection, lower sensitivity in early infection, cross-reactivity possible.
CRISPR-Cas System Detection	Nasopharyngeal swab, sputum	Extremely high specificity, single-base discrimination, portable readout, rapid detection post-amplification.	Often requires pre-amplification step, complex assay design, high cost, limited commercial availability.
Biosensor-coupled technology	Nasopharyngeal swab, sputum, saliva	Ultra-rapid potential, label-free detection, miniaturizable for portability, continuous monitoring possible.	High development and unit cost, stability and reproducibility challenges in complex samples, early stage of commercialization.

Comparative Diagnostic Performance of Major POCT Platforms

The clinical implementation of rapid MPP diagnostics requires strategic selection aligned with disease progression dynamics, critically enabling early diagnosis, treatment monitoring, and antimicrobial resistance management. Immunological approaches demonstrate robust severe MPP prediction through clinical-cytokine-flow cytometry models, though limitations include potential sampling time bias, unvalidated biomarkers, and absent resistance gene analysis.[36] Real-time quantitative PCR offers superior early sensitivity in febrile-phase respiratory specimens but cannot distinguish active from past infections nor detect resistance genes, impacting precision therapy.[37] Serological testing shares these limitations while adding reliability concerns from single-sample versus paired-serum designs.[38] CRISPR-based diagnostics show exceptional specificity without cross-reactivity but exhibit marginally lower sensitivity than fluorescence methods, risking very low pathogen load misses, with large-scale stability unverified. Collectively, these findings advance optimization pathways for MPP immunological management, serodiagnosis, and precision treatment despite validation needs.[39]

Clinical Utility in Special Populations and Treatment Optimization

Rapid Mp diagnostics must account for population differences. Multimodal testing is crucial for accurate diagnosis in elderly and immunocompromised patients. Special protocols for pregnant women and infants must ensure biosafety and efficacy. Innovations in biosensors and nucleic acid technologies improve diagnostic precision.

POCT's accuracy and speed are vital for optimizing treatment decisions and evaluating efficacy. Monitoring pathogens and antibodies allows for personalized treatment adjustments, improving outcomes and reducing costs. Rapid resistance testing aids in early identification of resistant strains, supporting targeted prevention strategies and protecting public health.

Cost Structures and Context-Specific Feasibility

To address the essential link between POCT adoption and real-world implementation, we have incorporated a dedicated analysis of cost structures and context-specific feasibility. Technologies are stratified into low-, medium-, and high-cost tiers, reflecting inherent trade-offs between expense and performance. Feasibility is critically evaluated across settings: high-resource laboratories prioritize accuracy with advanced systems; primary care balances speed, cost, and ease of use; Beyond direct costs, operational variables such as sample type, turnaround time, and supply-chain resilience determine practical viability. Future development must therefore shift toward designing equitable, equipment-free, and environmentally robust assays to enable truly accessible point-of-care diagnostics.

Conclusion

As a transformative innovation overcoming traditional laboratory limitations, MP POCT significantly enhances early diagnosis and clinical management of respiratory infections through rapid operation, portability, and user-friendliness. This review systematically examines principles, performance, and clinical applications of three major POCT categories: immunological, molecular biological, and biosensor technologies. Immunological methods show that while colloidal gold immunochromatography enables 15-minute screening, its sensitivity and interference tolerance remain limited. Silver amplification technology elevates detection limits to trace levels via signal amplification, demonstrating high specificity and clinical utility. Fluorescence immunoassay balances sensitivity and convenience through quantum dot labeling with multimodal outputs. In molecular biology, microfluidics-integrated isothermal amplification achieves single-copy sensitivity within 30 minutes, ideal for primary care. The CRISPR-Cas system attains 100% clinical concordance through multiplexed gene editing and cascade amplification. Biosensor technology enables femtogram-level detection limits and automated integration via nanomaterial-modified electrochemical/optical transduction, advancing precision diagnostics.

Despite substantial progress in POCT technologies, clinical translation faces persistent challenges. These include balancing sensitivity with cost-effectiveness in immunological methods, overcoming standardization hurdles and complex sample inhibitor interference in molecular techniques, and addressing limited multiplexing capability in biosensors. Current technologies also inadequately differentiate active from past infections and lack integrated real-time resistance gene profiling. Future research should prioritize multimodal detection integrating host inflammatory markers with pathogen nucleic acid analysis, coupled with artificial intelligence-assisted interpretation to precisely identify infection stages and resistance phenotypes. Concurrent efforts must advance reagent lyophilization, device miniaturization, and process automation to lower technical barriers and costs, enabling widespread POCT adoption in primary care and home settings. Through interdisciplinary integration and multicenter validation, POCT could become an essential component of respiratory infection control systems, delivering efficient solutions for global public health security.

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Disclosure

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