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Review

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Emerging point-of-care technologies for bacterial pathogen detection

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Abstract: Bacterial infections remain a significant threat to public health worldwide, driving an urgent need for rapid, accurate, field-deployable diagnostic techniques. Point-of-care testing (POCT) has emerged as a transformative strategy, providing timely detection, operational simplicity and portability. Recent studies have aimed at enhancing sensitivity, specificity, multiplexing capability, and automation through the integration of molecular diagnostics with microfluidics and lab-on-chip technologies, alongside the development of low-cost, portable devices equipped with smartphone-based readout and cloud connectivity for real-time surveillance in resource-limited settings. Nonetheless, evidence-based frameworks for selecting optimal detection targets, such as genomic sequences, conserved protein epitopes, or viable whole cells, and matching them to appropriate POCT modalities remain notably underrepresented in the literature. This review systematically summarizes recent advances in POCT strategies for bacterial detection, categorized according to three major types of detection targets, including cellular phenotypic characteristics, surface antigens, and nucleic acids. We discuss the principles, advantages, limitations, and representative applications of key POCT platforms, which include microscopy-based visualization, immunoassays, isothermal amplification, clustered regularly interspaced short palindromic repeats-associated protein Cas (CRISPR-Cas) systems, and microfluidic biosensors. Critical challenges such as sample pretreatment, detection sensitivity and operational simplicity have been partially addressed through recent innovations. Finally, we outline the main future research directions focused on the development of integrated, automated and intelligent POCT systems for clinical deployment.

Key words: Point-of-care testing (POCT); Bacterial detection; Microfluidic; Immunoassay; Nucleic acid amplification; Clustered regularly interspaced short palindromic repeats-associated protein Cas (CRISPR-Cas) systems

1 Introduction

Pathogenic microorganisms represent a persistent and significant threat to global public health, causing severe clinical bacterial infections and widespread environmental contamination, resulting in substantial economic and social burdens (Murray et al., 2022; Walker et al., 2023; Lei et al., 2024). Thirty-one major pathogens are known to cause foodborne diseases (e.g., *Campylobacter*, non-typhoidal *Salmonella*, or

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pathogenic *Escherichia coli* (*E. coli*)), significantly contributing to global gastrointestinal illness (World Health Organization, 2024). Beyond foodborne pathogens, numerous highly pathogenic respiratory microorganisms, such as *Mycobacterium tuberculosis* (*M. tuberculosis*), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), spread rapidly through airborne droplets in densely populated environments. These pathogens pose a heightened risk to vulnerable populations, including children and older adults with weakened immune function, often leading to severe clinical manifestations and fatal outcomes. According to global health data, approximately 1.23 million people died from tuberculosis in 2024 (World Health Organization, 2025). What is more, since 2019, SARS-CoV-2 has resulted in more than 777 million confirmed cases and approximately 7 million deaths globally (World Health Organization, 2025). The propensity of these diseases to trigger clustered outbreaks within a short period highlights the critical importance of rapid pathogen identification for timely intervention and effective containment. Polymerase chain reaction (PCR) has high specificity and sensitivity, thus is widely regarded as the gold standard for pathogen detection in clinical diagnostics (Kubiak et al., 2023). However, its application in primary healthcare settings and remote regions is often hindered by the lack of standardized laboratory equipment, such as biochemical incubators and analyzers, as well as a shortage of trained personnel (Abayasekara et al., 2017). Consequently, when PCR is required to confirm the pathogen-induced diseases in these settings, the total turnaround time from sample collection to result delivery can extend to 4-5 days (Gracias and McKillip, 2004; Santana, 2025). Such delays raise the risk of disease progression in patients and hinder public health efforts to control pathogen transmission. To address these challenges, a series of technological platforms have been developed that enable the rapid, accurate, cost-effective, and facile on-site detection of pathogenic microorganisms (Zhao et al., 2023). These advances demonstrate substantial potential for practical application in improving clinical decision-making and enhancing population-level health security.

Point-of-care testing (POCT) facilitates the rapid, on-site detection of target analytes using portable devices and simple reagents outside conventional laboratory settings, attracting growing attention in biomedicine, food safety and environmental monitoring (Dabla and Dabas, 2025) (Fig. 1). POCT relies on high-sensitivity and specific recognition technologies including visual imaging (Wang et al., 2020), immunoassays (Song et al., 2020), and nucleic acid analyses (Li et al., 2022). These approaches typically integrate portable analytical devices, including handheld microscopes (Nguyen et al., 2025), paper-based chips (Rink and Baeumner, 2023), and microfluidic chips (Jung et al., 2015). They also frequently employ mobile phones as all-in-one readout systems to deliver diagnostics functionality independent of centralized laboratory infrastructure (Peng et al., 2024). As a result, POCT can be operated with minimal training by healthcare or frontline personnel, making it well-suited for use in community health centers, outbreak sites, and even remote or resource-limited settings. Notably, POCT reduces detection times to minutes or a few hours, significantly enhancing diagnostic efficiency and enabling earlier diagnosis, the timely treatment of bacterial infections, and the rapid tracing of environmental contamination sources (Lu et al., 2025; Chen et al., 2025). A recent study of a POCT platform for monkeypox virus reported 94.1% sensitivity and specificity, revealing its utility in outbreak response for emerging infectious diseases (Cavuto et al., 2025). The key advantages of POCT, including simple operation, short turnaround time, low cost, intuitive readouts, and high portability, align with the WHO's ASSURED criteria (affordable, sensitive, specific, user-friendly, rapid, equipment-free, and delivered). These developments highlight the essential role of POCT in modern healthcare and public health systems, particularly in areas with limited medical resources and underserved populations (Wang et al., 2023; Liu et al., 2025).

In recent years, advances at the intersection of nanotechnology, molecular biology and information technology have significantly enhanced the sensitivity, specificity, multiplexing capability, miniaturization, and automation of POCT systems. These analytical platforms are now widely employed in clinical diagnostics for a range of diseases, including cancer (Hollaender et al., 2020), diabetes (Kristensen et al., 2020), and bacterial infections (Lin et al., 2023; Pang et al., 2024; Rong et al., 2025; Huang et al., 2025). Various nanoparticles enable diverse signal transduction and readout mechanisms in POCT, owing to their distinct optical, electrical and magnetic properties (Thongmee et al., 2024). Clustered regularly interspaced short palindromic

repeats-associated protein Cas (CRISPR-Cas) systems, as a revolutionary genome editing technology, have not only reshaped genetic engineering but also spurred innovations in bioanalysis, leading to the creation of novel molecular detection platforms (Gu et al., 2024). Concurrently, the identification of new molecular detection targets has laid the groundwork for enhanced analytical performance and functional versatility. For instance, resistance genes such as vanA/vanB in vancomycin-resistant *Enterococcus* are now routinely used as detection targets. When coupled with nuclease-mediated signal amplification strategies, these genetic markers allow the rapid identification of resistant strains without time-consuming culture or conventional antimicrobial susceptibility testing, thereby supporting more precise and timely antibiotic stewardship (Mori et al., 2025). Similar approaches have been extended to other clinically relevant resistance and virulence determinants, enabling syndromic testing and multiplex POCT panels in comprehensive clinical diagnostics (Chen et al., 2023). In this review, we systematically summarize recent advances in POCT for bacterial detection, focusing on three major detection targets, including bacterial phenotypic characteristics, surface antigens, and nucleic acids. We also examine the key enabling technologies that support their detections, such as isothermal amplification, clustered regularly interspaced short palindromic repeats-associated protein Cas (CRISPR-Cas) systems, microfluidic platforms, and various biosensors. The underlying principles, analytical performance, operational advantages, and practical applications of these approaches are discussed, along with current limitations in standardization, quality control, quantitative accuracy, and integration with data management systems. Finally, we address the prevailing and implementation barriers, as well as emerging trends such as fully integrated sample-to-answer systems, multiplex and syndromic testing panels, digital connectivity, and scalable deployment in resource-limited regions.

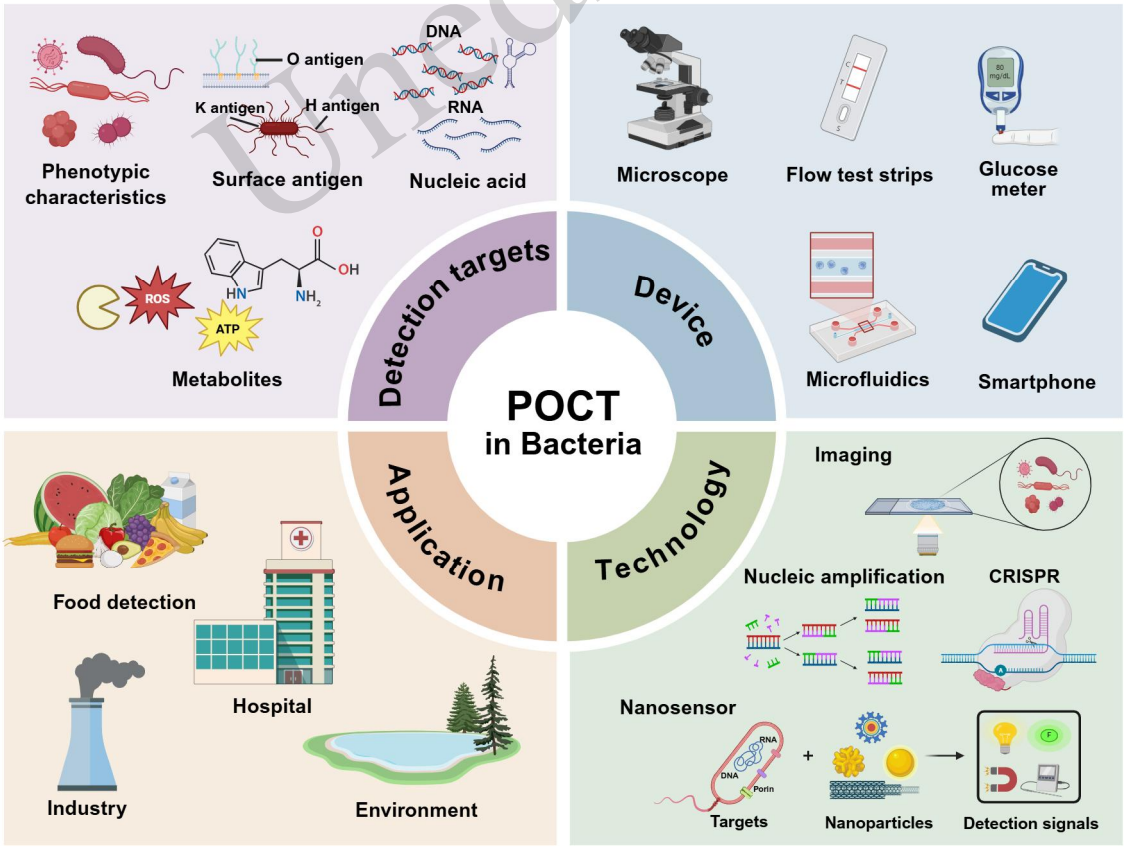

Fig. 1 Overview of point-of-care testing (POCT) in bacteria. Created with BioRender.com.

2 Detection targets for POCT in bacterial pathogens

Bacterial detection relies on the identification of distinctive biological features or molecular markers for qualitative and quantitative analysis. The most widely used detection targets can be classified into three primary categories: cellular phenotypic characteristics, surface antigens, and nucleic acids (Qureshi and Niazi, 2020; Zasada, 2020; Antunes and Ferreira, 2023). Additionally, bacterial metabolites and enzymes are increasingly explored as auxiliary or complementary targets involved in novel detection strategies (Chen et al., 2022; Wang et al., 2024; Li et al., 2024). Ideal detection targets should exhibit facile recognizability, good stability and high specificity (Table 1). Therefore, the selection of appropriate targets is critical for developing POCT platforms that are simultaneously sensitive, specific, rapid, and suitable for point-of-care use.

2.1 Bacterial phenotype

Different bacterial species exhibit distinct morphological features in terms of shape, size, arrangement, and staining properties. These characteristics allow preliminary identification through microscopic observation or physical-property-based methods (e.g., size and surface charge). For example, *Staphylococcus aureus* (*S. aureus*) is spherical and typically arranged in grape-like clusters (Santana, 2025); *Streptococcus pneumoniae* (*S. pneumoniae*) appears as diplococci (Grangeasse, 2016); *M. tuberculosis* is a slender and slightly curved bacillus characterized by acid-fast-positive rods (Alva et al., 2013). Although morphology provides a classical and rapid means for preliminary screening in some POCT formats (e.g., microscopy-integrated devices), its low specificity and reliance on bacterial viability limit its standalone use. Thus, phenotypic detection is often combined with other molecular methods to improve accuracy.

2.2 Surface antigens

Bacterial surface biomacromolecules serve as critical biomarkers for the capture and identification of pathogens, including peptidoglycan in Gram-positive bacteria (Han et al., 2020; Zhang et al., 2024), capsular polysaccharide in *S. aureus* (Østerlid et al., 2025), and the capsular antigen of *Klebsiella pneumoniae* (*K. pneumoniae*) (Ravenscroft et al., 2025). These surface antigens can be specifically recognized by antibodies, ligands, or aptamer, thereby providing a highly specific molecular basis for immunodetection. Surface antigen-based detection technologies enable the direct detection of intact bacterial cells without lysis, making them among the most widely adopted approaches in POCT, such as lateral flow immunochromatographic assays (Huang et al., 2025) and immunosensors (Bai et al., 2022). However, they may suffer from cross-reactivity with related species and reduced sensitivity in the presence of antigenic variation or low bacterial loads.

2.3 Nucleic acids

Nucleic acid-based detection targets species-specific genetic sequences, offering superior stability and specificity compared to antigen-based approaches (Phuong et al., 2025). Amplification techniques such as classic PCR, loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and CRISPR-Cas systems exhibit high sensitivity, even at low bacterial concentrations (Ahmadi et al., 2025; Baig et al., 2025). Furthermore, nucleic acid testing allows for strain typing, virulence-gene detection and the identification of drug-resistance markers, supporting precision treatment and epidemiological surveillance (Silva et al., 2025; Azizian et al., 2025). For POCT, challenges include the need for sample preprocessing, inhibitor resistance, and simplified amplification-readout integration. Recent advances in microfluidics and paper-based nucleic-acid tests can address these limitations.

2.4 Other detection targets: metabolites and enzymes

Bacterial-specific metabolites (e.g., hydrogen peroxide, organic acids) and enzymes (e.g., catalase, β-lactamase) can serve as auxiliary detection targets of viable bacteria (Yu et al., 2023; Chen et al., 2025). For instance, catalase, an enzyme commonly found in aerobic bacteria, can be functionally assessed through its

activity to enable the rapid screening of viable cells (Zeng et al., 2025). Similarly, acidic metabolites generated during bacterial glucose metabolism can induce colorimetric changes in pH indicators, allowing for both qualitative and quantitative bacterial analysis (Zhou et al., 2024). Furthermore, leveraging the catalytic decomposition of H_2O_2 by catalase-positive bacteria, researchers developed a platinum nanoparticle-based electrochemical sensor for rapid phenotypic antibiotic susceptibility testing (AST). This sensor exhibits a sensitivity of approximately 382.2 $\mu A \cdot cm^{-2} \cdot mM^{-1}$ toward H_2O_2, achieving detection time within 60 min for *E. coli* and 45 min for *S. aureus* (Li et al., 2025). In a complementary approach, Ding et al. created an AI-assisted point-of-care detection system targeting β-lactamase activity, which responds within 20 seconds and has a detection limit of 0.13 nmol/L. By integrating a broad-spectrum fluorescent probe, a 3D microfluidic paper-based device, and a smartphone-AI cloud platform, the system enables the ultrafast, quantitative identification of diverse drug-resistant bacteria, including *S. aureus* and *E. coli* (Ding et al., 2024). These metabolic targets are closely linked to bacterial viability, rendering them valuable for the detection of live bacteria and conducting antimicrobial susceptibility testing. However, due to their relatively low specificity, they are typically used in combination with other biomarkers in multiplex or sequential detection schemes to improve overall accuracy (Ding et al., 2024; Zhou et al., 2024).

The choice of detection target depends heavily on the specific application context, balancing the need for speed, specificity, sensitivity, and ease of use. For instance, food safety monitoring prioritizes the rapid screening of viable bacteria, making enzyme- or metabolism-based methods particularly advantageous. In clinical diagnostics, high specificity for pathogenic species and strain-level discrimination are often required, which is typically best met by selecting nucleic acid-based targets. Outbreak investigations and field testing may prioritize portability and rapid turnaround time, favoring antigen-detection strips or simplified nucleic acid amplification platforms.

Next-generation POCT for bacterial pathogens increasingly rely on multiplex strategies integrating complementary detection targets (e.g., genomic sequences and surface antigens) to simultaneously improve diagnostic accuracy, expand pathogen coverage, and mitigate target variability (Golichenari et al., 2019; Vincenzo et al., 2025). Concurrent advances in microfluidic automation, smartphone-based readout systems, and the AI-powered interpretation of assay signals will collectively reduce hands-on time, minimize user error, and accelerate time-to-result (Tseng et al., 2023; Chen et al., 2025). Critically, the design of an optimal POCT platform must be driven not by technological capability alone, but rather by a clinically or epidemiologically grounded target selection strategy.

3 Bacterial morphology-based POCT

The rapid identification and quantification of target bacteria rely primarily on bacterial phenotypic characteristics, such as cell shape (Alva et al., 2013), size (Zhou et al., 2025), arrangement (Caccamo and Brun, 2018), and staining properties (Wang et al., 2024). These bacterial morphology-based analytical methods may represent one of the earliest forms of POCT, characterized by simplicity, low cost, and intuitive results. As a result, they are widely used in primary healthcare settings, resource-limited areas, and on-site rapid screening applications (Tavakoli et al., 2022). Herein, POCT based on bacterial morphology is classified into three types depending on the need for staining/labeling and the integration of other technologies, including microscope-based visualization (Fig. 2a), staining and chromogenic techniques (Fig. 2b), and microfluidic-integrated morphological analysis systems (Fig. 2c).

3.1 Direct imaging-based pathogen detection

Since Leeuwenhoek's pioneering observation using his homemade microscope, microscopy has remained a cornerstone of bacterial analysis, since its direct visualization of samples with minimal preparation offers an intuitive and rapid means of assessment (Teng et al., 2020). It is routinely employed in the evaluation of urinary tract or vaginal samples (Yang et al., 2021). Despite their utility, conventional microscopes can only identify bacterial morphological characteristics and relative abundance, and are unable to reliably distinguish between

bacterial subtypes (e.g., pathogenic versus non-pathogenic *E. coli*). Moreover, microscopic imaging requires a high bacterial concentration, often necessitating the preculture and amplification of bacteria, significantly delaying diagnosis and increasing the risk of false-negative results in low-abundance infections (Zasada, 2020). The convergence of recent technologies is overcoming these constraints (Shao et al., 2024). For instance, advances in wearable devices, holographic imaging and 3D surface reconstruction have enabled the development of a portable holographic microscope integrated into contact lenses. This platform allows for the direct visualization and quantification of *S. aureus* with a detection limit of (LOD) approximately 16 bacteria/μL (Veli and Ozcan, 2018). Simultaneously, the integration of smartphones with 3D-printed optical attachments has established a promising foundation for POCT. However, the most significant recent evolution in these systems is the integration of deep learning algorithms (Molani et al., 2024). Convolutional neural networks (CNNs) are now trained not only to acquire bacterial images but also to automate critical steps, including background suppression, cell segmentation and species classification. Furthermore, they can perform viability assessment based on morphological characteristics and behavior (Quintana et al., 2025). This enables the direct quantification of *E. coli* and *S. aureus* from complex biological matrices with high specificity (Cao et al., 2024).

Table 1 POCT methods for pathogenic bacteria detection

Detection Target		Method	Specificity & Sensitivity	Speed	Sample	Reference
Bacterial phenotype	Shape	Microfluidic, Imaging	*S. aureus*, *E. coli*, 500 CFU/mL	7 s	Vaginitis	Yang et al., 2021
	Arrangement	Imaging	Bacteria, dividing, cluster, 0.89 - 0.91 mAP	NA	Soil	Quintana et al., 2025
Surface Antigen	*S. aureus* cells	LFIA	*S. aureus*, 4 CFU/mL	NA	Urine	Liu et al., 2024
	Peptidoglycan layer	Immuno-sensors	Gram-positive bacteria, 42 CFU/mL	20-80 min	Urine	Han et al., 2020
	PBPs	Enzyme-catalyzed sensor	*L. monocytogenes*, 10 CFU/μL	7 s	Food	Bai et al., 2022
Nucleic Acid	*Khe*, *Sp2020*, *cop. B*	RPA；ELISA	*K. pneumoniae*, *S. pneumoniae*, *M. catarrhalis*, 100%	1.15-1.5 h	Respiratory	Azizian et al., 2025
	SE2313	LAMP；Microfluidic	*S. epidermidis*, 10 copies/μL	45 min	Aqueous humor	Zhang et al., 2019
	ITS	RPA；CRISPR-Cas	*Cryptococcus*, 10^2 copies/μL	50 min	Cerebrospinal fluid	Liu et al., 2024
Others	Lipids	Colorimetric	*S. aureus*, *P. aeruginosa*, 10^4 CFU/mL	4 h	Laboratory	Zhou et al., 2024
	Catalase	Staining	*S. aureus*, 72 CFU/mL	90 min	Food	Zeng et al., 2025
	β-lactamase	AI; Microfluidic	*S. aureus*, *E. coli*, *K. pneumoniae*, *E. cloacae* 0.13 nmol/L	20 s	Peritonitis, skin wound infection	Ding et al., 2024
	Metabolism-mediated acidification	Colorimetric	*S. aureus*, *E. coli*, *L. monocytogenes et al.,* 10^3 - 10^7 CFU/mL	NA	Food	Zhou et al., 2024

Abbrevatinos: *S. aureus*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; LFIA, lateral flow immunoassays; PBPs, penicillin binding proteins; RPA, recombinase polymerase amplification; ELISA, Enzyme-Linked Immunosorbent Assay; LAMP, loop-mediated isothermal amplification; ITS, internal transcribed spacer; CRISPR-Cas, clustered regularly interspaced short palindromic repeats-associated protein Cas; *L. monocytogenes*, *Listeria monocytogenes*; *K. pneumoniae*, *Klebsiella pneumoniae*; *S. pneumoniae*, *Streptococcus pneumoniae*; *M. catarrhalis*, *Moraxella catarrhalis*; *S. epidermidis*, *Staphylococcus epidermidis*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *E. cloacae*, *Enterobacter cloacae*.

3.2 Staining-based pathogen identification

Staining and colorimetric detection technologies, which employ specific dyes to enhance the contrast of bacterial cells, play a fundamental role in microbiology and POCT (Hwang et al., 2025). Classical methods like Gram staining, which exploits differences in cell wall structure to classify bacteria as Gram-negative or Gram-positive, remains a preliminary method of bacterial identification (Klimko et al., 2021). To align these principles with POCT, recent innovations have focused on simplifying staining reagents and workflows (Wang et al., 2020). This includes pre-encapsulating staining agents within test strips or microfluidic chips, enabling the automated mixing and reaction of samples and dyes without the need for specialized technical expertise. The evolution of this field is evident in the development of novel colorimetric and fluorometric assays. For example, Zeng et al. developed a dual-mode detection system using starch-KI test strips, where endogenous catalase from *S. aureus* triggers a visible color reaction, allowing for rapid visual assessment and quantitative colorimetry with a LOD of 72 CFU/mL (Zeng et al., 2025). Busra et al. engineered a phage-gated sensor by encapsulating sulforhodamine B in mesoporous silica particles. Upon exposure to *E. coli*, phage detachment releases the dye, generating a fluorescent signal within 5 minutes (sensitivity of 10^1 CFU/mL) using a simple LED lamp (Aslan et al., 2025). Further integrating biochemical sensing with portable hardware, Yin et al. constructed a smartphone-based fluorescent platform by synthesizing three distinct fluorescence probes that cause *S. aureus*, *E. coli* and *Pseudomonas aeruginosa* (*P. aeruginosa)* to emit unique fluorescence signatures. Using molecularly imprinted materials, the system achieves simultaneous bacterial separation and detection with a sensitivity of 10^2 CFU/mL within 40 minutes (Yin et al., 2023). Zhang et al. developed a whole-cell imprinted microarray platform based on three-dimensional photonic microspheres, which specifically capture and quantify pathogens via fluorescence intensity at a remarkably low detection threshold (1-20 CFU/mL). This method operates without the need for antibodies, aptamers or pre-enrichment culture, establishing a powerful tool for high-throughput, rapid screening of foodborne pathogens (Zhang et al., 2025). Taken together, these advances represent a significant transition from subjective and manual operational procedures towards a new generation of automated, high-sensitivity, field-deployable diagnostic systems.

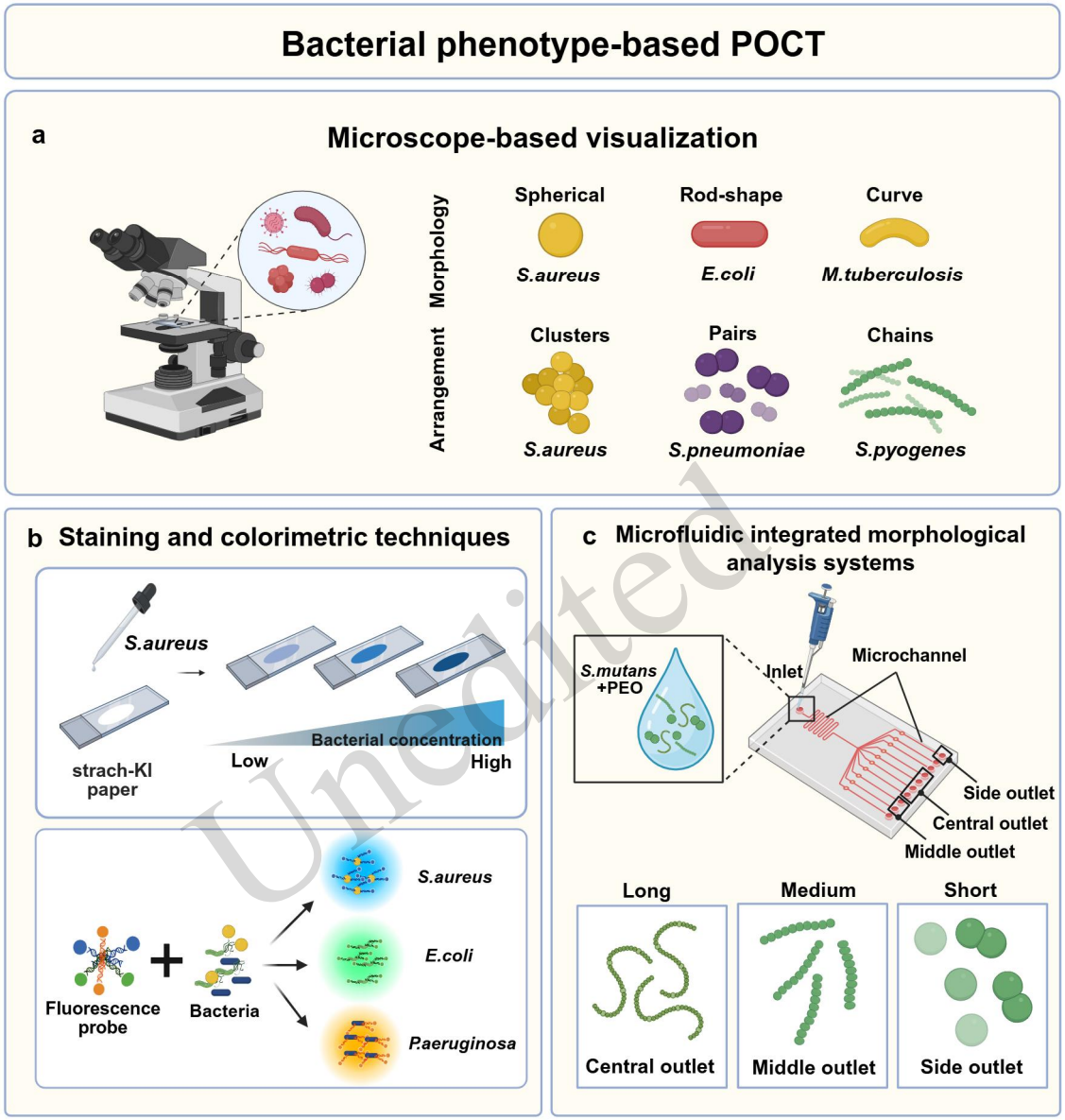

Fig. 2 Bacterial morphology-based point-of-care testing (POCT) in bacteria. (a) Identification via inherent bacterial morphology and arrangement. (b) Detection enabled by staining and chromogenic reactions. (c) Platform incorporating microfluidics technology for automated sorting analysis. Created with BioRender.com. Abbrevatinos: *S. aureus*, *Staphylococcus aureus*; *E. coli*, *Escherichia coli*; *M. tuberculosis*, *Mycobacterium tuberculosis*; *S. pneumoniae*, *Streptococcus pneumoniae*; *S. pyogenes*, *Streptococcus pyogenes*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *S. mutans*, *Streptococcus mutans*; PEO, polyethylene oxide.

3.3 Microfluidics strategies for morphology-based analysis

Microfluidics-based morphological analysis represents a transformative approach in POCT, integrating sample pretreatment, bacterial separation and microscopic observation into a single and automated platform (Wang et al., 2018; Guo et al., 2023). This synergy significantly enhances detection efficiency, minimizes user intervention and improves analytical accuracy, positioning it as an ideal solution for rapid on-site diagnostics (Khan et al., 2023). The core of this technology lies in its microchannel architecture, which enables the precise manipulation of fluids for automated transport, mixing and separation, thereby reducing reagent volumes and

operational complexity (Khalaf et al., 2023; Yin et al., 2024). When microfluidics technology is integrated with imaging systems and sensors, it enables fully automated detection workflows. Lee et al. developed a "count-on-a-cartridge" (COC) platform that combines a magnetic concentrator with a fluorescence image reader and a built-in counting algorithm, which achieves the quantitative detection of foodborne *S. aureus* with 92.9% sensitivity and 100% specificity within a few hours (Lee et al., 2020). A self-powered sensor functionalized with vancomycin-coated fluorescent microbeads monitors the Brownian motion of resulting bacteria-based complexes. This strategy allows for the label-free, real-time tracking of bacterial growth and antibiotic susceptibility, achieving results in as little as two hours (Wang et al., 2018). For ultimate portability, a handheld zoom microscopic imaging system has been coupled with microfluidics for vaginitis diagnosis. This device incorporates a 37× magnification optical system and a smartphone-based imaging module, where a dedicated application automatically identifies coccus morphology and assesses inflammation levels with 95% accuracy in just seven seconds, achieving a LOD of 500 CFU/mL (Yang et al., 2021).

Morphology-based POCT technologies advance rapidly towards higher sensitivity, greater integration, and automated, intelligent data interpretation (Liu et al., 2025). Their ability to provide rapid answers without complex lysis or amplification step demonstrates their unique advantage in field-deployed applications.

4 Surface antigen-based POCT

Attributed to the high specificity and accuracy of immunorecognition, surface antigen-based POCT has emerged as pivotal tools for the rapid, on-site screening of pathogenic bacteria (Pasquardini et al., 2023; Wang et al., 2024; Ji et al., 2025). This technology mainly relies on specific capture probes, such as antibodies, antigen-binding fragments and aptamers, which can specifically recognize and bind to bacterial surface antigens (Fig. 3a), and then analyze bacteria qualitatively and quantitatively by combining signal conversion and amplification technologies (Su et al., 2023; W. Zhao et al., 2025). Typical antigen-based POCT includes the lateral flow assay (LFA) (Fig. 3b), immunosensor (Fig. 3c) and microfluidic immunoassay (Ma et al., 2024; Han et al., 2025; Wang et al., 2025). These technologies are driving the evolution of POCT from rudimentary qualitative screening toward precise, quantitative and fully integrated "sample-to-answer" systems, offering robust solutions for clinical diagnostics and food safety (Mi et al., 2023; B. Hong et al., 2024; Priyadarshi et al., 2025).

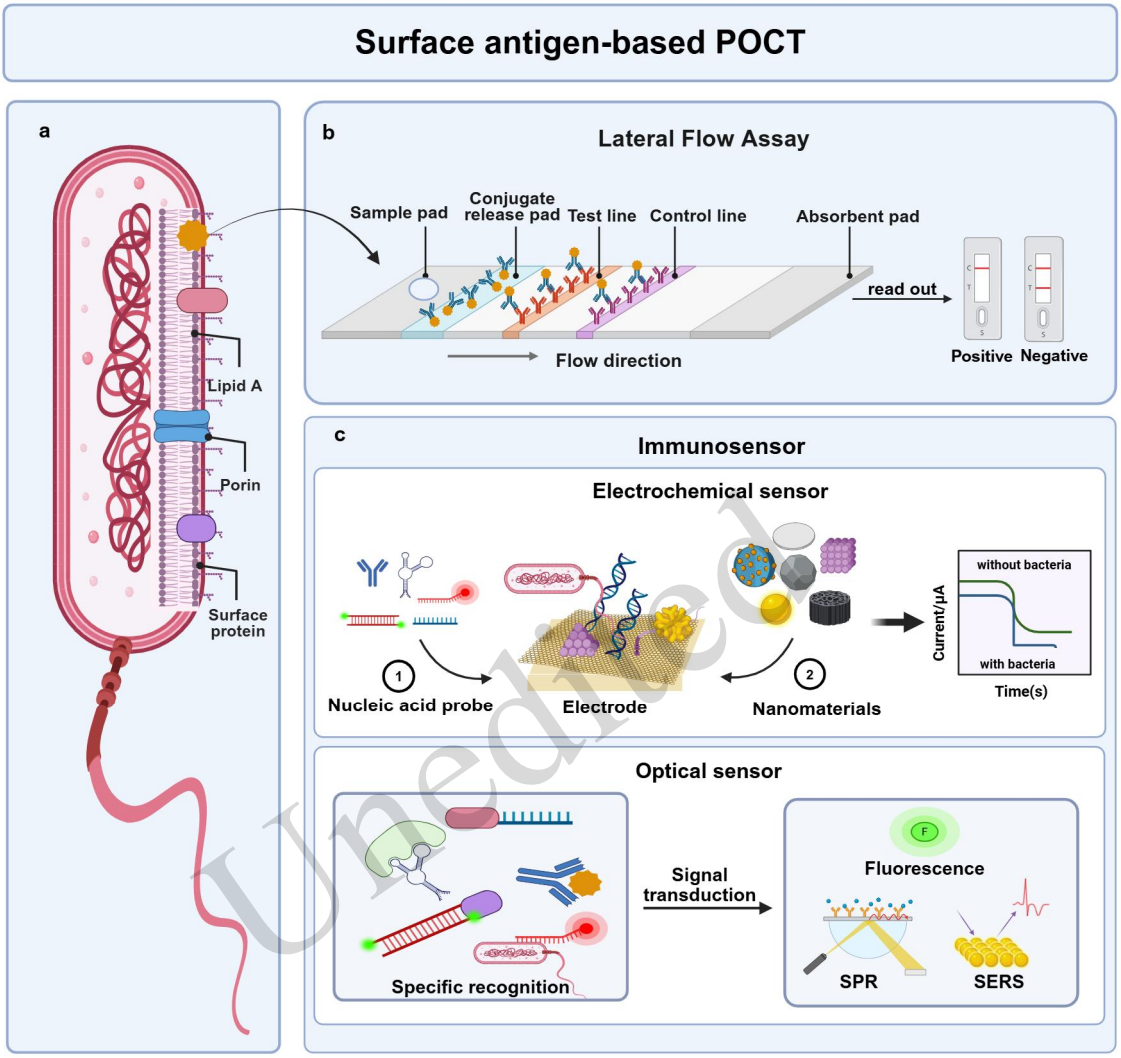

Fig. 3 Surface antigen-based point-of-care testing (POCT) in bacteria. a. Representative antigens located on the bacterial surface. b. Schematic of a lateral flow immunoassay platform. c. Overview of immunosensor technology, categorized into electrochemical sensor (based on changes in electrical signals upon antigen binding) and optical sensor (e.g., fluorescence or SERS). Created with BioRender.com. Abbrevatinos: SPR, surface plasmon resonance; SERS, surface-enhanced Raman scattering.

4.1 Lateral flow immunoassays (LFIAs)

LFIAs utilize paper-based matrices and capillary action to facilitate equipment-free detection with visual readouts (Du et al., 2025; Ran et al., 2025). The core principle involves the immobilization of antibodies on a nitrocellulose membrane to capture target antigens as the sample migrates, along with the generation of a detection signal by labeled reporters such as colloidal gold, fluorescent microspheres, or nanozymes (Fig. 3b). Conventional colloidal gold-based LFIAs constitute a foundational POCT platform, as evidenced by their widespread commercial success in pregnancy testing and SARS-CoV-2 antigen detection (Jin et al., 2025). However, the intrinsic optical signal amplification capacity of colloidal gold nanoparticles is limited. Each antigen-antibody binding event generates only modest absorbance, resulting in low signal-to-noise ratios. Consequently, when target bacterial concentrations fall below the nanogram-per-milliliter (or even picogram-per-milliliter) threshold, the assay signal is obscured by nonspecific background interference, severely compromising analytical sensitivity and quantitative reliability (Mandal et al., 2022; Miyamura et al.,

2023). To address this problem, advanced signal amplification strategies employing novel labels have been developed. For example, Liu et al. constructed a "visual-colorimetric-photothermal" three-in-one multimodal LFIA platform based on palladium/platinum nanoparticles (Pd/Pt NPs), which enables the visual detection of *S. aureus* by utilizing the sandwich capture mechanism. Moreover, relying on the photothermal properties of Pd/Pt NPs to catalyze 3,3',5,5'-tetramethylbenzidine (TMB) into oxidized TMB (oxTMB), an ultra-sensitive quantitative analysis with a LOD as low as 4 CFU/mL was achieved (Liu et al., 2024). Huang et al. developed a label-free colorimetric/photothermal dual-modal lateral flow biosensor based on tannic acid-tantalum ditelluride (TA-$TaTe_2$)@lysozyme nanocomposites, which realizes the antibody-free label detection of *S. aureus* through combining with the specific recognition of bacterial cell walls by lysozyme. Its photothermal LOD shows a 68-fold improvement in sensitivity compared with the colorimetric detection (Huang et al., 2025). These studies demonstrate a pivotal shift in LFIA from simple qualitative screening toward highly sensitive, multiparameter quantitative analysis. Apparently, the integration of sophisticated nanomaterials and multimodal detection paradigms is significantly expanding the frontiers of POCT.

4.2 Immunosensors

Immunosensors require the immobilization of specific biorecognition elements on solid interfaces (e.g., electrodes, resonators) and the transduction of binding events into measurable physical signals (Jiang et al., 2023; Li et al., 2024; Rong et al., 2024; Sankar et al., 2024). Based on the transduction mechanism involved, immunosensors can be categorized into three types (Fig. 3c). The first include electrochemical immunosensors, which monitor changes in current, potential, or impedance, offering exceptional compatibility with miniaturization and portable systems. The second encompass optical immunosensors, which rely on surface plasmon resonance (SPR), fluorescence, and surface-enhanced Raman scattering (SERS) and provide high sensitivity and multiplexed capacity. The third are mass-sensitive sensors, such as quartz crystal microbalance (QCM), which detect mass changes through resonant frequency shifts. An enzyme-free electrochemical biosensor using peptidoglycan-functionalized Pt-Ni-Cu nanocubes achieved a wide linear range (1.5×10^2-1.5×10^8 CFU/mL) and 90.4-107% recovery rates for Gram-positive bacteria in urine (Han et al., 2020). Bai et al. (2022) developed a cascade reaction signal amplification strategy using alkaline phosphatase (ALP) and glucose oxidase (GOD) in a robust sandwich immunoassay that repurposes commercial blood glucose meters (BGMs) to detect *Listeria monocytogenes* with 10 CFU/mL sensitivity in juice, drastically reducing the cost of POCT. Thus, POCT based on next-generation immunosensors may rely on the deeper integration of consumer electronics and the rational design of robust, multifunctional nanomaterial interfaces.

4.3 Microfluidic immunoassays

Microfluidic immunoassays significantly advance POCT by fully integrating sample processing, immunoreactions, and detection within monolithic chips, leveraging advantages including minimal sample consumption, accelerated reaction kinetics, and automated operation to enhance analytical efficiency and accuracy. Song et al. established a microfluidic system using antibody-modified microspheres for the fluorescent quantification of *S. aureus*, achieving a LOD of 15 CFU/μL and greatly reducing the duration of analysis compared to traditional culture methods (24-72 hours) (Song et al., 2020). Wang et al. developed a microfluidic chip that integrates bifunctional manganese dioxide nanoclusters (MnO_2 NCs) as both fluid-driving components and signal amplifier. The dissolution of MnO_2 NCs by H_2O_2 generates oxygen, which actively promotes the mixing and reaction of immunomagnetic beads, target samples and immune complexes in the chip. The catalytic process induces a colorimetric response through TMB oxidation, quantifying via smartphone-based application. This method enables the quantitative detection of *Salmonella* at 63 CFU/mL within 30 minutes (Wang et al., 2023).

Beyond conventional antibodies, alternative biorecognition elements are expanding the capabilities of bacterial detection. For instance, Li et al. isolated a *P. aeruginosa*-specific phage and fixed it on agarose microspheres within a 3D-printed device to construct a POCT platform. This system enables the quantification of the target pathogen in just 25 minutes via colorimetric and pressure dual-signal outputs (Li et al., 2024). Zeng

et al. prepared activated macrophage membrane-coated magnetic silica nanoparticles (aM-MSNPs), which utilize natural membrane receptors for broad-spectrum pathogen capture, achieving visual LOD of 10 CFU/mL for various bacteria when coupled with a tyramide signal amplification strategy (Zeng et al., 2024).

Surface antigen-based POCT offers high specificity, simple operation and rapid detection, making this the most commercially successful bacterial POCT platform to date. However, several challenges remain, including limited antibody stability, high production cost, interference from complex sample matrices, and insufficient sensitivity for low-concentration bacteria (Zhou et al., 2020). Future advancements may be realized through the development of highly specific and stable recognition elements, such as engineered antibodies, aptamers or bacteriophages, improved sample pretreatment strategies to minimize the matrix effect, and the integration of nanomaterials with signal amplification technologies to improve detection sensitivity. Addressing these aspects will significantly advance the performance and practical applicability of antigen-based POCT systems.

5 Nucleic acid–based POCT

Nucleic acids, serving as the genetic material of living organisms, provide intrinsically specific targets for detection. The integration of nucleic acid extraction, amplification and signal readout into compact platforms enables nucleic acid-based POCT to identify low-abundance pathogens, discriminate drug-resistant strains, and support rapid clinical decision-making (Hong et al., 2024; Choi et al., 2025). Recent advances in isothermal amplification techniques, CRISPR-Cas systems, and microfluidic engineering drive remarkable improvements in detection speed, analytical sensitivity and operational simplicity (Liu et al., 2024; S. Liu et al., 2025) (Fig. 4). The ongoing miniaturization and functional integration of nucleic acid analysis modules are consolidating this approach as a cornerstone of next-generation bacterial POCTs, delivering exceptional sensitivity and specificity (Jayakanthan et al., 2025; Hua et al., 2026).

5.1 Nucleic acid amplification technologies

Although nucleic acids comprise the most specific biomarker for bacterial detection, their concentrations are typically very low during early-stage infection. Consequently, nucleic acid amplification is essential in nucleic acid-based POCT to enable the rapid enzymatic amplification of target sequences, enhance detection signals and allow the reliable identification of low-abundance pathogens (Hu et al., 2024). Conventional PCR-based methods provide excellent sensitivity but depend on precision thermal cycling instruments, which constrains their use in POCT (Chen et al., 2025). These limitations have driven the adoption of isothermal amplification methods, which efficiently amplify nucleic acids at constant temperatures, thereby eliminating the need for complex instrumentation (Falco et al., 2022).

Recombinase polymerase amplification (RPA) utilizes recombinase-primer complexes that specifically bind to target DNA, facilitating strand separation at a constant temperature of 37-42°C. Upon strand displacement, single-strand binding proteins (SSBs) immediately stabilize the exposed DNA templates, preventing reannealing and secondary structure formation, thereby enhancing primer accessibility and extension efficiency. This is followed by polymerase-mediated DNA synthesis with strand displacement capability (Fig. 4a). The entire reaction can be completed within 20 minutes, significantly reducing turnaround time while maintaining high specificity and sensitivity. (Shang et al., 2024; Li et al., 2025). A fully integrated paper-based platform has been developed that incorporates nucleic acid extraction, RPA amplification and lateral flow immunoassay, driven simply by a hand warmer at 45℃. This system achieved the equipment-free detection of *Neisseria gonorrhoeae* with a sensitivity of 1 CFU/mL (Chu et al., 2025). Similarly, a RPA-based platform coupled with Enzyme-Linked Immunosorbent Assay (ELISA) enabled the rapid diagnosis of bacterial respiratory pathogens at 39℃ within 30 minutes, demonstrating 100% detection sensitivity for *Klebsiella pneumoniae* and *S. pneumoniae* (Azizian et al., 2025).

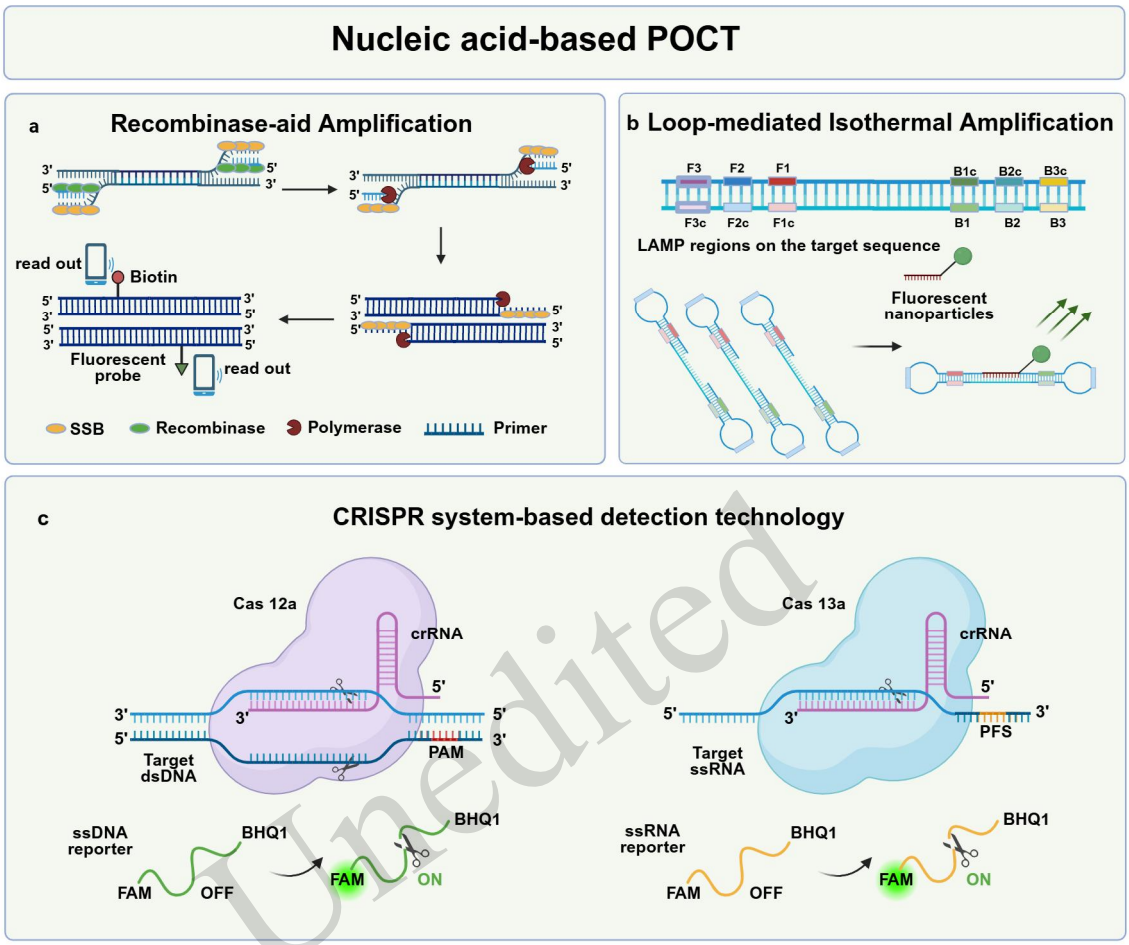

Fig. 4 Nucleic acid-based point-of-care testing (POCT) in bacteria. a. Recombinase polymerase amplification technology. b. Loop-mediated isothermal amplification strategy. c. CRISPR-Cas systems-based assay. Created with BioRender.com. Abbrevatinos: SSB, single-stranded DNA-binding protein; CRISPR-Cas, clustered regularly interspaced short palindromic repeats-associated protein Cas; PAM, protospacer adjacent motif; PFS, protospacer flanking site.

LAMP, as another prominent isothermal application technology, employs four or six distinct primers that recognize multiple regions of the target DNA, enabling auto-cycling strand displacement synthesis and the formation of loop structures, which collectively drive exponential amplification at around 65°C (Fig. 4b) (Zou et al., 2020; Lee et al., 2024; Cho et al., 2025). Zhang et al. designed a microfluidic chip integrating sample pretreatment, reagent introduction, and LAMP amplification, enabling the detection of *Staphylococcus epidermidis* with a LOD below 10 copies/μL while maintaining strong correlation with traditional methods (Zhang et al., 2019). WU et al. also established a simplified tube-type-oil-bath PCR method that provides a stable and cost-effective alternative for portable nucleic acid detection (Wu et al., 2020).

5.2 CRISPR-based diagnostic strategies

CRISPR-Cas systems, originally developed as revolutionary genomic editing tools, have been widely used for disease therapy owing to their high precision and efficacy (Habimana et al., 2022; Y. Li et al., 2023). More recently, they have also been used to develop state-of-the-art nucleic acid-based POCT platforms (Fig. 4c) (He et al., 2025). CRISPR-Cas12 (particularly Cas12a) specially recognizes both double-stranded and single-stranded DNA and binds to dsDNA targets, which enables the high-sensitivity detection of specific DNA sequences (Lee et al., 2023). In contrast, Cas13a selectively recognizes and cleaves RNA sequences, making it

well suited for RNA detection (Li et al., 2023). For instance, Liu et al. developed RPA-Cas12a fluorescence and immunochromatographic assays for *Cryptococcus neoformans* and *Cryptococcus gattii*, achieving a LOD of 10^2 copies/μL within 50 minutes and 100% concordance with culture results for 36 clinical samples (Liu et al., 2024). Similarly, Jiang et al. developed the "Cas12aVIP" platform, combining RPA, CRISPR-Cas12a, and a polythiophene derivative (PMNT) for the visual detection of *E. coli* O157:H7 within 40 minutes (Jiang et al., 2023). In addition, Zhou et al. reported a light-controlled single-tube RPA-CRISPR-Cas12a system with a LOD of 10 copies/μL and full agreement with PCR results for *Acinetobacter baumannii*. This platform integrates amplification and detection in a closed single-tube format, reducing operational procedures and the risk of cross-contamination (Zhou et al., 2025). Pang et al. designed a CRISPR-Cas12a-mediated surface-enhanced Raman scattering (SERS) LFA that directly quantifies HIV-1 dsDNA without any pre-amplification, achieving a LOD of 0.3 fM and completing the entire assay within 1 hour (Pang et al., 2022). These advances highlight the potential of CRISPR-Cas systems for developing remarkably specific and sensitive molecular diagnostics in formats amenable to POCT.

5.3 Integrated and portable nucleic acid detection platforms

Beyond the detection technologies discussed above, microfluidic systems represent a paradigm shift by miniaturizing and integrating the entire analytical process, from sample preparation and nucleic acid amplification to signal detection, onto a single portable chip (Megarity et al., 2022; Zhao et al., 2024). This integration offers a powerful solution for rapid, on-site pathogen diagnosis. Nguyen et al. developed a compact gene analyzer (28 cm×28 cm×26 cm, 10 kg) that combines a centrifugal disc with a nucleic acid extraction column and multiple reaction chambers, enabling multiplex LAMP and PCR amplification for feline respiratory pathogens and completing the entire process within 1.5 hours (Nguyen et al., 2021). Similarly, Wang et al. designed a 3D-printed microfluidic chip for norovirus that integrates sample pretreatment, the RTRPA-CRISPR cascade reaction, and a glucose biosensing readout via a personal glucose meter (PGM). This cascade strategy provides a valuable blueprint for bacterial nucleic acid detection (Wang et al., 2026). Furthermore, the concept of fully inkjet-printed, multi-channel paper-based sensors, through currently used for food additives, showcases a low-cost, visually interpretable platform with clear potential for the multiplexed screening of bacterial targets (Deng et al., 2021). Portability and the digitization of signal readout are equally critical for advancing nucleic acid POCT. To reduce dependence on bulky laboratory instruments, recent efforts have focused on leveraging ubiquitous or low-cost devices such as smartphones, handheld fluorometers, and portable colorimeters. For example, Lin et al. developed a handheld fluorometer coupled with a smartphone RGB detection mode to quantify *E. coli*. Their assay, which combines specific protease lysis with cascade amplification, achieved a LOD of 50 CFU/mL (fluorometer) and 15 CFU/mL (smartphone), respectively, showing strong concordance with clinical culture counts (Lin et al., 2024).

Nucleic acid-based POCT offers high specificity, rapid turnaround times that circumvents lengthy culture steps, and operational simplicity, making it suitable for primary care and field settings (Ma et al., 2025). However, several challenges of this technology remain, including relatively high costs, susceptibility to sample matrix interference, variable nucleic acid extraction efficiencies, and limitations in the quantitative accuracy and coverage of variant strains (Kulkarni et al., 2023). Specifically, major interfering constituents in the whole blood include hemoglobin, abundant plasma proteins, endogenous nucleases, and lipids. Hemoglobin inhibits nucleic acid amplification by competitively binding to polymerase enzymes or sequestering essential reaction substrates. Endogenous nucleases directly cleave target nucleic acid amplicons, predisposing assays to false-negative results. Besides, lipids, particularly free fatty acids and phospholipids, can reduce emulsification or phase separation in aqueous reaction mixtures, thereby impairing hybridization kinetics and probe-target binding efficiency (Amuran et al., 2024). Although sample pretreatment methods, including centrifugation, proteinase K digestion, and commercial nucleic acid extraction kits, can lower interference by removing bulk proteins and inactivating nucleases, residual hemoglobin and trace lipids often persist at functionally relevant concentrations. Critically, the residual interference correlates strongly with the initial abundance of these

substances in the specimen, leading to substantial inter-sample variability in assay performance (Qamar et al., 2017). Future progress will depend on the continued optimization of amplification technology, the development of miniaturized detection devices, and the implementation of effective cost-reduction strategies. The field is expected to advance toward higher sensitivity, multiplexed detection, and fully automated, integrated systems. As these innovations mature, nucleic acid-based POCT is likely to become a cornerstone technology for rapid infectious disease screening and public health emergency response, further promoting the implementation of precision medicine.

6 Conclusions and perspectives

The accurate and timely detection of bacterial pathogens is essential for safeguarding public health, ensuring food safety, and facilitating the effective clinical diagnosis of infectious diseases (Deng et al., 2023; Gharbi et al., 2025). Traditional detection methods are often hindered by prolonged turnaround times, complex procedures, and dependence on specialized laboratory infrastructure, making them ill-suited for rapid on-site testing (Lagier et al., 2015). In this context, POCT has emerged as a compelling alternative, characterized by operational simplicity, rapid results, cost efficiency, and straightforward interpretation (Qin et al., 2022). This review systematically categorizes and summarizes various POCT approaches according to their different detection targets, including bacterial morphology, surface antigens, and nucleic acids. Morphological analysis provides the most direct form of identification and is particularly useful for preliminary screening. Surface antigens, owing to their high immunogenicity and specificity, form the basis of immunoassay-based POCT platforms. Nucleic acids offer unparalleled specificity when combined with advanced amplification technologies, allowing for highly sensitive detection and precise strain identification. The intrinsic properties of these detection targets determine the assay principles, performance, and application scenarios. In practice, the selection of appropriate targets or the combination of multiple targets primarily depends on the specific detection requirement. POCT technologies have evolved from simple LFAIs to sophisticated systems integrating microfluidic chips, isothermal amplification and CRISPR-Cas-based detection. These advances have progressively improved the sensitivity, specificity, multiplex capability, and degree of automation of POCT, highlighting its transformative potential in clinical diagnostics, food safety monitoring, and environmental surveillance.

Despite this promising outlook, the advancement of POCT continues to face significant challenges. In terms of detection performance, the complex composition of clinical specimens (e.g., blood, sputum, urine) and food samples often cause signal interference, making efficient and rapid sample pretreatment a critical bottleneck for practical application (He et al., 2018). Future efforts should focus on developing novel target molecules, optimizing signal amplification strategies, and refining sample preparation workflows to further enhance sensitivity and specificity, ultimately enabling single-bacterium detection and the accurate identification of low-abundance bacteria in complex matrices. With respect to technical integration and automation, a deeper integration of microfluidics systems with POCT platforms will be crucial to achieve full-process automation from sample input, amplification and detection to result readout, thereby reducing human intervention and improving reproducibility.

The convergence of AI and POCT is poised to drive a paradigm shift in bacterial detection, moving beyond qualitative readouts toward quantitative, interpretable, and self-correcting diagnostics. At the signal processing level, deep learning algorithms can overcome fundamental limitations in optical signal-to-noise ratio (Zhou et al., 2025). For example, for weak signal bands in LFIAs, U-Net-based CNNs enable pixel-level segmentation and enhancement of faint test-line signals embedded in high-background, low-contrast chromatographic images, extracting grayscale gradient features imperceptible to human observers, thereby extending the effective analytical sensitivity by one to two orders of magnitude (Wang et al., 2021; Jin et al., 2025). At the intelligent interpretation layer, the AI system integrates multimodal clinical data, including longitudinal microbial isolate records, AST profiles, and resistance surveillance, to construct dynamically updated knowledge graphs. Upon pathogen identification by a POCT device, this system delivers not only binary (positive/negative) results but

also real-time, context-aware predictions of resistance genotypes and evidence-informed empirical therapy recommendations (Cavuto et al., 2025). Future integration with electronic health records and pharmacogenomic data will further support individualized dosing and regimen selection. At the quality assurance level, AI enables closed-loop, real-time monitor across the entire POCT workflow. By embedding microsensors (e.g., for temperature, pH and fluidic flow rate) within microfluidic cartridges, machine learning models can continuously monitor operational parameters and autonomously adjust reaction kinetics. In one validated implementation, a deep learning-driven feedback controller reduced the detection CV value from 12% to < 7% (Lu et al., 2025). Moreover, AI-powered batch calibration algorithms preemptively characterize lot-to-lot reagent variability and auto-generate adaptive standard curves, ensuring result accuracy and traceability across manufacturing batches (Davis and Tomitaka, 2025). Collectively, this self-optimizing capability significantly enhances robustness, reproducibility and operational reliability, particularly under resource-constrained or field-deployed settings.

Furthermore, the successful translation of POCT from research to routine use requires greater emphasis on standardization and regulatory science. Firstly, key operational parameters in LAMP, such as primer design criteria (e.g., stem-loop structure stability, GC content distribution, and avoidance of dimerization), reaction temperature (typically 60-65℃), and incubation time (15-60 min), remain methodologically heterogenous across studies, with no consensus guidelines established by international standardization. Likewise, the quantitative evaluation of trans-cleavage activity in CRISPR-Cas systems lacks universally accepted metrics. Establishing universally accepted methodological protocols, interpretative criteria, and quality control frameworks is essential to ensure the accuracy, reliability and comparability of results across diverse settings and devices. Secondly, an underdeveloped quality control infrastructure represents a critical constraint on the analytical reliability of POCT. Unlike centralized laboratory assays, POCT devices are frequently deployed in decentralized, resource-limited settings (e.g., community health centers, mobile clinics, or field epidemiological investigations). In such contexts, preanalytical variables, including ambient temperature and humidity, inter-operator variability in assay execution, and suboptimal reagent storage, have not been comprehensively characterized or quantitatively linked to assay performance degradation, thereby introducing uncontrolled resources of bias and imprecision. Therefore, concerted efforts are needed to accelerate the bench-to-bedside translation of these technologies. To this end, strengthening the collaboration among academic, industrial and clinical stakeholders will be critical for improving the commercialization and broad adoption of POCT: this synergy will enable these innovative tools to realize their full potential in safeguarding public health and supporting more effective earlier intervention and precision therapy in the management of bacterial infections.

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Author contributions

Wen ZHANG and Yundong HANG contributed equally to the work.

Wen ZHANG: conceptualization, writing – original draft, and visualization. Yundong HANG: writing – original chart, and visualization. Sisi ZHAN, and Weiyao SONG: proofreading, and literature searching. Binxiao LI, and Nan CHEN: revising the manuscript. Min LV: writing – review & editing. All authors have read and approved the final version.

Compliance with ethics guidelines

All authors declare that they have no conflict of interest. This review does not contain any studies with human or animal subjects.

Declaration on the use of generative AI tools

DeepSeek was employed as a supplementary language refinement tool to identify and rectify grammatical inaccuracies and enhance textual coherence during the preparation of the manuscript. Subsequently, all authors critically reviewed, revised, and

finalized the work.

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