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Recombinase Polymerase Amplification-Based Analytical Platforms for Rapid Detection of Foodborne Pathogenic Bacteria: Recent Advances, Challenges, and Perspectives

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Research Article

Keywords: Recombinase polymerase amplification, Foodborne pathogens, Isothermal amplification, Lateral flow assay, CRISPR/Cas, Food safety

Posted Date: September 2nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10519636/v1

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Additional Declarations: No competing interests reported.

Abstract

Foodborne pathogenic bacteria remain a major threat to food safety and public health, highlighting the need for rapid, sensitive, and field-deployable detection technologies. Conventional culture-based methods are reliable for confirmatory testing but are time-consuming, labor-intensive, and dependent on laboratory infrastructure. Polymerase chain reaction (PCR)-based methods provide high sensitivity and specificity, yet their reliance on thermal cycling instruments and trained personnel limits decentralized use. Recombinase polymerase amplification (RPA), a low-temperature isothermal amplification technique, has attracted increasing attention because of its rapid reaction, simple instrumentation, and compatibility with diverse signal readouts. Recent RPA-based assays have progressed from single-target amplification toward integrated analytical platforms, including real-time fluorescence RPA, lateral flow RPA, multiplex RPA, RPA coupled with clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) systems, viable-cell-specific RPA, and sample-to-answer devices. These platforms have been applied to major pathogens such as *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Vibrio* spp., *Staphylococcus aureus*, *Shigella* spp., *Bacillus cereus*, *Clostridium perfringens*, and *Cronobacter sakazakii*. This review evaluates RPA-based foodborne pathogen detection from a food analytical workflow perspective, covering amplification principles, analytical formats, pathogen-specific applications, current challenges, and future perspectives. Particular attention is given to matrix-compatible pretreatment, signal-readout design, nonspecific amplification, carryover contamination, multiplexing, viable/dead cell discrimination, and real-sample validation. The practical value of RPA depends less on amplification speed alone than on sample preparation, contamination-resistant formats, and standardized matrix-level validation, while culture-based methods remain necessary for confirmation and isolate recovery.

1. Introduction

Foodborne pathogenic bacteria remain a major threat to food safety, public health, and global food supply chains. They contribute substantially to the burden of foodborne diseases and require continuous surveillance across food production, processing, distribution, and consumption systems (Havelaar et al. 2015; World Health Organization n.d.; EFSA and ECDC 2025). Major bacterial hazards include *Salmonella* spp., *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli* (STEC), *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Staphylococcus aureus*, *Shigella* spp., *Bacillus cereus*, *Clostridium perfringens*, and *Cronobacter sakazakii*. These pathogens can contaminate meat, seafood, dairy products, eggs, ready-to-eat foods, fresh produce, infant formula, and other matrices, requiring rapid, reliable detection for risk monitoring, outbreak prevention, inspection, and quality control.

Conventional culture-based methods remain indispensable because they allow recovery of viable isolates for biochemical identification, serotyping, antimicrobial susceptibility testing, and epidemiological investigation. However, culture workflows generally require pre-enrichment, selective enrichment, isolation, and confirmatory testing, resulting in long turnaround times and high labor demand (ISO 2017; U.S. Food and Drug Administration n.d.-a, n.d.-b). PCR and quantitative PCR have improved sensitivity and specificity, but their dependence on thermal cycling instruments, fluorescence systems, and trained operators limits their use in resource-limited or on-site settings (Law et al. 2015; Ndraha et al. 2023).

Isothermal nucleic acid amplification technologies offer alternatives for rapid pathogen detection because they avoid repeated thermal cycling and can be integrated with portable readout systems. However, food-derived inhibitors, including proteins, fats, polysaccharides, salts, phenolic compounds, and other matrix components, may affect nucleic acid extraction, enzymatic amplification, and signal readout (Moon et al. 2022; Ndraha et al. 2023). Thus, rapid amplification chemistry must be evaluated together with sample preparation and matrix compatibility.

Among isothermal amplification methods, RPA is especially attractive. It typically operates at approximately 37–42°C and relies on recombinase, single-stranded DNA-binding protein, and strand-displacing DNA polymerase. Recombinase-primer complexes search for homologous target sequences, promote primer invasion, and initiate strand-displacement synthesis, enabling exponential amplification under constant-temperature conditions (Piepenburg et al. 2006; Lobato and O'Sullivan 2018). These features make RPA suitable for low-power devices, field-deployable assays, and point-of-need food safety testing.

RPA-based assays have recently moved beyond single-target amplification toward visual, multiplexed, CRISPR-assisted, viable-cell-specific, and integrated platforms. Lateral flow RPA provides instrument-light visual readout (Li et al. 2020; Zhao et al. 2024). Multiplex RPA enables simultaneous detection of multiple pathogens or serovars (Ma et al. 2020; Jin et al. 2022; Zhan et al. 2024). RPA-CRISPR/Cas systems add guide RNA-mediated sequence recognition and collateral cleavage-based signal amplification (Chen et al. 2018; Li et al. 2018; Ji et al. 2025). PMA/PMAxx-assisted RPA improves viable-cell relevance by reducing amplification from dead-cell DNA (Zeng et al. 2016; Gao et al. 2021; Rani et al. 2022; Chen et al. 2022). In addition, magnetic separation, paper-based extraction, microfluidics, smartphone-assisted readout, and closed devices are being incorporated into RPA workflows to improve pretreatment, portability, and contamination control (Ahn et al. 2018; Chen et al. 2023; Shang et al. 2024; Trinh and Nam 2024).

Despite these advances, routine use of RPA in food safety monitoring remains constrained by food matrix interference, sample pretreatment requirements, nonspecific amplification, primer-dimer formation, carryover contamination, multiplex assay instability, and limited validation in naturally contaminated samples (Moon et al. 2022; Wang et al. 2020; Zhao et al. 2024). Unlike general reviews of rapid pathogen detection or isothermal amplification, this review evaluates RPA-based platforms from a food analytical workflow perspective, emphasizing matrix-compatible pretreatment, signal-readout design, viable-cell relevance, contamination control, and validation. The aim is to clarify not only what RPA can detect, but also what is required for RPA assays to operate reliably in food matrices.

2. Principle and Characteristics of RPA

2.1 Basic principle of RPA

Recombinase polymerase amplification is an isothermal nucleic acid amplification technique first reported by Piepenburg et al. (2006). Unlike PCR, which depends on repeated thermal denaturation, primer annealing, and extension, RPA uses recombinase-mediated strand invasion to initiate primer binding under constant-temperature conditions. The reaction mainly involves recombinase, single-stranded DNA-binding protein, and strand-displacing DNA polymerase (Piepenburg et al. 2006; Lobato and O'Sullivan 2018).

In a typical reaction, recombinase binds to primers to form recombinase-primer complexes. These complexes scan double-stranded DNA, identify homologous target sequences, and promote primer invasion. The displaced single-stranded DNA is stabilized by single-stranded DNA-binding proteins, while strand-displacing polymerase extends the primer and synthesizes new DNA strands. Repeated primer invasion and extension from opposing primers result in exponential amplification under isothermal conditions.

RPA usually operates at approximately 37–42°C and can generate detectable products within 10–30 min, depending on template concentration, assay format, reaction chemistry, and sample matrix (Lobato and O'Sullivan 2018). Its mild reaction temperature makes it compatible with simple heaters, portable instruments, disposable cartridges, and integrated field-testing devices. The RPA reaction mechanism is illustrated in Fig. 1.

2.2 Primer and probe design considerations

Primer and probe design is critical for RPA performance. RPA primers are usually longer than conventional PCR primers, often approximately 30–35 nucleotides, which facilitates recombinase binding and strand invasion. Long primers, however, may also increase secondary structure formation and primer-dimer risk. RPA is generally more suitable for short amplicons, commonly around 100–200 bp, although longer fragments can be amplified under optimized conditions (Lobato and O'Sullivan 2018).

RPA primer design should consider moderate GC content and avoid long homopolymeric stretches, repeated sequences, strong hairpins, and primer-primer complementarity. The 3' end is particularly important because nonspecific extension may cause false-positive amplification. Since RPA primer performance is difficult to predict using conventional PCR design rules, multiple primer pairs usually require experimental screening (Lobato and O'Sullivan 2018; Zhao et al. 2024).

Probe design is also important for fluorescence RPA, lateral flow RPA, and CRISPR-assisted RPA. Poor probe design can cause weak signals, nonspecific amplification, or false-positive lateral flow lines. Primer/probe screening should therefore be performed together with matrix-specific validation rather than only using purified genomic DNA (Moon et al. 2022; Zhao et al. 2024).

2.3 Advantages for foodborne pathogen detection

Several features make RPA relevant to food analytical workflows. First, its low reaction temperature reduces equipment requirements. Unlike PCR and qPCR, RPA does not require precise thermal cycling and can be performed using a simple heater, water bath, portable incubator, or integrated microdevice (Lobato and O'Sullivan 2018).

Second, the amplification step is short. Detectable products can often be generated within 10–30 min, which is valuable for high-risk sample screening, processing-environment monitoring, and emergency response. Total assay time, however, must include sample preparation, enrichment, lysis, extraction, and signal readout (Moon et al. 2022; Ndraha et al. 2023).

Third, RPA is compatible with multiple signal readout formats, including real-time fluorescence, lateral flow readout, colorimetric systems, electrochemical sensors, CRISPR/Cas systems, and microfluidic devices (Chen et al. 2018; Li et al. 2018; Lobato and O'Sullivan 2018; Trinh and Nam 2024; Zhao et al. 2024). Fourth, RPA can be integrated with magnetic separation, immunomagnetic capture, rapid lysis, PMA/PMAxx viability pretreatment, paper-based extraction, and microfluidic processing (Ahn et al. 2018; Hice et al. 2019; Gao et al. 2021; Rani et al. 2022; Chen et al. 2023; Shang et al. 2024).

2.4 Comparison with other nucleic acid amplification methods

Compared with PCR and qPCR, RPA has lower equipment dependence and shorter amplification time. PCR-based assays are mature and specific but require thermal cycling instruments. qPCR provides quantitative and closed-tube detection but requires fluorescence detection systems and trained operators. RPA, in contrast, can be performed at low constant temperatures and integrated with visual or portable readout platforms (Law et al. 2015; Lobato and O'Sullivan 2018; Ndraha et al. 2023).

Compared with loop-mediated isothermal amplification (LAMP), RPA generally operates at a lower temperature and requires fewer primers. LAMP is efficient and widely used, but its primer design is more complex and nonspecific amplification or carryover contamination may occur. RPA also faces false-positive risks, especially in endpoint formats, but its low-temperature operation and compatibility with lateral flow and CRISPR/Cas systems make it attractive for portable analytical platforms (Law et al. 2015; Lobato and O'Sullivan 2018; Zhao et al. 2024).

Recombinase-aided amplification (RAA) is another low-temperature recombinase-based method. Although RAA shares similarities with RPA, RPA has been more widely reported in international studies on foodborne pathogen detection. For food testing, method selection should consider not only amplification speed but also matrix compatibility, pretreatment requirements, and robustness in real samples (Moon et al. 2022; Ndraha et al. 2023). A comparison of RPA with other amplification methods is summarized in Table 1.

Table 1
Comparison of RPA with other nucleic acid amplification methods for foodborne pathogen detection

Method	Principle	Typical reaction condition	Approximate detection time	Instrument requirement	Main advantages	Main limitations	Suitability for foodborne pathogen detection
Conventional PCR	Thermal cycling-based amplification with repeated denaturation, annealing, and extension	Cyclic temperature changes, commonly including denaturation, annealing, and extension	Usually 1–2 h for amplification, excluding sample preparation	Thermal cycler; electrophoresis or endpoint detection system	Mature method; high specificity; widely used	Thermal cycling required; post-amplification handling may increase contamination risk	Laboratory confirmation and method comparison
qPCR	Real-time fluorescence monitoring during PCR amplification	Thermal cycling with fluorescent probes or dyes	Usually 1–2 h, excluding sample preparation	Real-time PCR instrument	High sensitivity; quantitative or semi-quantitative; closed-tube detection	Expensive instrument and trained personnel required	Laboratory screening, quantification, and validation
LAMP	Auto-cycling strand-displacement amplification using multiple primers	Usually 60–65°C	Usually 30–60 min	Heating block, water bath, fluorescence reader, or visual readout	High amplification efficiency; visual detection possible	Complex primer design; nonspecific amplification and carryover risk	Rapid screening when primer design and contamination control are robust
RPA	Recombinase-mediated primer invasion, SSB stabilization, and strand-displacing polymerase extension	Usually 37–42°C	Usually 10–30 min	Simple heater, portable incubator, fluorescence reader, lateral flow readout, or integrated device	Low reaction temperature; rapid amplification; flexible readout; portable platform compatibility	Sensitive to primer/probe design; nonspecific and primer-dimer signals; reagent stability concerns	Point-of-need screening and field-deployable food safety testing
RAA	Recombinase-assisted low-temperature isothermal amplification	Usually around 37–42°C	Usually 10–30 min	Simple heating device or portable detection system	Rapid and field-compatible	Reagent availability and international foodborne applications are less extensive than RPA	Related low-temperature isothermal option
RPA-CRISPR/Cas	RPA pre-amplification followed by guide RNA-mediated CRISPR/Cas recognition and reporter cleavage	RPA usually 37–42°C; Cas reaction often around 37°C	Usually 30–60 min for combined workflow	Heater plus fluorescence, lateral flow, colorimetric, electrochemical, or smartphone-assisted readout	Improved specificity through secondary sequence recognition; flexible output	Additional enzymes and guide RNAs increase cost and optimization complexity	High-specificity rapid screening and integrated analytical platforms

Note: PCR, polymerase chain reaction; qPCR, quantitative PCR; LAMP, loop-mediated isothermal amplification; RPA, recombinase polymerase amplification; RAA, recombinase-aided amplification; CRISPR, clustered regularly interspaced short palindromic repeats; Cas, CRISPR-associated protein. Detection times and reaction conditions are approximate and may vary by target organism, primer/probe design, matrix, pretreatment, and detection format.

2.5 Limitations and technical concerns

Despite its advantages, RPA has important limitations. Nonspecific amplification can occur because the reaction operates efficiently at relatively low temperatures and uses long primers. This may cause false-positive results, particularly in endpoint visual assays using lateral flow readout (Lobato and O'Sullivan 2018; Zhao et al. 2024). Primer-dimer formation can also produce false-positive lateral flow signals when labeled primers or probes are captured on test strips, as shown in *Listeria monocytogenes* lateral flow RPA assay development (Wang et al. 2020).

Carryover contamination is another major concern because RPA rapidly generates large amounts of amplicons. Opening tubes after amplification, especially for lateral flow readout, may contaminate subsequent reactions. Closed-tube fluorescence detection, sealed lateral flow cartridges, one-pot RPA-CRISPR/Cas systems, and microfluidic compartmentalization are promising solutions (Gong et al. 2024; Chen et al. 2025; Zhao et al. 2024).

Food matrix interference may affect bacterial recovery, lysis, nucleic acid extraction, enzyme activity, and signal readout (Moon et al. 2022). In addition, most RPA assays detect DNA and cannot inherently distinguish viable bacteria from dead cells. PMA, EMA, PMAxx, RNA targets, short enrichment, and other viability-oriented strategies may improve biological relevance, but they require pathogen- and matrix-specific validation (Elizaquivel et al. 2014; Taylor et al. 2014; Zeng et al. 2016; Han et al. 2020; Chen et al. 2022). Overall, RPA is best positioned as a rapid, flexible, and field-compatible screening method that complements culture-based reference methods.

3. RPA-Based Analytical Strategies for Foodborne Pathogen Detection

RPA can be coupled with multiple signal readouts to meet different analytical requirements in foodborne pathogen detection. Early assays often relied on agarose gel electrophoresis for amplicon verification, whereas recent studies have focused on fluorescence monitoring, lateral flow readout, multiplex detection, CRISPR/Cas-assisted signal amplification, viable-cell-specific analysis, and integrated sample-to-answer platforms (Lobato and O'Sullivan 2018; Zhao et al. 2024; Ji et al. 2025; Trinh and Nam 2024). These strategies differ in sensitivity, specificity, equipment requirement, contamination risk, and suitability for complex food matrices. The following subsections distinguish analytical formats rather than pathogen-specific applications, which are discussed in Section 4. Representative RPA-based detection formats are shown in Fig. 2.

3.1 Real-time fluorescence RPA

Real-time fluorescence RPA monitors target amplification during the reaction, reducing post-amplification tube opening and providing more objective interpretation than endpoint visual assays. Yang et al. developed a real-time RPA assay targeting the empV gene of *Vibrio vulnificus* in seafood. The assay was performed at 39°C, generated positive signals within approximately 2–14 min, and achieved a reported limit of detection of 17 gene copies or 1 CFU per reaction (Yang et al. 2020).

Real-time RPA has also been reported for *Salmonella* detection in multiple food matrices, including egg products, chocolate products, meat products, cereal products, and soy products (Li et al. 2025). Beyond tube-based assays, real-time RPA has been incorporated into integrated microfluidic systems and has been applied to pathogens such as *Campylobacter jejuni* (Geng et al. 2019; Shang et al. 2024). Its main limitation is the need for optical detection equipment, although portable fluorescence readers can partly address this issue.

3.2 RPA coupled with lateral flow readout

Lateral flow RPA is one of the most widely used formats for field-oriented detection. Labeled amplicons are captured on lateral flow membranes and converted into visible test and control lines, enabling rapid interpretation with minimal equipment (Zhao et al. 2024). Zhan et al. developed a multiplex lateral flow RPA method for simultaneous detection of *Salmonella* spp., *S. Enteritidis*, and *S. Typhimurium* (Zhan et al. 2024). Bai et al. reported a lateral flow RPA method for *E. coli* O157, *Salmonella*, and *Shigella* in meat and used a disposable lid-sealed detection box to reduce contamination risk (Bai et al. 2024).

Lateral flow RPA is convenient but vulnerable to endpoint contamination, primer-dimer-derived false positives, and subjective interpretation of weak lines. Improved probe design, sealed strip devices, internal controls, dual-mode signal output, and smartphone-assisted readout can improve reliability (Li et al. 2020; Li et al. 2021; Feng et al. 2025; Zhao et al. 2024).

3.3 Multiplex RPA

Food samples may contain multiple pathogens, and separate single-target assays are inefficient. Multiplex RPA improves screening efficiency by detecting several pathogens, serovars, or virulence genes within one workflow. A representative integrated system combined a mini automatic nucleic acid extractor with lateral flow immunoassay-integrated RPA to detect *V. parahaemolyticus*, *S. aureus*, *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes* (Jin et al. 2022). Multiplex lateral flow RPA has also been applied to *Salmonella* genus- and serovar-level detection (Zhan et al. 2024) and to seafood-associated pathogens (Ma et al. 2020).

Multiplex RPA remains technically challenging. Multiple primer pairs may compete for enzymes, nucleotides, magnesium ions, and template access. High-abundance targets may suppress low-abundance targets, and signal discrimination becomes difficult as target number increases. Robust multiplex validation therefore requires mixed pathogens, different concentration ratios, non-target background organisms, and representative food matrices.

3.4 RPA-CRISPR/Cas systems

RPA-CRISPR/Cas systems combine rapid isothermal amplification with guide RNA-mediated recognition. Cas12a-mediated assays are particularly attractive because target recognition activates collateral cleavage of single-stranded DNA reporters, enabling fluorescence, lateral flow, colorimetric, electrochemical, or dual-mode readout (Chen et al. 2018; Li et al. 2018). CRISPR-based diagnostic platforms have been developed toward portable and multiplexed detection (Gootenberg et al. 2017, 2018; Kellner et al. 2019; Ji et al. 2025).

In foodborne pathogen detection, RPA-CRISPR/Cas12a has been applied to *Salmonella*, *V. parahaemolyticus*, *S. aureus*, *Shigella flexneri*, and *B. cereus* (Liu et al. 2022; Wang et al. 2024; Ma et al. 2024; Xu et al. 2024; Li et al. 2024; Meng et al. 2024). These systems add a sequence-recognition step after RPA, but they also introduce Cas enzymes, guide RNAs, reporters, and buffer-compatibility requirements. Development priorities include one-pot or closed-tube formats, lyophilized reagents, simplified guide RNA design, and validation in real food matrices (Chen et al. 2025; Gong et al. 2024; Ji et al. 2025).

3.5 RPA for viable bacteria detection

DNA-based amplification assays, including RPA, cannot inherently distinguish viable bacteria from dead cells because residual DNA may persist after heat treatment, sanitization, or environmental stress (Elizaquivel et al. 2014; Zeng et al. 2016). Viability-oriented RPA is therefore important for processed foods, sanitized surfaces, beverages, and low-moisture products.

PMA, EMA, and PMAxx pretreatments suppress amplification from membrane-compromised dead cells by binding DNA after light activation. Rani et al. developed a PMAxx-assisted lateral flow RPA assay for viable and viable-but-nonculturable *E. coli* O157:H7 in milk, apple juice, and drinking water (Rani et al. 2022). Gao et al. reported PMAxx-qRPA for viable *C. sakazakii* in powdered infant formula (Gao et al. 2021). These approaches improve risk relevance but are affected by species, cell envelope structure, injury status, turbidity, light exposure, and matrix composition (Taylor et al. 2014; Chen et al. 2022). VBNC cells further complicate interpretation because they may retain membrane integrity while failing to grow under standard culture conditions (Han et al. 2020).

3.6 Integrated and field-deployable RPA platforms

Amplification speed alone is insufficient for real food testing. The complete workflow includes sampling, bacterial concentration, enrichment or recovery, lysis, nucleic acid extraction, amplification, signal readout, and result interpretation. Therefore, recent RPA studies increasingly emphasize integrated platforms that combine sample preparation with amplification and detection.

Miniaturized extractors, magnetic separation, membrane-based extraction, paper-based devices, microfluidic chips, electrochemical sensors, and smartphone-assisted readers have been introduced to improve portability and usability (Ahn et al. 2018; Hice et al. 2019; Chen et al. 2023; Shang et al. 2024; Trinh and Nam 2024). Magnetic enrichment can increase local target concentration and reduce matrix interference (Hice et al. 2019; Ma et al. 2024). The field is moving from stand-alone RPA assays toward sample-to-answer analytical platforms with closed operation, preloaded reagents, and digital readout.

4. Applications in Major Foodborne Pathogenic Bacteria

RPA-based assays have been developed for a broad range of foodborne pathogenic bacteria. Target selection, readout format, and pretreatment strategy vary among pathogens because food reservoirs, contamination routes, virulence determinants, physiological states, and risk interpretation differ. Representative applications are summarized in Table 2. The table should be interpreted as a workflow-level comparison because matrix type, pretreatment, enrichment, LOD definition, and readout format strongly influence reported performance and limit direct cross-study comparison.

Table 2
Representative RPA-based assays for foodborne pathogenic bacteria

Pathogen or target group	RPA-based format	Matrix or sample type	Pretreatment or workflow	Reported analytical performance or condition	Reference
Salmonella spp., *S. Enteritidis*, and *S. Typhimurium*	Multiplex RPA-LFD	Artificially contaminated chicken; retail chicken, pork, and duck meat	Artificial contamination and retail meat testing	38°C for 25 min; pure-culture LODs of 2.8 × 10^2, 5.9 × 10^2, and 7.6 × 10^2 CFU/mL for *Salmonella* spp., *S. Enteritidis*, and *S. Typhimurium*, respectively.	Zhan et al. (2024)
E. coli O157, *Salmonella*, and *Shigella*	RPA-LFD with disposable lid-sealed detection box	Meat products	Meat sample processing followed by closed-lid visual detection	37°C for 30 min; lateral flow readout within 5–10 min; reported detection limits of 0.168, 0.72, and 1.25 fg/μL; contamination-reducing device design	Bai et al. (2024)
Salmonella spp.	RPA-CRISPR/Cas12a	Chicken and egg samples	Boiling pretreatment; optional short enrichment	genomic DNA LOD of 1 × 10^{-4} ng/μL and bacterial suspension LOD of 10^2 CFU/mL; chicken and egg samples initially detected at 10^3 CFU/mL, improving to 10^2 and 10^1 CFU/mL, respectively, after 3 h enrichment at 37°C	Liu et al. (2022)
Viable and VBNC *E. coli* O157:H7	PMAxx-RPA-LFA	Milk, apple juice, and drinking water	PMAxx pretreatment before RPA-LFA	PMAxx-RPA-LFA completed at 39°C within 40 min; viable/VBNC *E. coli* O157:H7 detected in spiked commercial beverages including milk, apple juice, and drinking water	Rani et al. (2022)
Listeria monocytogenes	Improved RPA-LFS	Artificially contaminated milk and bacterial cultures	Direct assay without DNA purification	37–42°C within 25 min; LOD of 1 CFU/reaction without DNA purification or 100 fg genomic DNA/50 μL; primer-dimer false-positive signals eliminated by probe design	Wang et al. (2020)
Vibrio vulnificus	Real-time RPA	Shrimp, fish, crab, and oysters	Seafood enrichment when needed	39°C; detection within 2–14 min; LOD of 17 gene copies or 1 CFU/reaction, and 1 CFU/10 g in spiked food after enrichment	Yang et al. (2020)
Vibrio parahaemolyticus	RPA-CRISPR/Cas12a with immunochromatographic strip	Salmon sashimi and seafood samples	Direct detection or 6 h enrichment	RPA at 40°C for 10 min; total detection approximately 30 min; LOD of 1.4 × 10^2 CFU/mL by immunochromatographic strip; salmon sashimi detected at 154 CFU/g without enrichment and 2 CFU/g after 6 h enrichment	Wang et al. (2024)
V. parahaemolyticus, *S. aureus*, *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes*	Mini automatic nucleic acid extractor combined with RPA-LFIA	Milk, pork, egg, chicken, cheese, shrimp, fish, broccoli, and fruit juice	Automatic magnetic-bead nucleic acid extraction	RPA at 37°C for 15 min; visual LODs of 2.4 × 10^1, 7.1 × 10^1, 4.5 × 10^1, 5.1 × 10^1, and 2.7 × 10^1 CFU/mL for *V. parahaemolyticus*, *S. aureus*, *S. enterica*, *E. coli* O157:H7, and *L. monocytogenes*, respectively; recoveries in spiked foods were 90.5–104.5%	Jin et al. (2022)
Shigella spp. and EIEC	RPA-LFD	Cucumber model samples	Artificial contamination	39°C within 25 min; plasmid LOD of 1.29 × 10^2 copies/μL; cucumber matrix LOD around	Bian et al. (2022)

Note: RPA, recombinase polymerase amplification; qRPA, quantitative RPA; PCR, polymerase chain reaction; qPCR, quantitative PCR; LAMP, loop-mediated isothermal amplification; RPA-LFD, RPA coupled with lateral flow dipstick; RPA-LFA, RPA coupled with lateral flow assay; RPA-LFS, RPA coupled with lateral flow strip; RPA-LFB, RPA coupled with lateral flow biosensor; LFIA, lateral flow immunoassay; PMAxx, improved propidium monoazide; VBNC, viable but non-culturable; EIEC, enteroinvasive Escherichia coli; CRISPR/Cas, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein system; LOD, limit of detection. Values are summarized from the corresponding studies and are not directly comparable because pathogen type, target gene, matrix, sample pretreatment, enrichment condition, readout format, and LOD definition differ among studies.

Pathogen or target group	RPA-based format	Matrix or sample type	Pretreatment or workflow	Reported analytical performance or condition	Reference
				10^3 CFU/mL; *Shigella*/EIEC not differentiated by ipaH alone	
S. aureus, *V. parahaemolyticus*, and *S. Enteritidis*	Multiplex RPA-LFD	Seafood	Seafood matrix validation	The whole process, including amplification and reading, was completed at 37°C within 15 min; LODs of 2.6×10^1, 7.6×10^1, and 1.29×10^1 CFU/mL for *S. aureus*, *V. parahaemolyticus*, and *S. Enteritidis*, respectively.	Ma et al. (2020)
Staphylococcus aureus	Magnetic enrichment cascade single-step RPA-CRISPR/Cas12a	Lake water, orange juice, pork, and lettuce	Antibody-functionalized magnetic beads and extraction-free lysis	Magnetic enrichment achieved 98% capture efficiency within 5 min; extraction-free single-step RPA-CRISPR/Cas12a detected 1 copy/μL DNA template or 10 CFU/mL *S. aureus* within 40 min	Ma et al. (2024)
Shigella flexneri	RPA-CRISPR/Cas12a-LFA	Artificially contaminated milk; bananas, milk, and salads	Artificial contamination and market sample comparison	RPA optimized at 36°C for 25 min; genomic DNA LOD of 8.3 copies/μL by fluorescence and approximately 83 copies/μL by LFA; in artificially contaminated milk, fluorescence detection reached 5.6×10^1 CFU/mL and LFA detection reached 5.6×10^2 CFU/mL	Xu et al. (2024)
Emetic and non-emetic *Bacillus cereus*	RPA-LFS	Raw milk, cream, and dairy products	Artificial contamination; enrichment when needed	37°C within 20 min; LODs of 10^4 copies/mL and 10^2 CFU/mL in pure cultures and milk; evaluated in cream with results consistent with standard culture; distinguishes emetic and non-emetic *B. cereus*	Wang et al. (2022)
Clostridium perfringens	RPA-LFB	Chicken meat and milk	Artificial contamination	RPA-LFB completed within 20 min; nucleic acid sample LOD of 100 pg/μL; stable detection in artificially contaminated chicken meat and milk at 1×10^4 and 1×10^3 CFU/mL, respectively	Tian et al. (2024)
Viable *Cronobacter sakazakii*	PMAxx-qRPA	Powdered infant formula	PMAxx viable-cell pretreatment	PMAxx-qRPA targeting gluA completed within 20 min at 37°C; longer amplicons improved suppression of dead-cell DNA; viable cells from 0 to 10^7 CFU/g were quantified in spiked powdered infant formula containing 10^7 CFU/g dead cells, with results consistent with culture	Gao et al. (2021)
E. coli O157:H7	RPA-LFA compared with PCR, qPCR, and LAMP	Ten food and beverage matrices	Nitrocellulose paper-based DNA extraction within less than 1 min	Nitrocellulose strip-based DNA extraction completed in < 1 min; RPA at 39°C for 15 min; RPA-LFA combined with field-deployable extraction identified contamination within < 25 min and showed higher sensitivity than PCR, qPCR, and LAMP in tested food and beverage matrices	Batra et al. (2024)

Note: RPA, recombinase polymerase amplification; qRPA, quantitative RPA; PCR, polymerase chain reaction; qPCR, quantitative PCR; LAMP, loop-mediated isothermal amplification; RPA-LFD, RPA coupled with lateral flow dipstick; RPA-LFA, RPA coupled with lateral flow assay; RPA-LFS, RPA coupled with lateral flow strip; RPA-LFB, RPA coupled with lateral flow biosensor; LFIA, lateral flow immunoassay; PMAxx, improved propidium monoazide; VBNC, viable but non-culturable; EIEC, enteroinvasive Escherichia coli; CRISPR/Cas, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein system; LOD, limit of detection. Values are summarized from the corresponding studies and are not directly comparable because pathogen type, target gene, matrix, sample pretreatment, enrichment condition, readout format, and LOD definition differ among studies.

Pathogen or target group	RPA-based format	Matrix or sample type	Pretreatment or workflow	Reported analytical performance or condition	Reference
Listeria monocytogenes	RPA with lateral flow readout	Food-processing surfaces	Surface sampling, enrichment, and lateral flow RPA	Food-contact surface sampling followed by enrichment and lateral flow RPA; amplification at 39°C for 40 min; naked-eye lateral flow detection for *L. monocytogenes* surface monitoring	Azinheiro et al. (2020)
Campylobacter jejuni	Real-time RPA	Food samples	Real-time fluorescence RPA targeting *hipO*	Real-time RPA targeting hipO; analysis time reduced to approximately 13 min; practical applicability evaluated in artificially contaminated milk and chicken breast samples, with performance compared with real-time PCR and culture-based methods	Geng et al. (2019)
Campylobacter jejuni	Integrated paper microfluidic RPA device	Chicken meat samples	Paper-based DNA extraction, RPA amplification, and lateral flow readout	Hybrid paper/polymer microfluidic device integrating paper-based DNA extraction, RPA, and LFD; RPA completed in 20 min; LOD of 46 CFU/mL after paper-based DNA extraction and 460 CFU/mL on the integrated device; in spiked chicken meat, > 10^2 CFU/g was detected without enrichment, whereas 10^1–10^2 CFU/g required 5–10 h enrichment	Chen et al. (2023)
Enterotoxigenic *Bacillus cereus*	RPA-CRISPR/Cas12a with fluorescence and lateral flow readout	Food-related samples	RPA amplification followed by Cas12a recognition	RPA-CRISPR/Cas12a dual-signal platform combining fluorescence and lateral flow readout for rapid detection of *B. cereus* with three-component enterotoxin-producing potential	Li et al. (2024)
Emetic *Bacillus cereus*	RPA-assisted CRISPR/Cas12a naked-eye platform	Food-related samples	RPA amplification and CRISPR/Cas12a-mediated readout	Universal naked-eye RPA-CRISPR/Cas12a platform for emetic *B. cereus* detection; suitable as a toxin-type-oriented visual detection example	Meng et al. (2024)

Note: RPA, recombinase polymerase amplification; qRPA, quantitative RPA; PCR, polymerase chain reaction; qPCR, quantitative PCR; LAMP, loop-mediated isothermal amplification; RPA-LFD, RPA coupled with lateral flow dipstick; RPA-LFA, RPA coupled with lateral flow assay; RPA-LFS, RPA coupled with lateral flow strip; RPA-LFB, RPA coupled with lateral flow biosensor; LFIA, lateral flow immunoassay; PMAxx, improved propidium monoazide; VBNC, viable but non-culturable; EIEC, enteroinvasive Escherichia coli; CRISPR/Cas, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein system; LOD, limit of detection. Values are summarized from the corresponding studies and are not directly comparable because pathogen type, target gene, matrix, sample pretreatment, enrichment condition, readout format, and LOD definition differ among studies.

4.1 *Salmonella spp.*

Salmonella spp. are among the most frequently targeted foodborne pathogens in RPA studies because they are associated with poultry, eggs, meat, dairy products, produce, and ready-to-eat foods. Common targets include conserved genes such as invA and fimY, as well as serovar-specific markers for *S. Enteritidis* and *S. Typhimurium* (Li et al. 2020; Liu et al. 2017; Zhan et al. 2024).

Recent assays have moved from single-target visual detection toward multiplex, CRISPR-assisted, colorimetric, and dual-readout formats. Zhan et al. developed a multiplex lateral flow RPA assay for simultaneous detection of *Salmonella spp.*, *S. Enteritidis*, and *S. Typhimurium* (Zhan et al. 2024). Liu et al. combined RPA with CRISPR/Cas12a for *Salmonella* detection in chicken and egg samples (Liu et al. 2022). One-pot RPA-CRISPR approaches and dual-readout lateral flow RPA systems further support contamination-resistant and field-oriented detection (Gong et al. 2024; Chen et al. 2025; Feng et al. 2025). For *Salmonella*, stronger risk interpretation requires integration of genus-level, serovar-specific, and virulence-associated targets, together with validation in naturally contaminated samples and mixed microbiota.

4.2 *Listeria monocytogenes*

Listeria monocytogenes is a major concern in ready-to-eat foods, dairy products, meat products, seafood, and food-processing environments. Wang et al. developed an improved lateral flow RPA assay that eliminated primer-dimer false-positive signals through probe design (Wang et al. 2020). This study is important because false-positive test lines are a common weakness of endpoint visual RPA assays.

RPA has also been applied to food-processing surface monitoring. Azinheiro et al. reported lateral flow RPA for naked-eye detection of *L. monocytogenes* on food-processing surfaces (Azinheiro et al. 2020). For *L. monocytogenes*, swabs, biofilm-associated cells, cold-chain environments, and ready-to-eat matrices require separate validation because environmental monitoring and product testing involve different sampling and recovery challenges.

4.3 STEC and *E. coli O157:H7*

STEC and *E. coli* O157:H7 are associated with meat products, raw milk, fresh produce, juice, and water. RPA assays often target serogroup- or virulence-associated genes such as rfbE, stx1, stx2, and eae. Batra et al. compared lateral flow RPA with PCR, qPCR, and LAMP across food and beverage matrices and incorporated a field-deployable nitrocellulose paper-based DNA extraction approach (Batra et al. 2024).

Viability-oriented detection is especially relevant for *E. coli O157:H7* because stressed cells in foods and processing environments may be underestimated by culture or overestimated by DNA assays. Rani et al. reported PMAxx-assisted lateral flow RPA for viable and VBNC *E. coli O157:H7* in milk, apple juice, and drinking water (Rani et al. 2022). Risk-relevant STEC assays therefore need to combine serogroup identification, virulence markers, and viable-cell information when interpretation depends on biological activity (Zeng et al. 2016; Han et al. 2020).

4.4 *Vibrio spp.*

Pathogenic *Vibrio* species are strongly associated with seafood and aquatic products. Yang et al. developed a real-time RPA assay targeting the *empV* gene of *V. vulnificus*, with detection within 2–14 min at 39°C and sensitive detection after enrichment in spiked seafood (Yang et al. 2020). Wang et al. developed an RPA-CRISPR/Cas12a platform coupled with an immunochromatographic strip for visual detection of *V. parahaemolyticus* in salmon sashimi and seafood samples (Wang et al. 2024). Seafood matrix compatibility and discrimination of pathogenic strains from non-pathogenic environmental strains remain central requirements for *Vibrio* assays.

4.5 *Staphylococcus aureus*

Staphylococcus aureus is associated with meat, dairy products, ready-to-eat foods, and processing environments. Because staphylococcal food poisoning may result from preformed enterotoxins, bacterial DNA detection alone does not fully represent toxin risk. Nevertheless, rapid detection is valuable for hygiene monitoring and prevention of bacterial proliferation before toxin formation.

Ma et al. developed a magnetic enrichment cascade single-step RPA-CRISPR/Cas12a assay for *S. aureus* detection in food samples (Ma et al. 2024). Jin et al. included *S. aureus* in a five-pathogen platform combining a mini automatic nucleic acid extractor with lateral flow immunoassay-integrated RPA (Jin et al. 2022), and Ma et al. reported multiplex RPA for *S. aureus*, *V. parahaemolyticus*, and *S. Enteritidis* in seafood (Ma et al. 2020). When risk assessment extends beyond bacterial presence, *S. aureus* assays should incorporate enterotoxin genes or toxin detection.

4.6 *Shigella spp.* and *enteroinvasive E. coli*

Shigella spp. and enteroinvasive *E. coli* are closely related enteric pathogens. Bian et al. developed a lateral flow RPA assay targeting *ipaH* for rapid detection of *Shigella spp.* and EIEC in cucumber model samples (Bian et al. 2022). This target supports rapid screening of invasion-associated contamination but cannot distinguish *Shigella* from EIEC alone. Xu et al. developed an RPA-CRISPR/Cas12a assay with lateral flow readout for *Shigella flexneri* in food samples using a conserved target and qPCR comparison (Xu et al. 2024). Multi-target panels are needed for better taxonomic and epidemiological resolution.

4.7 *Bacillus cereus* and other spore-forming bacteria

Bacillus cereus is a spore-forming pathogen associated with emetic and diarrheal disease. Wang et al. developed lateral flow RPA assays for detecting *B. cereus* and distinguishing emetic and non-emetic types using species- and toxin-associated targets (Wang et al. 2022). More recent RPA-CRISPR/Cas12a assays have expanded detection toward enterotoxigenic and emetic strain identification with fluorescence, lateral flow, and naked-eye readouts (Li et al. 2024; Meng et al. 2024).

RPA has also been applied to *Clostridium perfringens*. Tian et al. developed an RPA-LFB assay targeting plc for chicken meat and milk (Tian et al. 2024). For spore-forming bacteria, sample pretreatment should account for spores, vegetative cells, and toxin-related genes because lysis requirements and risk interpretation differ.

4.8 *Cronobacter sakazakii*

Cronobacter sakazakii is a critical pathogen in powdered infant formula and other low-moisture foods. Gao et al. developed a PMAxx-qRPA assay for viable *C. sakazakii* in powdered infant formula, using PMAxx pretreatment to suppress dead-cell DNA signals (Gao et al. 2021). For *C. sakazakii*, low-moisture matrix compatibility, viable-cell discrimination, and validation against recognized methods are especially important because infant formula testing has high public health sensitivity.

4.9 *Campylobacter jejuni*

Campylobacter jejuni is frequently associated with poultry products, raw milk, water, and cross-contaminated foods. RPA applications for *C. jejuni* are fewer than those for *Salmonella* or *E. coli O157:H7*, but they extend the method to fastidious and microaerophilic bacteria. Geng et al. developed a real-time RPA assay targeting *hipO* for rapid detection of *C. jejuni* in food samples (Geng et al. 2019). Chen et al. later integrated paper-based DNA extraction, RPA amplification, and lateral flow readout into a sample-to-answer paper microfluidic device (Chen et al. 2023). *C. jejuni* assays need stronger evaluation in poultry-related matrices, low-level contamination, injured cells, and comparison with reference culture methods.

4.10 Summary of pathogen-specific applications

Across pathogens, the reported assays indicate that RPA is most informative when the analytical format is matched to the biological question and food matrix. *Salmonella* and STEC detection benefit from multi-target and virulence-oriented strategies; *L. monocytogenes* detection requires separate attention to product and environmental samples; *Vibrio* assays depend strongly on seafood matrix compatibility and pathogenic strain discrimination; *S. aureus* assays should consider toxin-related risk; *B. cereus* assays should distinguish toxigenic types; *C. sakazakii* assays require viable-cell relevance in low-moisture matrices; and *C. jejuni* assays need stronger evaluation in poultry samples and recovery conditions. RPA development should therefore move beyond simple presence/absence screening toward matrix-adapted and risk-relevant analytical platforms.

5. Current Challenges in Food Matrix Analysis and Field Deployment

Translation of RPA-based assays from proof-of-concept studies to routine food safety monitoring remains challenging. Food testing requires reliable sample pretreatment, amplification, contamination control, signal interpretation, and validation in realistic matrices. These limitations should therefore be evaluated at the workflow level rather than only at the amplification step. Key challenges and possible solutions are summarized in Table 3.

Table 3
Key challenges and possible solutions for RPA-based foodborne pathogen detection

Challenge	Main cause	Potential impact	Possible solutions	Future research priority
Food matrix interference	Proteins, fats, salts, polysaccharides, polyphenols, preservatives, endogenous enzymes, and background microbiota	Reduced recovery, amplification inhibition, weak signals, and false-negative results	Matrix-specific pretreatment, inhibitor removal, optimized lysis, magnetic separation, dilution, and internal controls	Validate assays in representative food matrices rather than only purified DNA or buffer systems
Sample pretreatment bottleneck	Low pathogen abundance, uneven distribution, large sample size, stressed cells, background flora, and inhibitors	Amplification is rapid, but total workflow remains long	Rapid lysis, immunomagnetic separation, magnetic-bead extraction, membrane extraction, paper-based extraction, and microfluidics	Report total workflow time including enrichment, extraction, amplification, and readout
Nonspecific amplification	Low-temperature primer binding, long primers, weak primer-template interactions, and primer-primer interactions	False-positive amplification and reduced specificity	Primer/probe screening, optimized reaction time, specificity testing, sequence confirmation, and CRISPR/Cas secondary recognition	Establish standardized RPA primer/probe design and validation criteria
Primer-dimer false-positive signals	Labeled primers or probes generating nonspecific products captured on lateral flow membranes	Visible test lines without true target amplification	Improved probe architecture, no-template controls, internal controls, optimized labeled-primer concentration, and CRISPR/Cas recognition	Minimize labeled primer-dimer capture in lateral flow RPA formats
Carryover contamination	High amplicon yield and post-amplification tube opening	Persistent false-positive results, especially in endpoint assays	Closed-tube fluorescence RPA, sealed cartridges, one-pot RPA-CRISPR/Cas, disposable closed devices, and microfluidics	Treat contamination resistance as a core performance indicator for field use
Multiplex instability	Primer competition, unequal amplification efficiency, different target abundance, and signal overlap	Missed low-abundance targets and unbalanced signals	Primer balancing, spatial separation, multichannel fluorescence, barcoded lateral flow, electrochemical arrays, and CRISPR/Cas reporters	Validate using mixed pathogens, concentration ratios, and matrix backgrounds
Viable/dead cell discrimination	DNA persistence after heat treatment, sanitization, drying, or cell death	Overestimation of viable pathogen risk	PMA, EMA, PMAxx, RNA targets, short enrichment, phage-assisted strategies, and metabolic indicators	Develop pathogen- and matrix-specific viable-cell RPA protocols
VBNC interpretation	Stressed cells may remain viable but fail to grow under standard culture conditions	Culture-negative but molecular-positive results may be difficult to interpret	Combine viability dyes, short resuscitation, RNA or metabolic markers, and culture confirmation where appropriate	Clarify how RPA signals relate to infectivity, recovery potential, and actual risk
Limited real-sample validation	Reliance on pure cultures, purified DNA, or artificially contaminated samples	Overestimated applicability and limited regulatory confidence	Naturally contaminated foods, processing-environment swabs, field samples, and reference method comparison	Conduct inter-laboratory and real-world validation studies
Incomplete reporting	Omission of sample size, enrichment condition, extraction protocol, total time, and LOD definition	Cross-study comparison becomes difficult	Standardized reporting of analytical sensitivity, matrix-specific sensitivity, total assay time, and workflow details	Develop RPA reporting guidelines for foodborne pathogen detection
Reagent stability and cost	Enzyme-based RPA and CRISPR/Cas reagents may require cold-chain storage and can be expensive	Limited field deployment and routine high-throughput use	Lyophilized reagents, room-temperature-stable pellets, preloaded tubes, and disposable cartridges	Evaluate shelf life, storage stability, transport stability, and per-test cost
Subjective visual interpretation	Weak lateral flow lines and variation in lighting or operator judgment	Inconsistent result interpretation	Smartphone imaging, strip readers, fluorescence readers, calibration lines, image-analysis algorithms, and standardized lighting boxes	Improve digital readout, traceability, and semi-quantitative interpretation

Note: The listed challenges should be considered in relation to the complete analytical workflow, including food sampling, homogenization, pathogen enrichment or concentration, bacterial lysis, nucleic acid extraction, inhibitor removal, RPA amplification, signal readout, result interpretation, and validation against reference methods. Proposed solutions may require pathogen-specific and matrix-specific optimization before practical application.

Challenge	Main cause	Potential impact	Possible solutions	Future research priority
Regulatory acceptance	Insufficient validation against recognized culture or molecular reference methods	Limited use in official control or confirmatory testing	Benchmarking against FDA BAM, ISO, GB, or other reference methods; inclusivity/exclusivity testing; reproducibility studies	Position RPA as a rapid screening and complementary method before full regulatory adoption

Note: The listed challenges should be considered in relation to the complete analytical workflow, including food sampling, homogenization, pathogen enrichment or concentration, bacterial lysis, nucleic acid extraction, inhibitor removal, RPA amplification, signal readout, result interpretation, and validation against reference methods. Proposed solutions may require pathogen-specific and matrix-specific optimization before practical application.

5.1 Food matrix complexity and pretreatment bottlenecks

Food matrices differ in composition, microbial background, and inhibitor content. Meat and seafood contain proteins, fats, salts, and endogenous enzymes; dairy products contain proteins, calcium, and emulsified fats; fresh produce contains polysaccharides, polyphenols, organic acids, and surface-associated microorganisms; and low-moisture foods present additional challenges in rehydration and uneven bacterial distribution. Such components may reduce bacterial recovery, inhibit amplification, weaken test lines, or increase false-negative risk (Moon et al. 2022).

Sample pretreatment is often the main bottleneck. RPA amplification may take only 10–30 min, but homogenization, enrichment, concentration, lysis, extraction, inhibitor removal, and readout can dominate total assay time. Direct amplification from crude homogenates is rarely reliable in complex foods. Magnetic enrichment, immunomagnetic separation, membrane extraction, paper-based extraction, and microfluidics can improve recovery and reduce inhibition (Ahn et al. 2018; Hice et al. 2019; Chen et al. 2023; Shang et al. 2024; Trinh and Nam 2024). Performance reports should therefore separate amplification time from total workflow time, including enrichment and sample preparation.

5.2 Nonspecific amplification and contamination risk

Nonspecific amplification is a major technical challenge because RPA operates at relatively low temperatures and uses long primers. Primer-primer interactions or weak primer-template binding may generate background products. In lateral flow assays, labeled primer-dimers can be captured on lateral flow membranes and produce false-positive lines, as demonstrated in *L. monocytogenes* assay development (Wang et al. 2020). Careful primer/probe screening, no-template controls, non-target controls, internal controls, and matrix controls are necessary.

Carryover contamination is particularly important for endpoint assays. RPA produces large amounts of amplicons rapidly, and tube opening after amplification can contaminate subsequent reactions. Practical control measures include closed-tube fluorescence RPA, sealed lateral flow devices, disposable cartridges, microfluidic compartmentalization, and one-pot RPA-CRISPR/Cas systems (Chen et al. 2025; Gong et al. 2024; Zhao et al. 2024). For field deployment, contamination resistance should be evaluated experimentally rather than treated only as a handling recommendation.

5.3 Multiplexing and viable-cell interpretation

Multiplex RPA improves testing efficiency but increases reaction complexity. Multiple primer pairs may compete for reagents, high-abundance targets may suppress low-abundance targets, and signal discrimination can become difficult. Multiplex fluorescence requires compatible probes and channels, while multiplex lateral flow requires spatially separated test lines or labels. Validation therefore needs to include mixed targets, different concentration ratios, background microbiota, and food matrix effects.

Viable/dead cell discrimination remains incomplete. DNA from dead bacteria may persist after heat treatment, drying, fermentation, or sanitation, causing molecular signals that overestimate viable risk (Elizaquivel et al. 2014; Zeng et al. 2016). PMA/PMAxx-based methods improve viable-cell relevance but depend on membrane integrity, dye penetration, light activation, cell injury, and matrix turbidity (Taylor et al. 2014; Chen et al. 2022). VBNC cells complicate interpretation because they may retain membrane integrity and potential virulence while failing to grow on routine media (Han et al. 2020).

5.4 Validation, reporting, and field usability

Many RPA studies still rely on purified DNA, cultures, or artificially contaminated samples. Such experiments are useful for development but may overestimate performance compared with naturally contaminated foods. Regulatory or industrial use requires validation against recognized methods, such as FDA BAM procedures and ISO horizontal methods, where applicable (ISO 2017; U.S. Food and Drug Administration n.d.-a, n.d.-b). Inclusivity, exclusivity, matrix-specific detection limits, robustness, repeatability, reproducibility, and inter-laboratory comparison should be included when practical.

Reporting standards are central to cross-study comparison. Sample size, matrix, enrichment condition, pretreatment, extraction method, amplification time, total workflow time, LOD definition, and result interpretation criteria need to be stated clearly. Field usability also depends on reagent stability, cost, and operator simplicity. Lyophilized reagents, preloaded cartridges, closed devices, portable heaters, strip readers, and smartphone-based interpretation may improve adoption, but shelf life and robustness should be tested under realistic conditions (Feng et al. 2025).

6. Future Perspectives

RPA-based foodborne pathogen detection needs to shift from assay development to platform development. The central objective is not simply lower detection limits under ideal conditions, but robust workflows that connect food samples to actionable results.

6.1 Sample-to-answer platforms and closed formats

The practical value of RPA will depend on sample-to-answer integration. Food samples require homogenization, pathogen concentration or enrichment, cell lysis, nucleic acid preparation, and inhibitor removal before amplification. Integrated platforms should therefore combine bacterial capture, lysis, extraction, amplification, signal readout, and interpretation within simplified devices. Paper-based devices, microfluidic biosensors, magnetic enrichment, and integrated extraction-amplification platforms provide practical routes toward this goal (Ahn et al. 2018; Hice et al. 2019; Chen et al. 2023; Shang et al. 2024; Trinh and Nam 2024).

Closed and contamination-resistant formats are also required. Real-time fluorescence RPA avoids post-amplification transfer, while one-pot RPA-CRISPR/Cas systems and sealed lateral flow cartridges can reduce tube opening (Chen et al. 2025; Gong et al. 2024; Zhao et al. 2024). For field applications, device engineering, leakage prevention, and user workflow may be as important as amplification chemistry. An integrated sample-to-answer workflow is outlined in Fig. 3.

6.2 RPA-CRISPR/Cas and multiplex risk-oriented detection

RPA-CRISPR/Cas systems remain a major direction because they combine rapid amplification with guide RNA-mediated specificity. Cas12a systems support fluorescence, lateral flow, colorimetric, electrochemical, and dual-mode readouts (Chen et al. 2018; Li et al. 2018). Development priorities include one-pot or sealed two-chamber systems, buffer compatibility, temporal reaction control, guide RNA stability, and lyophilized reagent formulation (Ji et al. 2025).

Multiplex panels should be risk-oriented. Poultry and eggs may prioritize *Salmonella* spp. and *C. jejuni*; seafood may prioritize *V. parahaemolyticus* and *V. vulnificus*; ready-to-eat foods and processing environments may prioritize *L. monocytogenes* and *S. aureus*; infant formula requires special attention to viable *C. sakazakii*; and STEC detection should combine serogroup and virulence targets. Microfluidic spatial separation, multichannel fluorescence, barcoded lateral flow, electrochemical arrays, and CRISPR/Cas reporters may improve multiplex performance (Gootenberg et al. 2018; Ji et al. 2025; Shang et al. 2024).

6.3 Viable-cell relevance, digital readout, and validation

Viable-cell-specific RPA needs to distinguish viable, dead, injured, and VBNC cells more explicitly. PMA/PMAxx pretreatment is practical but not universally reliable and requires optimization for each pathogen-matrix combination (Elizaquivel et al. 2014; Taylor et al. 2014; Zeng et al. 2016; Chen et al. 2022). Combining viability dyes with short enrichment, RNA targets, metabolic indicators, or phage-assisted recognition may strengthen biological relevance.

Digital readout can reduce subjectivity in visual assays. Smartphone imaging, strip readers, fluorescence readers, and dual-mode lateral flow systems can support objective interpretation, time and location records, and data traceability (Feng et al. 2025). However, digital tools require validated imaging conditions, calibration, internal controls, and robust algorithms.

Standardized validation will determine whether RPA platforms move from research tools to routine food safety applications. Naturally contaminated foods, processing-environment swabs, and comparison with recognized reference methods should be included whenever possible (EFSA and ECDC 2025; ISO 2017; U.S. Food and Drug Administration n.d.-a, n.d.-b). In this role, RPA is best viewed as a rapid screening and decision-support technology that complements, rather than replaces, culture-based confirmation and isolate recovery.

7. Conclusions

The practical value of RPA in foodborne pathogen detection depends less on amplification speed alone than on matrix-compatible pretreatment, contamination-resistant formats, and standardized validation. Recent studies show that RPA-based methods have evolved

from simple amplification assays into analytical platforms integrating fluorescence monitoring, lateral flow readout, multiplex detection, CRISPR/Cas recognition, viability pretreatment, magnetic separation, paper-based extraction, microfluidics, and portable interpretation.

Applications now cover major foodborne pathogens, including *Salmonella* spp., *L. monocytogenes*, STEC and *E. coli* O157:H7, *Vibrio* spp., *S. aureus*, *Shigella* spp., *B. cereus*, *C. perfringens*, *C. sakazakii*, and *C. jejuni*. However, assay design must remain pathogen-specific and matrix-adapted. Important barriers include food matrix inhibition, sample pretreatment, nonspecific amplification, primer-dimer signals, carryover contamination, multiplex instability, viable/dead cell discrimination, reagent stability, and insufficient real-sample validation.

Further progress should focus on complete workflow optimization rather than amplification performance alone. Closed-tube formats, sample-to-answer integration, one-pot RPA-CRISPR/Cas systems, risk-oriented multiplex panels, viable-cell-specific detection, reagent stabilization, digital readout, and standardized validation remain key priorities. With improved specificity, contamination control, device integration, and matrix-level validation, RPA-based platforms can support decentralized food safety screening while culture-based methods remain necessary for confirmation and isolate recovery.

Statements and Declarations

Funding: This work was supported by the 2025 Zhumadian Science and Technology Research Project “Development of RPA-based POCT technology for foodborne pathogenic bacteria based on flocculation–precipitation phenomena” (Grant No. ZMDSKJGG2025007).

Competing interests: The authors declare no competing interests.

Data availability: No new datasets were generated or analyzed during the current study.

Author contributions: W.Z. contributed to conceptualization, literature searching and screening, evidence synthesis, visualization, and writing the original draft. W.L. contributed to literature searching, data extraction, and critical revision of the manuscript. Y.Y. contributed to literature screening, verification of the summarized evidence, and critical revision of the manuscript. X.F. contributed to data curation, figure preparation, and critical revision of the manuscript. Y.W. contributed to conceptualization, supervision, project administration, funding acquisition, and critical revision of the manuscript. All authors read and approved the final manuscript.

Ethics approval: Not applicable.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

References

1. Ahn H, Batule BS, Seok Y, Kim MG (2018) Single-step recombinase polymerase amplification assay based on a paper chip for simultaneous detection of multiple foodborne pathogens. Anal Chem 90:10211-10216. https://doi.org/10.1021/acs.analchem.8b01309
2. Azinheiro S, Carvalho J, Prado M, Garrido-Maestu A (2020) Application of recombinase polymerase amplification with lateral flow for a naked-eye detection of Listeria monocytogenes on food processing surfaces. Foods 9:1249. https://doi.org/10.3390/foods9091249
3. Bai W, Chen J, Chen D, Zhu Y, Hu K, Lin X, Chen J, Song D (2024) Sensitive and rapid detection of three foodborne pathogens in meat by recombinase polymerase amplification with lateral flow dipstick (RPA-LFD). Int J Food Microbiol 422:110822. https://doi.org/10.1016/j.ijfoodmicro.2024.110822
4. Batra AR, Dike CC, Mantri N, Ball AS (2024) Recombinase polymerase amplification-lateral flow assay as a rapid and sensitive test for Escherichia coli O157:H7 detection in food and beverage: a comparative study. Food Control 155:110076. https://doi.org/10.1016/j.foodcont.2023.110076
5. Bian Z, Liu W, Jin J, Hao Y, Jiang L, Xie Y, Zhang H (2022) Development of a recombinase polymerase amplification assay with lateral flow dipstick (RPA-LFD) for rapid detection of Shigella spp. and enteroinvasive Escherichia coli. PLoS One 17:e0278869. https://doi.org/10.1371/journal.pone.0278869
6. Chen JS, Ma E, Harrington LB, Da Costa M, Tian X, Palefsky JM, Doudna JA (2018) CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science 360:436-439. https://doi.org/10.1126/science.aar6245
7. Chen Q, Wang H, Xu H, Peng Y, Yao B, Chen Z, et al (2025) One-pot RPA-CRISPR/Cas12a integrated dual-mode electrochemical lateral flow strip for ultrasensitive and precise detection of Salmonella. Biosens Bioelectron 285:117529. https://doi.org/10.1016/j.bios.2025.117529

8. Chen Y, Hu Y, Lu X (2023) An integrated paper microfluidic device based on isothermal amplification for simple sample-to-answer detection of Campylobacter jejuni. Appl Environ Microbiol 89:e00695-23. https://doi.org/10.1128/aem.00695-23
9. Chen Y, Chen Y, Shi C, Li Z, Li X, Liu Y, et al (2022) PCR-mediated nucleic acid molecular recognition techniques for viable/dead foodborne pathogen detection. Foods 11:2675. https://doi.org/10.3390/foods11172675
10. EFSA and ECDC (2025) The European Union One Health 2024 zoonoses report. EFSA J 23:e09759. https://doi.org/10.2903/j.efsa.2025.9759
11. Elizaquivel P, Sanchez G, Aznar R (2014) Recent developments in the use of viability dyes and quantitative PCR in the food microbiology field. J Appl Microbiol 116:1-13. https://doi.org/10.1111/jam.12365
12. Feng J, Xiong M, Qiao Y, Wang P, Lin X, Kang L, Duan N, Wang Z, Wu S (2025) Ultrasensitive point-of-care testing of Salmonella in lettuce and milk samples using a recombinase polymerase amplification-based colorimetric/fluorescent dual-readout lateral flow assay. Food Chem 481:144058. https://doi.org/10.1016/j.foodchem.2025.144058
13. Gao S, Sun C, Hong H, Gooneratne R, Mutukumira A, Wu X (2021) Rapid detection of viable Cronobacter sakazakii in powdered infant formula using improved propidium monoazide (PMAxx) and quantitative recombinase polymerase amplification (qRPA) assay. Food Control 124:107899. https://doi.org/10.1016/j.foodcont.2021.107899
14. Geng Y, Liu G, Liu L, Deng Q, Zhao L, Sun X, Wang J, Zhao B, Wang J (2019) Real-time recombinase polymerase amplification assay for the rapid and sensitive detection of Campylobacter jejuni in food samples. J Microbiol Methods 157:31-36. https://doi.org/10.1016/j.mimet.2018.12.010
15. Gong J, Zhang D, Fu L, Dong Y, Wu K, Dou X, Wang C (2024) A one-pot convenient RPA-CRISPR-based assay for Salmonella enterica serovar Indiana detection. Microorganisms 12:519. https://doi.org/10.3390/microorganisms12030519
16. Gootenberg JS, Abudayyeh OO, Lee JW, Essletzbichler P, Dy AJ, Joung J, Verdine V, Donghia N, Daringer NM, Freije CA, Myhrvold C, Bhattacharyya RP, Livny J, Regev A, Koonin EV, Hung DT, Sabeti PC, Collins JJ, Zhang F (2017) Nucleic acid detection with CRISPR-Cas13a/C2c2. Science 356:438-442. https://doi.org/10.1126/science.aam9321
17. Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F (2018) Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science 360:439-444. https://doi.org/10.1126/science.aaq0179
18. Han L, Wang K, Ma L, Delaquis P, Bach S, Feng J, Lu X (2020) Viable but nonculturable Escherichia coli O157:H7 and Salmonella enterica in fresh produce: rapid determination by loop-mediated isothermal amplification coupled with a propidium monoazide treatment. Appl Environ Microbiol 86:e02566-19. https://doi.org/10.1128/AEM.02566-19
19. Havelaar AH, Kirk MD, Torgerson PR, Gibb HJ, Hald T, Lake RJ, Praet N, Bellinger DC, de Silva NR, Gargouri N, Speybroeck N, Cawthorne A, Mathers C, Stein C, Angulo FJ, Devleesschauwer B (2015) World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. PLoS Med 12:e1001923. https://doi.org/10.1371/journal.pmed.1001923
20. Hice SA, Clark KD, Anderson JL, Brehm-Stecher BF (2019) Capture, concentration, and detection of Salmonella in foods using magnetic ionic liquids and recombinase polymerase amplification. Anal Chem 91:1113-1120. https://doi.org/10.1021/acs.analchem.8b04751
21. ISO (International Organization for Standardization) (2017) 6579-1:2017. Microbiology of the food chain-horizontal method for the detection, enumeration and serotyping of *Salmonella*-Part 1: detection of *Salmonella* spp. International Organization for Standardization, Geneva
22. Ji T, Li Y, Wang X, Zhang Y, Liu H, et al (2025) Research progress on the application of RPA-CRISPR/Cas12a in the rapid visual detection of pathogenic microorganisms. Front Cell Infect Microbiol 15:1640938. https://doi.org/10.3389/fcimb.2025.1640938
23. Jin B, Ma B, Li J, Hong Y, Zhang M (2022) Simultaneous detection of five foodborne pathogens using a mini automatic nucleic acid extractor combined with recombinase polymerase amplification and lateral flow immunoassay. Microorganisms 10:1352. https://doi.org/10.3390/microorganisms10071352
24. Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO, Zhang F (2019) SHERLOCK: nucleic acid detection with CRISPR nucleases. Nat Protoc 14:2986-3012. https://doi.org/10.1038/s41596-019-0210-2
25. Law JWF, Ab Mutalib NS, Chan KG, Lee LH (2015) Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. Front Microbiol 5:770. https://doi.org/10.3389/fmicb.2014.00770
26. Li J, Ma B, Fang J, Zhi A, Chen E, Xu Y, Yu X, Sun C, Zhang M (2020) Recombinase polymerase amplification combined with lateral flow immunoassay for rapid detection of Salmonella in food. Foods 9:27. https://doi.org/10.3390/foods9010027
27. Li L, Song L, Wang Q, Yin H, Suo Y, Zhao Y, Li Q, Cao Y, Xing C, Wang D, Hu Z, Jiang J (2025) A rapid detection method for Salmonella based on real-time recombinase polymerase amplification in food. Foodborne Pathog Dis. https://doi.org/10.1177/15353141251380364

28. Li PR, Wang ZX, Xu ZK, Wang J, Li B, Shen X, Xu ZL (2024) An RPA-assisted CRISPR/Cas12a assay combining fluorescence and lateral flow strips for the rapid detection of enterotoxigenic Bacillus cereus. J Agric Food Chem 72:14000-14010. https://doi.org/10.1021/acs.jafc.4c03601
29. Li X, Zheng T, Xie YN, Li F, Jiang X, Hou X, Wu P (2021) Recombinase polymerase amplification coupled with a photosensitization colorimetric assay for fast Salmonella spp. testing. Anal Chem 93:6559-6566. https://doi.org/10.1021/acs.analchem.1c00791
30. Li SY, Cheng QX, Wang JM, Li XY, Zhang ZL, Gao S, Cao RB, Zhao GP, Wang J (2018) CRISPR-Cas12a-assisted nucleic acid detection. Cell Discov 4:20. https://doi.org/10.1038/s41421-018-0028-z
31. Liu HB, Zang YX, Du XJ, Li P, Wang S (2017) Development of an isothermal amplification-based assay for the rapid visual detection of Salmonella bacteria. J Dairy Sci 100:7016-7025. https://doi.org/10.3168/jds.2017-12566
32. Liu L, Zhao G, Li X, Xu Z, Lei H, Shen X (2022) Development of rapid and easy detection of Salmonella in food matrics using RPA-CRISPR/Cas12a method. LWT 162:113443. https://doi.org/10.1016/j.lwt.2022.113443
33. Lobato IM, O'Sullivan CK (2018) Recombinase polymerase amplification: basics, applications and recent advances. TrAC Trends Anal Chem 98:19-35. https://doi.org/10.1016/j.trac.2017.10.015
34. Ma B, Fang J, Chen E, Xu Y, Zhi A, Yu X, Sun C, Zhang M (2020) Multiplex recombinase polymerase amplification assay for the simultaneous detection of three foodborne pathogens in seafood. Foods 9:278. https://doi.org/10.3390/foods9030278
35. Ma Y, Wei H, Wang Y, Cheng X, Chen H, Yang X, Zhang H, Rong Z, Wang S (2024) Efficient magnetic enrichment cascade single-step RPA-CRISPR/Cas12a assay for rapid and ultrasensitive detection of Staphylococcus aureus in food samples. J Hazard Mater 465:133494. https://doi.org/10.1016/j.jhazmat.2024.133494
36. Meng JN, Xu ZK, Li PR, Zeng X, Liu Y, Xu ZL, Wang J, Ding Y, Shen X (2024) Universal and naked-eye diagnostic platform for emetic Bacillus cereus based on RPA-assisted CRISPR/Cas12a. J Agric Food Chem 72:8823-8830. https://doi.org/10.1021/acs.jafc.3c06744
37. Moon YJ, Lee SY, Oh SW (2022) A review of isothermal amplification methods and food-origin inhibitors against detecting food-borne pathogens. Foods 11:322. https://doi.org/10.3390/foods11030322
38. Ndraha N, Lin HY, Wang CY, Hsiao HI, Lin HJ (2023) Rapid detection methods for foodborne pathogens based on nucleic acid amplification: recent advances, remaining challenges, and possible opportunities. Food Chem Mol Sci 7:100183. https://doi.org/10.1016/j.fochms.2023.100183
39. Piepenburg O, Williams CH, Stemple DL, Armes NA (2006) DNA detection using recombination proteins. PLoS Biol 4:e204. https://doi.org/10.1371/journal.pbio.0040204
40. Rani A, Dike CC, Mantri N, Ball AS (2022) Point-of-care lateral flow detection of viable Escherichia coli O157:H7 using an improved propidium monoazide-recombinase polymerase amplification method. Foods 11:3207. https://doi.org/10.3390/foods11203207
41. Shang Y, Xing G, Lin H, Sun Y, Chen S, Lin JM (2024) Development of nucleic acid extraction and real-time recombinase polymerase amplification (RPA) assay integrated microfluidic biosensor for multiplex detection of foodborne bacteria. Food Control 155:110047. https://doi.org/10.1016/j.foodcont.2023.110047
42. Taylor MJ, Bentham RH, Ross KE (2014) Limitations of using propidium monoazide with qPCR to discriminate between live and dead Legionella in biofilm samples. Microbiol Insights 7:15-24. https://doi.org/10.4137/MBI.S17723
43. Tian R, Xie F, Liu Y, Liu G, Li Q, Wang J, Zhang H, Dai L, Zhang W (2024) Recombinase polymerase amplification combined with lateral flow biosensor for rapid visual detection of Clostridium perfringens in chicken meat and milk. Front Vet Sci 11:1395188. https://doi.org/10.3389/fvets.2024.1395188
44. Trinh TND, Nam NN (2024) Isothermal amplification-based microfluidic devices for detecting foodborne pathogens: a review. Anal Methods 16:1150-1157. https://doi.org/10.1039/D3AY02039H
45. U.S. Food and Drug Administration (n.d.-a) Bacteriological Analytical Manual. U.S. Food and Drug Administration. Accessed 5 July 2026
46. U.S. Food and Drug Administration (n.d.-b) Bacteriological Analytical Manual Chapter 5: *Salmonella*. U.S. Food and Drug Administration. Accessed 5 July 2026
47. Wang J, Xu D, Liu H, Liu J, Zhu L, Zeng H, Wu W (2024) A visual, rapid, and sensitive detection platform for Vibrio parahaemolyticus based on RPA-CRISPR/Cas12a and an immunochromatographic test strip. Food Qual Saf 8:fyae008. https://doi.org/10.1093/fqsafe/fyae008
48. Wang L, Zhao P, Si X, Li J, Dai X, Zhang K, Gao S, Dong J (2020) Rapid and specific detection of Listeria monocytogenes with an isothermal amplification and lateral flow strip combined method that eliminates false-positive signals from primer-dimers. Front Microbiol 10:2959. https://doi.org/10.3389/fmicb.2019.02959
49. Wang L, Yang H, Wang K, Yang H, Zhao M, Shang Y, Wang F, Dong J, Zhao W, Li L, Liang W, Wang Y (2022) A visualized isothermal amplification method for rapid and specific detection of emetic and non-emetic Bacillus cereus in dairy products. Front Microbiol

13:802656. https://doi.org/10.3389/fmicb.2022.802656
50. World Health Organization (n.d.) Food safety. World Health Organization. Accessed 5 July 2026
51. Xu J, Zhang T, Lv X, Shi L, Bai W, Ye L (2024) An RPA-based CRISPR/Cas12a assay in combination with a lateral flow assay for the rapid detection of Shigella flexneri in food samples. Foods 13:3200. https://doi.org/10.3390/foods13193200
52. Yang X, Zhang X, Wang Y, Shen H, Jiang G, Dong J, Zhao P, Gao S (2020) A real-time recombinase polymerase amplification method for rapid detection of Vibrio vulnificus in seafood. Front Microbiol 11:586981. https://doi.org/10.3389/fmicb.2020.586981
53. Zeng D, Chen Z, Jiang Y, Xue F, Li B (2016) Advances and challenges in viability detection of foodborne pathogens. Front Microbiol 7:1833. https://doi.org/10.3389/fmicb.2016.01833
54. Zhan Z, He S, Cui Y, Yang J, Shi X (2024) Development of a multiplex recombinase polymerase amplification coupled with lateral flow dipsticks for the simultaneous rapid detection of Salmonella spp., Salmonella Typhimurium and Salmonella Enteritidis. Food Qual Saf 8:fyad059. https://doi.org/10.1093/fqsafe/fyad059
55. Zhao Y, Wei Y, Ye C, Cao J, Zhou X, Xie M, Qing J, Chen Z (2024) Application of recombinase polymerase amplification with lateral flow assay to pathogen point-of-care diagnosis. Front Cell Infect Microbiol 14:1475922. https://doi.org/10.3389/fcimb.2024.1475922

Figures

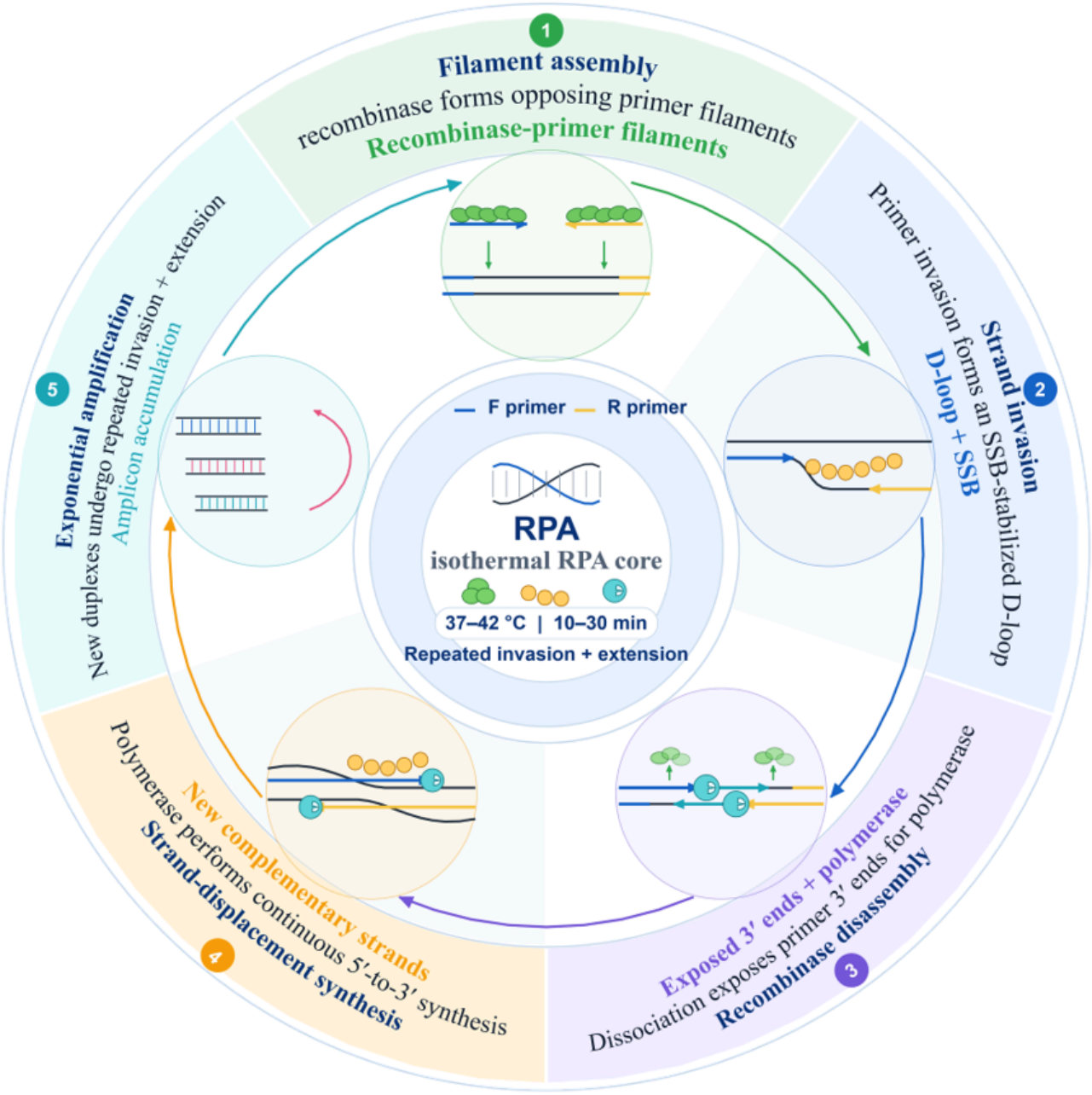


Figure 1

Schematic representation of the cyclic mechanism of recombinase polymerase amplification (RPA). Recombinase assembles with opposing primers to form recombinase-primer filaments, which recognize homologous sequences and mediate strand invasion into double-stranded DNA. Single-stranded DNA-binding proteins stabilize the displaced strands and D-loop intermediates. Following recombinase disassembly, strand-displacing polymerase extends the exposed primer 3' ends and synthesizes complementary strands in the 5'-to-3' direction. Repeated cycles of strand invasion and extension generate new duplex templates, enabling exponential amplification at 37–42 °C within approximately 10–30 min

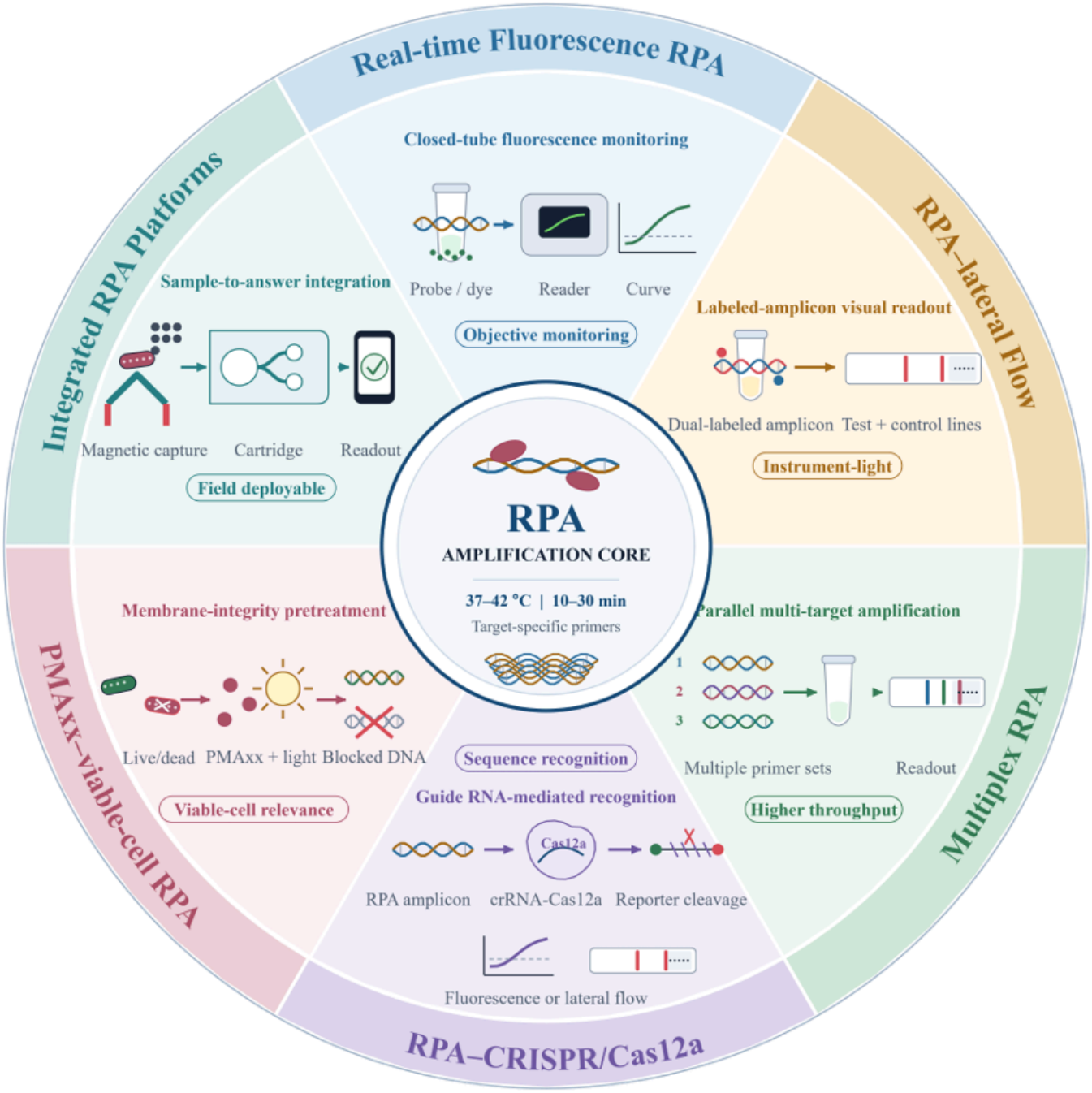


Figure 2

Representative recombinase polymerase amplification (RPA)-based analytical formats for foodborne pathogen detection. A shared low-temperature RPA core can be coupled with closed-tube real-time fluorescence monitoring, labeled-amplicon lateral flow readout, parallel multiplex amplification, CRISPR/Cas12a-mediated sequence recognition, improved propidium monoazide (PMAxx) pretreatment for viable-cell-relevant detection, and integrated sample-to-answer platforms. These configurations provide objective monitoring, instrument-light visualization, higher throughput, enhanced sequence specificity, reduced interference from dead-cell DNA, and field-deployable analysis, respectively

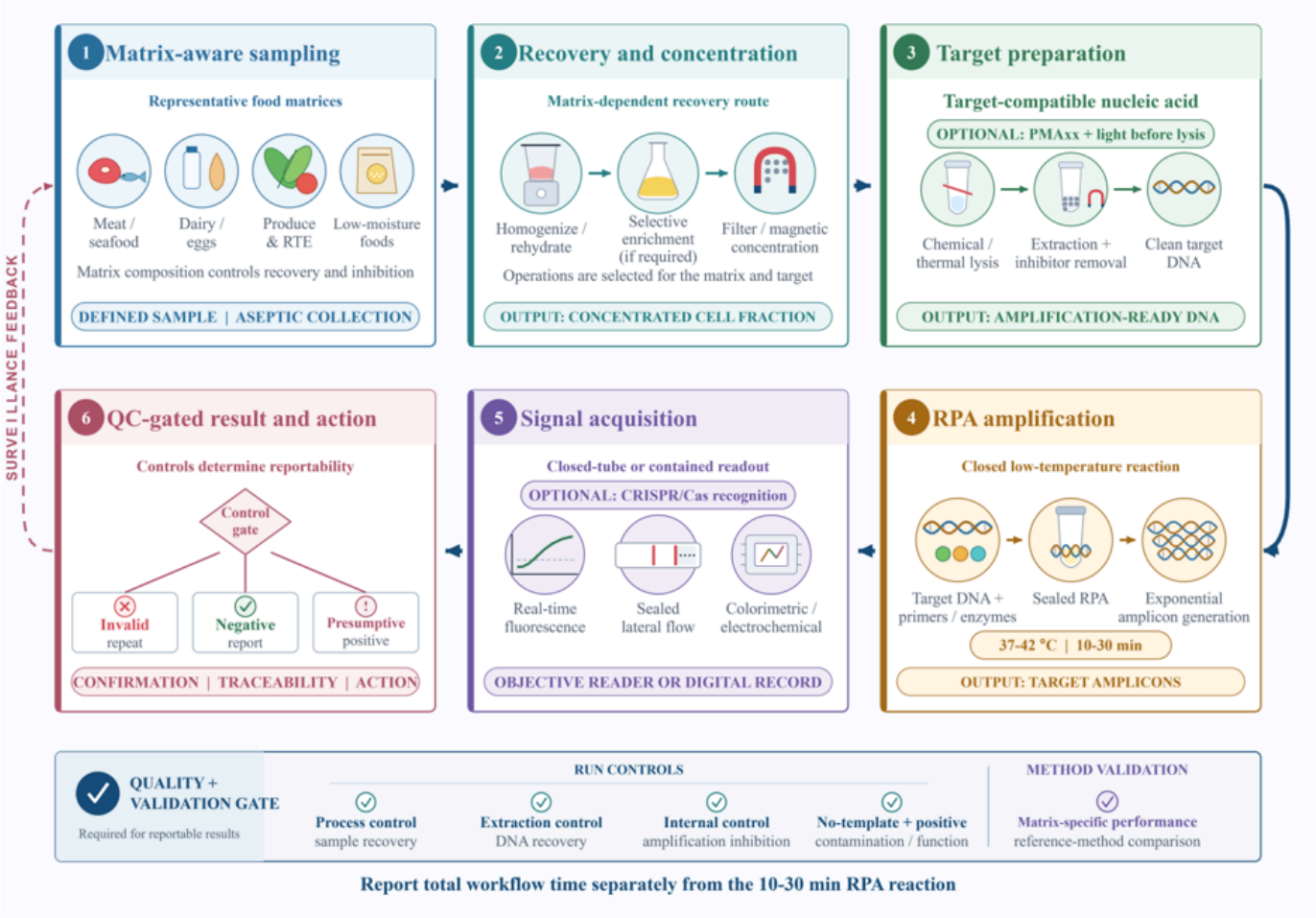


Figure 3

Integrated and quality-controlled recombinase polymerase amplification (RPA) workflow for foodborne pathogen detection. The workflow comprises matrix-aware sampling, target recovery and concentration, nucleic acid preparation, closed-tube RPA amplification, signal acquisition, and quality-control-gated result interpretation. Improved propidium monoazide (PMAxx) treatment before cell lysis and CRISPR/Cas-mediated recognition during signal acquisition may be incorporated when viable-cell discrimination or enhanced sequence specificity is required. Process, extraction, internal, no-template, and positive controls, together with matrix-specific method validation, determine whether results are reported as invalid, negative, or presumptive positive. Total sample-to-result time should be reported separately from the 10–30 min RPA reaction time