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CRISPR-Cas12a-Based Biosensing Platform for Rapid Detection of Foodborne Salmonella Contamination

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ABSTRACT

Background: Nontyphoidal Salmonella is a leading cause of foodborne illness, and culture-based confirmation is too slow for modern supply-chain decisions. Objective: To synthesise published evidence on CRISPR-Cas12a (and related Cas13/Cas9-family) diagnostic platforms for detecting Salmonella and other foodborne pathogens. Methods: Narrative review of peer-reviewed studies identified via PubMed, Google Scholar and Crossref using terms combining "CRISPR," "Cas12a," "Cas13," "Salmonella," "foodborne," "RPA/LAMP," and "lateral flow." Findings: Amplification-coupled Cas12a assays (RPA-, LAMP- or RAA-CRISPR) reliably reach limits of detection of 1-100 CFU/mL within 30-90 minutes, with fluorescent, colorimetric, lateral-flow and glucose-meter readouts demonstrated for Salmonella; amplification-free formats trade sensitivity for speed. Conclusions: CRISPR-Cas12a platforms now approach performance suitable for field deployment, though standardisation and multiplexing remain barriers to routine adoption.

Keywords: *CRISPR-Cas12a; Salmonella; foodborne pathogen detection; biosensor; lateral flow assay*

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Abstract

Background: Nontyphoidal *Salmonella* is a leading cause of foodborne illness, and culture-based confirmation is too slow for modern supply-chain decisions [1]. **Objective:** To synthesise published evidence on CRISPR-Cas12a (and related Cas13/Cas9-family) diagnostic platforms for detecting *Salmonella* and other foodborne pathogens. **Methods:** Narrative review of peer-reviewed studies identified via PubMed, Google Scholar and Crossref using terms combining "CRISPR," "Cas12a," "Cas13," "Salmonella," "foodborne," "RPA/LAMP," and "lateral flow." **Findings:** Amplification-coupled Cas12a assays (RPA-, LAMP- or RAA-CRISPR) reliably reach limits of detection of 1-100 CFU/mL within 30-90 minutes, with fluorescent, colorimetric, lateral-flow and glucose-meter readouts demonstrated for *Salmonella*; amplification-free formats trade sensitivity for speed. **Conclusions:** CRISPR-Cas12a platforms now approach performance suitable for field deployment, though standardisation and multiplexing remain barriers to routine adoption.

Keywords: CRISPR-Cas12a; Salmonella; foodborne pathogen detection; biosensor; lateral flow assay

Introduction

Nontyphoidal *Salmonella enterica* causes an estimated 93.8 million illnesses and 155,000 deaths annually, a substantial share attributable to contaminated food [1]. Poultry, eggs, produce and processed foods are recurrent vehicles, and outbreak investigations routinely implicate delayed detection as a driver of prolonged exposure and larger recalls. Conventional culture-based confirmation — pre-enrichment, selective enrichment, plating and biochemical or serological confirmation — requires 3-5 days, which is incompatible with perishable-food release timelines and just-in-time supply chains. Molecular methods such as PCR and quantitative PCR shortened confirmation to hours, but still require centralised laboratory infrastructure, trained personnel and thermal cyclers, limiting their use at ports of entry, processing lines or in low-resource settings. The discovery that class 2 CRISPR effectors such as Cas12a (Cpf1) and Cas13a possess target-activated collateral (trans) nuclease activity against bystander single-stranded nucleic acids [2,3,4] enabled a new diagnostic paradigm: guide-RNA-programmed recognition of a pathogen sequence triggers indiscriminate cleavage of a quenched reporter, generating a fluorescent, colorimetric or lateral-flow signal

without requiring specialised optics. Platforms built on this principle — SHERLOCK [5,6,7,8], DETECTR [3,9] and HOLMES [10] — were first validated against viral targets, including Zika, Dengue and SARS-CoV-2 [11,9], before groups adapted the same collateral-cleavage chemistry to bacterial foodborne pathogens, most extensively to *Salmonella*. This review synthesises the published literature specifically on CRISPR-Cas12a-based biosensing for *Salmonella* detection in food, situating it against the broader Cas13- and Cas12b-based diagnostic ecosystem from which it draws its core mechanism.

Table 1: Foundational CRISPR-Cas diagnostic platforms underlying current Salmonella biosensors.

Platform	Effector	Key reference	Original target
SHERLOCK	Cas13a	[5]	Zika/Dengue RNA
SHERLOCKv2	Cas13/Cas12a/Csm6	[6]	Multiplexed nucleic acids
DETECTR	Cas12a	[3]	HPV DNA
HOLMES	Cas12a	[10]	SNP genotyping, viral DNA

Methods

This is a narrative review of published studies on CRISPR-Cas12a-based diagnostic platforms for detection of *Salmonella* and other foodborne pathogens. Relevant articles were identified through structured searches of PubMed, Google Scholar and Crossref (through 2026) using combinations of "CRISPR," "Cas12a," "Cas13," "Cas12b," "Salmonella," "foodborne pathogen," "recombinase polymerase amplification," "loop-mediated isothermal amplification" and "lateral flow assay." Original research reporting analytical performance (limit of detection, assay time, specificity) for *Salmonella*-targeting or closely related foodborne-pathogen CRISPR-Cas12a assays were prioritised, supplemented by foundational mechanistic reports and prior review articles [12,13,14] for context. No meta-analysis or formal risk-of-bias scoring was performed; findings are synthesised narratively by assay format.

Cas12a Collateral-Cleavage Mechanism and Diagnostic Principle

Cas12a is a single RNA-guided nuclease that, upon binding a double-stranded DNA target flanked by a TTTV protospacer-adjacent motif, undergoes a conformational change that activates a second, non-specific single-stranded DNase activity in the same catalytic pocket [3,2]. This behaviour distinguishes Cas12a from the genome-editing-oriented Cas9 nuclease: whereas Cas9 primarily performs a single site-specific double-strand break, Cas12a's activated state persists and processively degrades any single-stranded DNA present in the reaction, including a deliberately added reporter oligonucleotide. In a diagnostic reaction, this "collateral cleavage" indiscriminately degrades a co-incubated single-stranded DNA reporter carrying a fluorophore-quencher pair or an antibody-hapten pair for lateral-flow capture, producing a binary signal proportional to target abundance. Related Cas13a/C2c2 collateral RNase activity underlies SHERLOCK's RNA-detection arm [4,5], while Cas12b variants extend the same logic in one-tube isothermal formats [15]. Because collateral cleavage is triggered stoichiometrically by a single bound target but reports catalytically through repeated reporter turnover, femtomolar-to-attomolar analytical sensitivity is achievable once combined with pre-amplification [10,6]. This two-step logic — amplify, then interrogate with a programmable nuclease — is what separates CRISPR diagnostics from simple isothermal amplification alone: the collateral-cleavage step adds a sequence-specific confirmation layer that suppresses false positives arising from primer-dimer or non-specific amplification products, a known limitation of amplification-only assays such as unconfirmed LAMP or RPA.

Guide RNA and Target Design for *Salmonella*-Specific Detection

Specificity depends on crRNA design against conserved, serovar-informative loci. Widely used targets include *invA*, *hilA*, *stn*, *pagN*, *siiD* and *sirA*, chosen for their conservation across *Salmonella enterica* subspecies and low cross-reactivity with other Enterobacteriaceae [16,17]. Because Cas12a requires an intact TTTV PAM immediately upstream of the protospacer, candidate amplicon regions must be screened computationally for compatible PAM sites before crRNA synthesis; sparse PAM availability in short, highly conserved regions can constrain design flexibility relative to PCR primer design. Iterative crRNA optimisation and PAM-independent design strategies have been used to sharpen single-nucleotide discrimination in Cas12a assays, a principle transferable to Salmonella serovar-level typing [18]. Dual- or triple-target crRNA panels, as used by Mao et al. and Akimbekova et al., reduce false negatives arising from single-locus deletion or mutation, since reliance on any one virulence gene risks missing serovars in which that locus is truncated or absent [16,17]. Guide length, mismatch tolerance and seed-region positioning further modulate the trade-off between sensitivity and single-nucleotide specificity, a balance that becomes especially important when an assay must distinguish a target serovar from closely related, non-pathogenic Enterobacteriaceae that share amplicon backbone sequence.

Amplification-Coupled and Amplification-Free Assay Formats

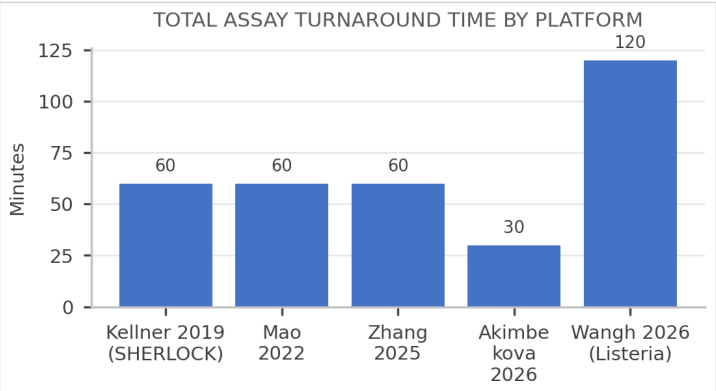
Most reported Salmonella-Cas12a assays couple isothermal pre-amplification with collateral-cleavage readout. RPA-CRISPR/Cas12a formats achieve limits of detection (LOD) around 50 CFU/mL within about one hour [16], with related RPA- or RAA-based designs reporting LODs from roughly 1-33 CFU/mL and total assay times of 30-90 minutes [19,20,17]. Recombinase polymerase and recombinase-aided amplification operate isothermally at 37-42°C, eliminating the thermal cycler and making them well suited to battery-powered or heat-block field devices. LAMP-CRISPR/Cas12a and Cas12b one-tube formats go a step further by combining amplification and detection in a single closed vessel, avoiding the aerosol-contamination risk of opening a tube between amplification and Cas12a addition, and report LODs near 2-100 CFU/mL with visual or lateral-flow readout [15,21]. Readout innovations diversify how the collateral-cleavage signal is captured: personal-glucose-meter quantification repurposes an inexpensive, ubiquitous consumer device for point-of-care numerical readout [22,20]; smartphone colorimetric imaging with metal-organic-framework-derived nanozyme signal enhancement enables quantification via a phone camera and app [23]; deoxyribozyme cascades provide label-free fluorescence amplification without antibody-based reporters [24]; and aptamer-based magnetic-bead enrichment ahead of lateral-flow Cas12a concentrates target bacteria from complex food matrices

before detection, a strategy demonstrated for the related pathogen *Listeria monocytogenes* [25]. Amplification-free formats, which couple Cas12a directly with Argonaute-protein cascade signal amplification, sacrifice some sensitivity for a further-reduced, single-step turnaround, targeting broader panels of foodborne pathogens without a separate pre-amplification step and thereby removing one common source of carry-over contamination [26]. Analogous lateral-flow Cas12a platforms validated against other pathogens, such as African swine-fever virus in whole blood, illustrate the same universal reporter-probe architecture applied across sample types and analyte classes, underscoring the modularity of the underlying chemistry [27].

Table 2: Representative CRISPR-Cas12a-based Salmonella detection platforms and reported performance.

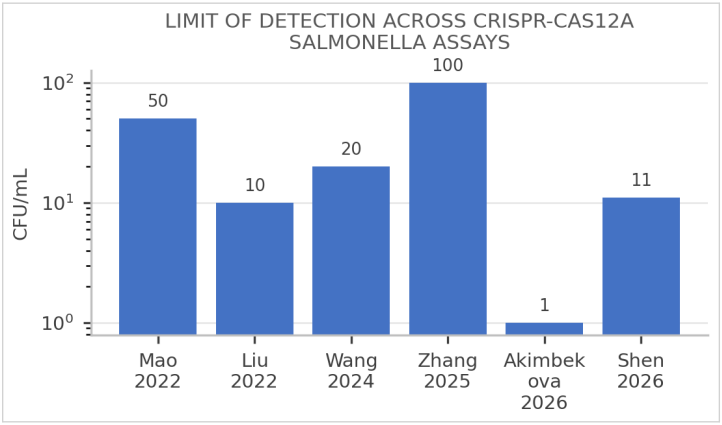
Platform/Study	Amplification	Readout	LOD	Assay time
Mao et al. [16]	RPA	Fluorescence	50 CFU/mL	~60 min
Liu et al. [19]	RPA	Colorimetric/ fluorescence	10 CFU/mL	~60 min
Zhou et al. [22]	None (direct)	Personal glucose meter	Not amplification-dependent	~40 min
Gong et al. [15]	LAMP (Cas12b)	One-tube fluorescence/lateral flow	2.3-23 CFU/mL	~60 min
Wang et al. [20]	RAA	Lateral flow / glucose meter	20-33 CFU/mL	~60 min
Zhang et al. [21]	LAMP	Fluorescence (portable device)	10^2 CFU/mL	~60 min
Akimbekova et al. [17]	RPA	Cas12a fluorescence	10^2 copies/reaction	~30 min

Figure 1: Total sample-to-result assay turnaround times reported for CRISPR-Cas-based diagnostic platforms [7,16,21,17,25].



Analytical Performance and Field-Deployment Readiness

Figure 2: Reported limits of detection (CFU/mL, log scale) for representative CRISPR-Cas12a Salmonella biosensing platforms [16,19,20,21,17,23].



Across the reviewed studies, amplification-coupled Cas12a assays for *Salmonella* consistently achieve LODs in the 1-100 CFU/mL range with total assay times of 30 minutes to 2 hours, approaching or matching qPCR sensitivity while avoiding thermal-cycling instrumentation [16,20,21,17]. This places them comfortably within the sensitivity window needed to flag contamination before short pre-enrichment steps would otherwise be required, and several studies report performance directly benchmarked against culture-based or qPCR reference methods with concordant results in spiked food matrices such as chicken, milk, egg and ready-to-eat produce. Lateral-flow and glucose-meter readouts enable equipment-light, point-of-need interpretation suited to processing-line or field settings, since neither requires a plate reader, fluorescence excitation source or networked computer [22,20]. Portable integrated DNA-extraction devices further shorten sample-to-answer time for on-site Salmonella Typhimurium testing by removing the need to ship samples to a laboratory extraction bench [21]. Remaining barriers to routine deployment include reagent cold-chain requirements for CRISPR ribonucleoprotein complexes and enzymatic amplification mixes, the need for standardised, harmonised crRNA panels across serovars and geographic strain collections, limited native multiplexing relative to PCR (most published assays detect a single target per reaction, whereas foodborne outbreaks often require simultaneous screening for *Salmonella*, *Listeria* and Shiga-toxin-producing *E. coli*), and sparse validation across the full range of real food matrices and inhibitory backgrounds encountered in routine sampling. Recent reviews catalogue these gaps across the broader CRISPR-Cas food-safety biosensor literature and note that regulatory acceptance as an official confirmatory method, rather than a rapid screening tool, will likely require larger inter-laboratory validation studies [12,13,14].

Conclusion

CRISPR-Cas12a collateral-cleavage biosensing, built on mechanistic foundations shared with SHERLOCK and DETECTR platforms, has matured over roughly a decade from a genome-editing curiosity into a practical toolkit for rapid *Salmonella* detection in food. Published assays now routinely combine isothermal amplification with Cas12a readout to reach single-digit-to-low-hundreds CFU/mL sensitivity within thirty minutes to two hours, using instrumentation ranging from smartphones and personal glucose meters to dedicated lateral-flow strip readers, and with amplification-free Argonaute-cascade designs beginning to close the gap in turnaround time further still. Taken together, the literature

indicates that the underlying chemistry is no longer the limiting factor; the remaining work is chiefly translational — harmonising crRNA panels across *Salmonella* serovars and geographic strain sets, validating assays across the breadth of real food matrices and inhibitory backgrounds encountered in routine sampling, extending multiplexing to cover co-occurring foodborne pathogens in a single reaction, and packaging amplification and detection reagents in ambient-stable, ready-to-use formats. Continued progress on these fronts will determine how readily CRISPR-Cas12a platforms move from published proof-of-concept studies into routine food-safety surveillance and regulatory-accepted testing pipelines.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Fabbri F conceived the review, conducted the literature search, and drafted the manuscript. Vogel K contributed to the literature screening and technical framing and critically revised the manuscript for important intellectual content. Delgado S contributed additional context and approved the final version for submission.

Ethics Statement

Not applicable. This is a narrative review of previously published literature and did not involve new experimental data, human participants, or animal subjects.

Data Availability

No new data were generated or analyzed for this review. All sources synthesized are cited in the reference list and are available through their original publishers.

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